An Exploration of Non-Syndromic Cleft Lip and Palate: The Australian and Filipino Experiences

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Thesis submitted January 2015

A thesis submitted for the degree of Doctor of Philosophy
of the Southern Cross University, Lismore, New South Wales, Australia

Declaration

I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the university's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. I certify that I have complied with the rules, requirements, procedures and policy of the University (as they may be from time to time).

Graeme H. Wallace OAM, KCSJ, MHSc.

Signature 

Date: January 12, 2015

Abstract

Cleft lip and palate is a birth anomaly affecting one in every 600-700 children born in Australia with even higher incidences in some developing countries. This thesis aimed to further the understanding of the possible cause and effect relationships, and also of the impact of having a cleft on the lives of the individual and their family. Being an explorative work the breadth of the research presented in this thesis was wide, eclectic and of an epidemiological nature. The literature suggested that a cleft may be either the result of a genetic aberration, a nutritional deficiency in the mother, or the mother being exposed to an environmental toxin during the pregnancy. Unfortunately none of the literature was conclusive and left significant areas open for further exploration.

In Australia there had been no studies conducted to determine what the general public knew about this birth anomaly. A study (Chapter 3) was conducted using both qualitative and quantitative methods, which showed that the level of knowledge was poor, and that this was independent of age, gender, and educational achievement.

Many studies have been undertaken by researchers focussing on the genes involved in facial morphogenesis, while other researchers considered nutrition, developing their work from the folic acid relationship with neural tube defects. Extensive research involving possible toxin involvement has been carried out but is limited mainly to tobacco use and alcohol. Unlike many previous studies, this thesis commenced by listening to people whose lives have been affected by clefting, and used that information to build a network of research questions to determine the direction for each study.

In particular this thesis presented the outcomes of nine studies that addressed the following aspects of clefting:

Young adults who were born with a cleft were interviewed to understand the issues that they faced growing up with this anomaly (Chapter 4). The study indicated that they were strong and confident in their adult life, and coped well with all of the issues they faced, being well supported by the medical practitioners they came in contact with, and most of all their family.

Parents of young adults born with a cleft faced difficult times, many not knowing what the future held for their child (Chapter 5). The surgical staff was crucial in providing support, but they also gained strength from a volunteer group called CleftPals. Seeing their child having to undergo yet another surgical procedure was always stressful, but all were now proud of their child's success in life.

A qualitative and quantitative study undertaken in the Philippines (Chapter 6) suggested that a nutritional deficiency may be involved, but the precise nature of that was difficult to determine as all of the women involved in the study were living in poverty, and many in highly polluted areas.

An Australian study (Chapter 7) which considered the lifestyle of mothers whose child was born with a cleft could not directly connect the mother's tobacco use, body mass index or the birth-weight of the child to clefting. Although the sample size was small, it did confirm, as in other research, that the anomaly affects more boys than girls (gender bias). This study did find a relationship between the stress level of the mother at or around conception with clefting, and thus modulation of the HPA axis causative effect.

A controlled study (Chapter 8) involving the analysis of a pregnant mother's hair blood and urine found that all pregnant mothers have elevated cortisol levels, and that mothers carrying a cleft fetus had even higher levels. This latter result may have arisen due to unplanned pregnancy and having available funds for another child, or just knowing that the child would be born with a cleft, leaving the women more anxious than other expectant mothers. While small in scope, this study did suggest that mothers who appeared not to be absorbing, or were underutilising the available zinc in their bodies, were more likely to have a cleft child.

A qualitative study (Chapter 9) undertaken with women who had already had a cleft child and who wanted another baby showed that if they changed their lifestyle and took the appropriate preconception supplementation it was possible to have a second baby free of this anomaly.

To try and duplicate a study undertaken by a researcher into neural tube defects, a controlled study, using mixed methods (Chapter 10) and analysing the hair of mothers whose cleft child was less than twelve months old, was undertaken in both Australia and the Philippines. The study failed to show that zinc deficiency was associated, probably because of the difficulty to accurately determine which part of the hair related to the point of conception.

A final study (Chapter 11) to ascertain whether hair mineral analysis was a useful tool, considered the case of a young woman who was having trouble becoming pregnant. This study, while not related to a cleft issue, involved all of the thoughts from the above studies. She took a broad-spectrum preconception supplement, additional zinc, and undertook activities to reduce her stress prior to conception. She was able to become

pregnant and nine months later had a female baby with no anomalies. This single case can not be considered conclusive regarding cause and effect, but merely introduces hair analysis as a potential diagnostic tool.

While larger studies will be required to substantiate the findings obtained in this thesis, the pathway leading to a child being born with a cleft appears to have multiple starting points, including

- traumatic stress (at or around conception) perhaps initiates a hormonal response which may alter the flow of nutrients to the developing fetus;
- environmental toxins which may include pharmacological drugs, agricultural chemicals and toxins present in drinking water, and;
- nutritional deficiencies, in particular zinc.

Recommendations have been provided for those planning pregnancy as well as for those seeking to pursue further research in clefting.

Acknowledgements

While this thesis covers work conducted over some years, the fact that it was done at all is credit to a large number of people, and I thank each and every one for their support and guidance.

Obviously without the support of my wife Sheila none of my pursuits would have been possible. She has never chided me for my involvement in all the activities that I have pursued and in many this meant total disruption to family life. Her patience with me has been heroic.

In my earlier years I was inspired by Dr Howard Bradbury, an eminent CSIRO scientist, and later Lecturer at the Australian National University, who insisted that the reason for our existence was to improve the life of others, and that research should be directed at this if it was to be of value.

Working in the United States Research Headquarters of W. R. Grace under the direct Supervision of William (Bill) Sturgis, later President of the Cryovac Division, I learned that creativity was the key to successful research, and he was instrumental in shaping my future career.

My supervisors, Professor Shi Zhou, Dr Jacinta Arellano, and the late Dr Tini Gruner (all at Southern Cross University), and Professor Claire Roberts (University of Adelaide) have been inspirational, demanding at times, but have challenged me to pursue my goal with enthusiasm and vigour.

Dr Heather Hancock, a supervisor from the University of Adelaide has given me great insight into the world of academia, and challenged me to present the best work that I can possibly produce.

Dr Simon Cichello reviewed the thesis with vigour and provided the moral support required to finalise this work.

My thanks go to Shalem Lee from the University of Adelaide, who painstakingly led me through the world of statistics, and enabled me to see the data from many different perspectives.

I am indebted to the participants who volunteered to be involved in this research, and who opened their hearts to me so that we could offer their stories to help others.

I acknowledge CleftPals and all their members for their untiring support.

My thanks are also extended to:

Mrs Wilma Dunne, Chair of Operation Rainbow Australia Limited and Mission Director.

Piet Treijtel in Cebu, in the Philippines, for his amazing working in assembling a database of children requiring cleft surgery, and in arranging for this to be undertaken.

Florita Monredondo (Mia), the amazing angel who travelled with us around Cebu and Bohol in the Philippines, and translated our English into all the varying dialects so that the women interviewed were well informed of the research being undertaken, and how the results may help to prevent cleft lip and palate occurring in the future.

Healthscope Limited, for their provision of the Gribbles Pathology Laboratory for blood and urine analyses, and to Dr David Deem for his quality management of these. Interclinical Laboratories, for providing financial assistance with the hair tissue mineral analysis, and to Zac Bobrov and Ian Tracton for their input in trying to make sense of the results.

Dr and Mrs Malvar and their staff at the General Malvar Memorial Hospital in Quezon City, for their support in allowing me access to their consenting staff and patients.

David Sison (now Professor of Nutrition at the Women's Hospital, Quezon City), and Melanie Cruz (now a General Practitioner at St. Luke's Hospital Quezon City), previously students at the University of the Philippines, who assisted with interviewing mothers whose children presented for cleft surgery in Quezon City, the Philippines.

Frank Parsons of Alternative Health Sciences, for his continued analytical questioning of what I was doing.

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Acronyms, Abbreviations and Glossary

ABS	Australian Bureau of Statistics
ACS	American Chemical Society (USA)
ACTH	Adrenocorticotrophic hormone
AD	Anno Domini
AIHW	Australian Institute of Health and Welfare
AKT	Protein kinase B
$AU	Australian currency – dollars
BCL3	B-cell leukemia/lymphoma-3
BMI	Body mass index
CleftPals	A support group located in the states and territories of Australia assisting families with a cleft child
CL	Cleft lip
CLP	Cleft lip and/or palate
cM	Genetic distance
CP	Cleft palate
CRH	Corticotrophin releasing hormone
CRP	C-reactive protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation in Australia
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DHA	Docosahexaenoic acid
DOH	Department of Health
DNA	Deoxyribonucleic acid
DVD	Digital video disc
ECN	Ethics committee number
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epitheliomesenchymal transformation
ERK	Extra cellular regulated kinase
FDA	Federal Drug Administration (United States of America)
FPA	Fertilizer and Pesticide Authority (the Philippines)
GABRB3	Gaba (A) receptor β3 subunit gene
GH	General health
GHW	Graeme Hamlyn Wallace, the researcher

GRB2	Growth factor receptor-bound protein 2
GTF	Glucose tolerance factor
HREC	Human Research Ethics Committee (Southern Cross University, Australia)
HPA	Hypothalamus, pituitary, adrenal axis
HTMA	Hair tissue mineral analysis
IR	Insulin resistance
IRF6	Interferon regulatory factor 6
IVF	In vitro fertilization
KCSJ	Knight Commander of St. John of Jerusalem, Knights Hospitaller
MAPK	Mitogena activated protein kinase
MBR	Medical birth records (Sweden)
Medicare	An Australian government health care scheme
MEK	Member of kinases in MAPK signalling pathway
MSX1	Muscle segment homeobox 1
MMP25	Matrix metalloproteinase 25
MMT	Methylcyclopentadienyl manganese tricarbonyl
ms	Millisecond
mTOR	Mammalian target of rapamycin
n =	Number equals
ND	Nutritional deficiency
NHMRC	National Health and Medical Research Council (Australia)
NSAID	Non-steroidal anti-inflammatory drug
NSW	New South Wales (state of Australia)
NTD	Neural tube defect
OAMFS	Oral and maxillofacial surgery
ORAL	Operation Rainbow Australia Limited
OAM	Order of Australia Medal
OS	Oxidative stress
PES	Physical/emotional stress
Peso	The currency of the Philippines
PhD	Doctor of Philosophy
PTSD	Post-traumatic stress disorder
PI3-K	Phosphoinositide 3-kinase inhibitor
RAF	Rapidly accelerated fibrosarcoma
RARA	Retinoic acid receptor alpha

RAS	Rat sarcoma (family of proteins involved in transmitting signals within cells)
RDI	Recommended daily intake
ROS	Reactive oxygen species
SHH	Sonic hedgehog
SOS	Son of Sevenless refers to a set of genes encoding guanine nucleotide exchange factors, cell signalling
SRCM	Swedish Registry of Congenital Malformations
STAT	Family of proteins activated by tyrosine phosphorylation
T	Toxins
TBX22	T-BOX 22, a transcription gene
TGA	Therapeutic Goods Administration (Australia)
TGF	Transforming growth factor
TGFR-A	Transforming growth factor receptor alpha
TGFB3	Transforming growth factor beta-3
USA	United States of America
$US	United States of America currency – dollars
WA	Western Australia (an Australian state)
XRF	X-Ray fluorescence spectrometry
8OHdOG	8-hydroxy d-oxy guanisine

Chapter 1 Surveying the Cleft Territory

1.1 Introduction

The theoretical framework for this primary health care research is eclectic, combining quantitative and qualitative approaches with a diversity of methodologies as appropriate for each research question investigated. The thesis represents a metaphorical rainbow with each investigation building the individual colours together through each chapter, and the thesis combining them to form the multi-coloured outcome. My journey from Operation Rainbow Australia Limited, a charity providing surgery to children born with facial anomalies and living in poverty in the Philippines, in which I had been involved for more than 20 years, has brought me to form another rainbow – this thesis.

Birth defects in humans[1] are common and indeed in Victoria, Australia, one in every 25 babies is affected by a birth defect (Riley and Halliday 2005; Riley and Halliday 2008). This represents approximately 2,700 babies each year born in Victoria with a defect of varying magnitude. A birth defect is defined as any abnormality that may be detected during pregnancy, at birth or in early childhood (Riley and Halliday 2005; Riley and Halliday 2008). This includes structural, functional, genetic, chromosomal and biochemical changes. Birth defects can range from minor to severe conditions and can affect fetuses and babies in a number of ways (Riley and Halliday 2008).

The most common congenital defects, as taken from the Victorian statistical report for the period 2002 to 2006 were (Riley and Halliday 2005; Riley and Halliday 2008):

- Hypospadias 70.1 :10,000
- Obstructive defects of the renal pelvis 39.7 :10,000
- Ventricular septal defect 34.7 :10,000
- Congenital dislocated hip[2] 30.2 :10,000
- Trisomy 21(Down Syndrome) 34.7 :10,000
- **Cleft Lip and Palate** **18.9 :10,000**
- Hydrocephalus 9.4 :10,000

[1] It is recognised that clefting may occur in some non-human species, however, this work did not attempt to investigate the extent to which this may occur nor the causal factors related in such cases.

[2] The data did not indicate whether this related to one hip joint or both.

- Trisomy 18 (Edward Syndrome) 8.6 :10,000
- Renal agenesis/dysgenesis 6.8 :10,000
- Neural Tube Defects 6.2 :10,000

A link has been well established between neural tube defects (NTD) and maternal nutritional deficiency of folate (Hibbard 1964; Tamura and Picciano 2006; Pitkin 2007). CLP which is the failure of the lip and palate to close is more prevalent in the community than the much more serious NTD (Riley and Halliday 2005; Riley and Halliday 2008). The question is if NTD's are related to a nutritional deficiency could CLP also be associated with the same, or another deficiency? In Australia there were a total of 1,803 live births between 1998 and 2008 affected by NTD (Macaldowie and Hilder 2011), however, as ultrasound scan resolution has improved over time some women may have elected to abort once their fetus was detected as likely to have an NTD, hence this figure could be understated. Since 1992 countries around the world have recommended that at least 0.4 mg of folic acid be taken daily prior to conception and for at least the first three months' gestation (Eichholzer, Tonz et al. 2006). The Australian government recommends 0.5 mg be taken daily for the same period (NHMRC 2013). In 2000 the Australian government mandated that folic acid be added to wheat flour. Prior to this, in 1997 there was voluntary fortification and earlier no fortification (FSANZ 2013). This policy followed that of other countries such as the USA, Canada, Chile and South Africa among others (CDC 2008). The Australian statistics (Macaldowie and Hilder 2011) indicate that the incidence has fallen from 6.6/10,000 in 1993 to 4.0/10,000 in 2006. Given the strong evidence for folate deficiency as a causal factor in NTD it is not surprising that researchers have also explored whether folate is implicated in other congenital anomalies such as CLP.

1.1.1 Clefting in Particular

Three forms of oral defects have been described in clefting: cleft lip (CL), cleft palate (CP) and CLP. The aetiology of non-syndromic CLP, a state where either the lip or palate or both have not been completely formed, has to date not been adequately determined. Non-syndromic clefting is where the clefts to the lip and/or palate are the only abnormalities, while in syndromic clefting the cleft occurs with other well documented abnormalities (Sayetta, Weinrich et al. 1989; Vallino-Napoli, Riley et al. 2004). Syndromes that are associated with clefting include Van der Woude (Van der Woude 1954), Aicardi (Umansky, Neidich et al. 1993), Treacher-Collins (So, Gonzales

et al. 2004) and Pierre Robin (Evans, Sie et al. 2011). In these cases the clefting is generally the least of the concerns (Sargent 2005).

CLP has been recognised for centuries. The earliest known reported reference to clefting comes from China (Boo Chai 1966; Rogers 1971) where it is claimed that in AD 390 an unknown surgeon successfully closed a cleft on an 18-year-old girl, Wei Yang-Chi, who later became the Governor General of several Chinese provinces. Shakespeare also refers to a "hare lip" in *A Midsummer Night's Dream*, (written between 1590 and 1596), suggesting that this condition was known at the time when this play was written (Rogers 1971). It has also been described (Wells 1971) that a physician in England wrote to medical colleagues in various parts of the world in 1904 asking them to report their observations on the incidence of clefts.

1.2 Background

The researcher and his family had not been affected by clefting nor had he been involved in other research in this area thus eliminating any bias in this regard.

It seemed to the researcher from a preliminary reading of the literature that many researchers were approaching the question of why birth anomalies, such as CLP, occurred, from possibly the wrong end – the anomaly or perhaps epigenetic change as the outcome – rather than seeking to find why this had occurred to begin with. Could there be a simple causal factor that initiated an epigenetic outcome? This thesis has been an investigative process to try and determine whether such a factor exists, and how having a CLP has been experienced by the individuals themselves, their families, and the broader Australian and international communities.

Considerable research has been conducted in the area of CLP, and many minds have considered the problem; indeed every parent who has had a child born with a derivative of this facial anomaly has asked, "Why has this occurred and why us?" Charitable groups have been sending teams of surgeons and nursing staff to developing countries such as the Philippines to perform surgery on the faces of CLP children whose families could not afford this. Organisations such as ORAL, Viet Kids Western Australia (WA), Interplast and Romac in Australia, Operation Smile in the United States of America (USA), and Smile Train in the United Kingdom (UK) are involved in this surgical work. There are no doubt other organisations that could also be mentioned. The results of the work of these organisations are clearly demonstrated in the before and after pictures of a young girl (See Figure 1.1) who lives in the Philippines. Unfortunately there is little

follow-up surgery or medical care for these children, and certainly no orthodontic work or speech therapy that would normally be required. Follow-up care is difficult for two reasons: the limited funds available, and the specialists required are not as accessible as the craniofacial surgeons who perform the repairs. It has been estimated by the ORAL Mission leader (Mrs Wilma Dunne, Mission Director, and Nurse in Charge of Operations – personal communication) that there are approximately 20,000 children in the Philippines whose cleft has not been treated in any way. This is also based on the information supplied to ORAL by groups such as Rotary and other service groups who seek out the children for surgery. In stark contrast, in Australia all children born with a cleft are provided with a Medicare card at birth to cover medical and dental expenses until they reach 26 years of age (Medicare 2012). Some Australian parents also make use of private health services and health professionals via their own health insurance but all children receive the care they need regardless.

Before the operation Immediately after the operation

Figure 1.1 A child with a unilateral cleft before and after surgery

Source: Photograph provided by ORAL with specific permission for publication from the girl's parents

Several of the ORAL surgeons had often been asked: "Why not undertake research rather than fix up the problem after the event?" Their answer in many ways was quite straightforward and logical: "We do what we do best, and that is to repair and correct the problems we see." Indeed, not only were these surgeons doing this in developing countries such as the Philippines, but by being able to undertake up to five procedures each day, in not necessarily optimum conditions, they were not only helping a child who previously had little future, but were also continuing to refine their own skills to enhance and improve their surgical techniques. An important by-product of the overseas

missions is that local nurses and surgeons receive training by working alongside these specialists, gaining invaluable skills and knowledge.

The purpose of this thesis has been to consider a number of potential associations which may lead to clefting, and possibly develop a plausible hypothesis that could be tested by others. In addition, there continue to be questions needing answers regarding how families deal with and respond to CLP, how children with a cleft have been affected during their developing years, and what the community knows about clefting. Perhaps more so in this last question, it should also be asked: Who actually cares about this problem?

An open-minded conjecture was made at the outset that the possible answer to why a cleft occurred could lie in one or in combination with the following three areas:

1. Genetics
2. Environmental toxins
3. Nutrition (initially assumed as maternal nutrition, perhaps a deficiency in micronutrients, however, both partners need to be nutritionally healthy.)

1.3 Research History

Research into CLP for the major part has described surgical procedures and the history of this is well presented in Perko's (1986) paper titled "The History of Treatment of Cleft Lip and Palate". Perko (1986) indicates that the first exact description of a cleft lip repair operation was given by Johan Yperman (circa 1325-1351). Operations continued spasmodically after that time but it was only after the introduction of anaesthetics that surgical procedures became the norm in treating CLP (Rogers 1971; Perko 1986).

Causal research into clefting appears to have started in the middle of the 20th century (Fraser 1969; Perko 1986) but no clear linkages between genetics, nutritional, or environmental factors have been established that point to definitive causal factors. Researchers (Fraser 1969; Zucchero, Cooper et al. 2004; Bille, Knudsen et al. 2005; Gahassibe, Bayet et al. 2005) refer to clefting as a multi-factorial problem meaning that the cause may be genetic, nutritional or environmental, or a mix of all three. Review of the literature indicates that genetics, familial associations, nutrition, maternal drug use, and environmental toxins have been associated with CLP. No conclusive connection to any one or a combination of these factors has been established (Cedergren and Kallen 2005; Hozyasz 2010).

In the following chapters of this thesis the issues relating to a child growing up with a cleft and the concerns their parents have in raising them are explored. In addition studies were conducted to try and ascertain why clefts were formed in the first instance. These quantitative studies involved taking blood, urine, and tissue samples from pregnant mothers for analysis and, in a later study, hair from mothers who had a child under 12 months old who was born with a cleft, for comparison with control mothers. Apart from the trauma (social, emotional, and psychological, as well as physical) that a family may go through caring for their child with a CLP, a financial burden must be borne either by the family or conversely by federal governments where national medical insurance systems are in place. For example, in the USA the estimated birth hospitalisation and post-birth costs for a child born with a cleft to the age of two years were between $US7,988 and $US30,869 in 2003 (Weiss, Kotelchuck et al. 2009). The lifetime cost of treatment for a child born with a craniofacial cleft could be as high as $US101,000 (Weiss, Kotelchuck et al. 2009). Obviously these costs have increased since then due to inflation. In Australia, as there is no official estimate for the cost, the likely costs were discussed with a craniofacial surgeon, a dental surgeon, and a speech therapist. Totalling the estimated costs for each, gave an approximate figure of $AU56,400 for each child until the age of 26 when the government support via Medicare ceases (Medicare 2012). The actual cost will vary greatly from child to child but does give some idea of the cost burden. Given that one in 700-800 children are born with a cleft in Australia (based on the total annual birth rate this equates to approximately 250 CLP children each year) the annual cost of treatment to society as a whole is approximately $AU141,000,000.[3] The psychological costs are immeasurable.

1.4 Literature Review

This research developed from a position of high-level curiosity, with little knowledge, to one where a greater level of understanding of the many issues, including possible causal factors evolved. Because of the evolutionary nature of this primary health care study, literature relating to each particular investigation is reviewed in the relevant chapters, rather than being incorporated in an all-encompassing section. This chapter therefore presents a literature review based on the researcher's introduction to clefting and the theoretical framework for the thesis.

[3] This figure is based on the information gained from personal contact with a craniofacial surgeon and other health professionals involved in treating children in Australia and then by multiplying that figure by 250, which is the estimated incidence of CLP based on the total Australian birth data.

1.4.1 Palate Formation

In order to understand clefting it is necessary to consider how the fetal palate forms and what the possible mechanisms are for malformation. There are several stages in the development of the mammalian palate. Initially there is a multiplication of the neural crest cells, and then their migration to the first visceral arch to form outgrowths of the maxillary process (Freni and Zapisek 1991; Moore and Persaud 2008).

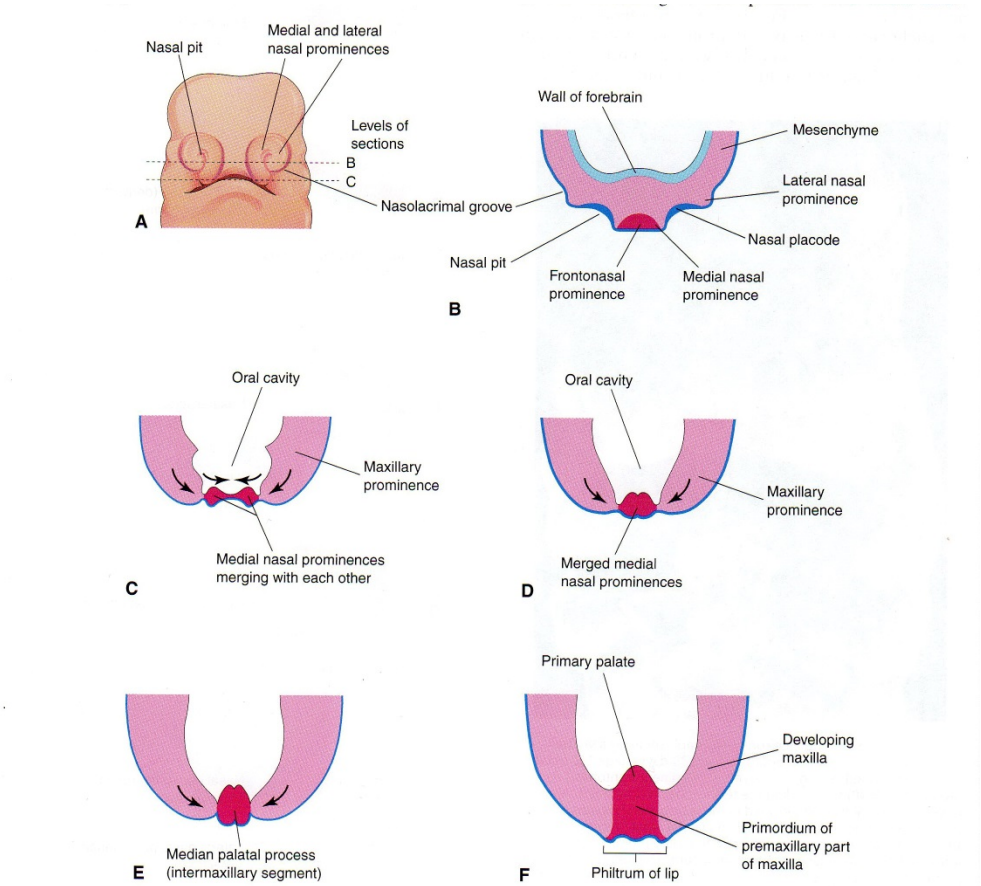


FIGURE 10-27. Illustrations of the early development of the maxilla, palate, and upper lip. **A,** Facial view of a 5-week embryo. **B** and **C,** Sketches of horizontal sections at the levels shown in **A**. The *arrows* indicate subsequent growth of the maxillary and medial nasal prominences toward the median plane and merging of the prominences with each other. **D** to **F,** Similar sections of older embryos showing merging of the medial nasal prominences with each other and with the maxillary prominences to form the upper lip.

Figure 1.2 Fetal lip and palate development

Source: This figure was published in the book *Before we are born* 7th. edition, Keith L. Moore and T.V.N. Persaud, illustrating fetal lip and palate development, p. 120. Permission has been granted by the publisher, Elsevier (November 21, 2014) for its reproduction in this thesis (Moore and Persaud 2008, p. 120).

Neural crest migration occurs from the middle of week three in human pregnancy to the beginning of week four (Freni and Zapisek 1991; Moore and Persaud 2008). In the next stage these buds grow alongside the tongue, forming vertical palatal shelves. Subsequently these shelves elevate above the tongue in a horizontal position, and then

finally the palatal epithelium differentiates to allow shelf fusion. Palatal shelves grow by cell replication in week six, and elevation occurs in week seven (Freni and Zapisek 1991; Moore and Persaud 2008). Interference in any one of these events is likely to result in the failure of fusion, and hence results in a cleft palate. What stops palatial fusion is unclear. Indeed, the blocking mechanism that appears to interfere with palatal growth may be the same cause of clefting in isolated CL (Freni and Zapisek 1991). It was hypothesised (Freni and Zapisek 1991) that an interruption in phospholipid metabolism may be the mechanism of action for some cleft palate teratogens, although this has not yet been substantiated. It is obvious however, that timing is extremely important in that the fetal face is being developed so early in the pregnancy, and in an apparently short time, thus creating a sensitive and time-critical window of effect.

1.4.2 Incidence of Clefting

1.4.2.1 Australia

In Australia birth defects registries are kept on a state by state and territory basis, with great variation in the quality of data recorded. In Victoria the incidence is set out in the table below.

Table 1.1 Victorian cleft data 1987-2006

Anomaly	**1987-1991 N/10,000**	**1992-1996 N/10,000**	**1997-2001 N/10,000**	**2002-2006 N/10,000**
Cleft Palate	6.6	8.0	7.8	8.9
Cleft Lip	4.0	3.6	4.1	3.9
Cleft Lip and Palate	6.6	6.2	7.1	6.1
Total	17.2	17.8	19.0	18.9

Source: (Riley and Halliday 2008)

As can be seen from Table 1.1 there has been no appreciable variation in total incidence from 1987 to 2006 in Victoria, except that there has been a variation in the type of cleft with CP having the largest increase over this period. These data appear to be consistent with those in many countries (Sayetta, Weinrich et al. 1989) where accurate statistics exist, and where no calamitous events such as earthquake, war or other pestilence have occurred (Sayetta, Weinrich et al. 1989).

Bell et al. (2013) determined that the incidence for CLP in Western Australia between the years 1980 and 2009 was 1.21 per 1,000 births and for isolated CP 1.012. They also found that the incidence among Aboriginal Australians was 1.9 and 1.3 times higher,

respectively. No reasons were given for the higher percentage of clefts within the Aboriginal community. Other anomalies were reported with 31% of CLP infants and 61% of those with an isolated CP.

1.4.2.2 CLP in the Philippines

The frequency of CLP in the Philippines has not yet been determined as the government does not keep a record of any birth defects. One analysis of Philippine hospital records (Murray, Daack-Hirsch et al. 1997) for 47,969 newborns over six sites between 1989 and 1996 found the overall rate was 1.94 per 1,000 for all clefting, with the higher risk of 2.3 per 1,000 for a second child with a CLP. This study is the only one to this date that indicates a risk level for a parent having two cleft children.

In the Philippines a total of 1,640,698 births were recorded in 2002. Sixty seven per cent of these births occurred in the home and only 28% in hospitals. Five per cent of births were not accounted for. Of the total births only 67% were attended by medical professionals who may have been health workers rather than midwives, doctors or nurses (Philippine DOH 2005). A health worker in the Philippines may not be a trained midwife, but a woman who has had experience in supporting women at birth, or who has assisted trained midwives to gain this experience (Grundy, Healy et al. 2003). This means that the hospital figures (Murray, Daack-Hirsch et al. 1997) represented births from parents of higher socioeconomic status who could afford to give birth in hospital. Information about place of birth in the Philippines is doubtful as hospital records provide only a part of the picture.

Anecdotal evidence, provided by Australian medical practitioners who have visited the Philippines on ORAL missions, and supported by local doctors at the General Malvar Memorial Hospital in Quezon City, has suggested that the occurrence of CLP is higher amongst the ‘poor’ (Dr P. L. Malvar, personal communication). This anecdotal evidence also suggests that the incidence in the Philippines is approximately three times higher than in countries such as Australia (ORAL 2006).

1.4.2.3 Other countries

The birth incidence of CLP has been reported to be 1/1,000 births among Caucasian[4] populations (Leck and Lancashire 1995). In Asian populations, the reported incidence ranges from 1.11 to as high as 2.06/1,000 births (Hu, Li et al. 1982; Natsume and Kawai 1986; Marazita, Hu et al. 1992; Natsume, Suzuki et al. 1998). In contrast, the incidence in African American populations is the lowest at 0.56/1,000 (Croen, Shaw et al. 1998). The researchers believe that the lower figure in this cohort was primarily explained by a lower prevalence of isolated cases when compared to the white population. Croen, Shaw et al. (1998) investigated variations in the prevalence of oral **cleft** anomalies according to parental race and ethnicity and maternal country of birth. They analysed a cohort of 2,221,755 live births and fetal deaths between 1983 and 1992 in residents of California, differentiated between races, and also confirmed where study participants were born. There were significant differences in occurrence of CLP between particular racial groups suggesting that there may be a genetic and/or socioeconomic factor involved.

A comprehensive review (Vanderas 1987) investigated the incidence of CLP in a wide range of countries and ethnic groups. The incidence varied from 0.9 to 2.69/1,000 live births in white populations and from 0.18 to 1.67/1,000 in black populations. In the American Indian population it ranged from 0.79 to 3.74/1,000 births and in the Chinese population from 1.56 to 4.04/1,000 births. They also found that clefting in stillbirths across all races ranged from 2.72/1,000 to 6.43/1,000 live births (Vanderas 1987).

Researchers in the USA (Parker, Mai et al. 2010) estimated that the incidence of CP without CL was 0.64/1, 000 births and for CL with and without palate clefting 1.06/1,000 births. This is consistent with other Western countries (Xiao 1989; Cooper, Stone et al. 2000; Marazita, Field et al. 2002; Carcini, Rullo et al. 2003; Elahi, Jackson et al. 2004; McLeod, Urioste et al. 2004; ABS 2005; Mathias, Fiorrester et al. 2006). Other researchers in the USA (Weiss, Kotelchuck et al. 2009) found that clefting was not associated with low birth weight (86%); that it was not associated with small for gestational age babies (80%); that the male to female ratio of incidence was 54:46; that it occurred predominantly in singleton births (95%) and mostly in white non-Hispanic races (75%).

[4] This study was undertaken in England and the data between 1963 and 1979 were divided into categories based on ethnicity, namely British, Irish other Europeans, and other 'whites'. After 1979 they were all grouped into one classification, this being 'Caucasian'.

In Poland the incidence of CLP has been found to be 1.7 per 1,000 live births (Hozyasz 2010). This figure is qualified in that the incidence is said to vary with geographic location, racial and ethnic background, and may be affected by environmental factors, exposure to toxins such as mercury, lead, cadmium and arsenic, and nutritional deficiencies such as zinc in combination with high copper (Hozyasz 2010).

In China (Xiao 1989) the CLP incidence is stated to vary between 1.34 cases per 1,000 births to 1.82. In rural areas the incidence was 2.08/1,000 compared to 1.69/1,000 in urban communities. In a sample of 2,265 Chinese children born with a cleft in hospitals, CLP represented 61.3% of all cases, with isolated CL 30.5% and isolated CP 8.2%. The sample was taken during a period of 12 months (October 1986 to September 1987), and 1,243,284 live and stillbirths were monitored from 945 hospitals of 29 provinces, cities, and autonomous regions of China (Xiao 1989).

In Pakistan (Elahi, Jackson et al. 2004) the incidence of CLP is 1.91/1,000 births of which isolated CL was 42%, isolated CP 24% and CLP 34%. Only 32% of mothers whose child was born with a cleft received any antenatal counselling or medical care during pregnancy. Only 28% of mothers who had a child with a cleft received any nutritional supplementation compared to 59% of mothers whose child did not have a cleft. While this study (Elahi, Jackson et al. 2004) provided added information regarding antenatal care, supplementations and counselling it was unable to clearly identify the type of supplementation given, the timing of it being given and therefore could not draw a conclusion on whether a different supplement or combination of nutritional elements may have helped to prevent the clefts.

Based on the above statistics, clefting would seem to occur from 1 in 800 live births to in excess of 2 per 1,000 live births overall globally. In some countries such as the Philippines the figures quoted above may be suspect due to poor statistical records or the fact that births are often not reported to authorities. Table 1.2 provides a summary of these statistics.

Table 1.2 Summary of some international incidence data of CLP

Country	Incidence of clefting per 1,000 births
African American	0.56
American Indigenous Indian	0.79-3.74
Asian populations (as a group)	1.11-2.06
Australia	1.2-1.8
Chinese (overall) Chinese, urban Chinese, rural	1.56-4.04 1.34 2.08
Pakistan	1.91
Poland	1.7
The Philippines	1.94

1.4.3 Genetic Links

1.4.3.1 Contributing chromosomes and/or genes

Research has been directed at both syndromic CLP and non-syndromic CLP with many researchers attempting to identify the major genes underlying these birth defects. Loci on chromosomes 2, 4, 6, 17, 19 and 22 have been studied with varied and inconclusive results. The results of these studies were reviewed by a number of researchers to assess the current state of knowledge at that time (Murray 1995; Wyszynski, Beaty et al. 1996; Carinci, Pezzetti et al. 2000) finding genetic associations in syndromic CLP, the most notable being in Van der Woude syndrome which appears to be caused by deletions in the chromosome band 1q32. Linkage analysis has confirmed this chromosomal region as the disruptive gene site (Cohen 2002). Linkage studies have also raised the possibility that the degree of phenotypic expression of a gene defect at this locus may be influenced by a second modifying gene that has been mapped to chromosome band 17p11 (Smith, McGavran et al. 2005).

Further studies have been carried out in relation to chromosome 17. Peanchitlertkajorn, Cooper et al. (2003) studied birth records for the years 1980 through to 1987 at ten hospitals in Shanghai (China) across eight city districts to identify CLP cases. Thirty six multiplex families (parents and/or children) were found with non-syndromic CLP, 13 with CL and 23 with CLP. The size of the families varied from 4 to 26 members (parents and children), with an average of 13. Blood samples were taken from 310 members of the 36 multiplex families, genomic DNA was then extracted and analysed by two independent laboratories for genetic markers between D17S1308 and D17S928

at ten cM[5] intervals. The analysis consisted of extracting the genomic DNA from the venous blood samples which the laboratories then amplified by polymerase chain reaction, using primers for the various genetic markers that had been chosen (Peanchitlertkajorn, Cooper et al. 2003). The results showed considerable variation, depending on the linkage or association method employed in the analysis. However, chromosome 17 in the region of the Retinoic Acid Receptor Alpha (*RARA*) locus seemed to yield consistently positive results (Peanchitlertkajorn, Cooper et al. 2003). The researchers therefore hypothesised that the genes on chromosome 17 play a role in the aetiology of non-syndromic CLP. As only a hypothesis resulted from this study further research is certainly required to confirm or refute the relationship, however, their work is promising.

Vieira, Orioli et al. (2003) studied a South American population from eight countries across Latin America and provided evidence of an association with genetic variation at two loci, *MSX1* and *TGFB3*. According to their study the locus at *MSX1* showed the strongest association in cases where there was only a CL. The locus at *TGFB3* appeared not to be associated with CL, however, it was suggested that there could be an interaction between the alleles at *MSX1* and *TGFB3* which could account for other variants of clefting (Vieira, Orioli et al. 2003). This work, while covering 217 cases involving CL, CLP and CP patients, supports other work that there may be differing aetiologies for each of the clefting anomalies. Further research on these two loci is required to confirm these findings.

A study in a Filipino population (Lidral, Murray et al. 1997) concluded that *TGFB2* and *MSX1* failed to show evidence of an association with non-syndromic CLP. This was a case-controlled study involving the collection of blood samples from children and adults awaiting surgery for CLP under the auspices of Operation Smile, a charity providing these services, with the DNA being subsequently extracted and analysed for allelic variants of the candidate genes. Initially it was thought that other genes may also have been involved, but these showed no association with either CLP or CP (Lidral, Murray et al. 1997). Two differing methods of collection were used: the first being liquid blood samples and the second using blood deposited on filter paper. This latter method is claimed to provide high quality results for the analysis of amino acids and DNA and is

[5] cM is the symbol for genetic distance, or map distance. One cM (centimorgan) is equivalent, on average, to a physical distance of approximately 1 megabase in the human genome (NIH. (2014). "Centimorgan definition." Retrieved June 12, 2014, 2014, from http://ghr.nlm.nih.gov/glossary=centimorgan.

said to have been used since the early 1960's (Mei, Alexander et al. 2001). Unlike Vieira, Orioli et al. (2003) mentioned above, there was no attempt to separate out the CL patients to ascertain if that group had an association with *TGFB3*. However, the researchers (Lidral, Murray et al. 1997) did not rule out that future studies might show a link with that allele. They did conclude that the collection of blood on filter paper was a cheaper alternative sampling method with similar reproducibility to fresh blood collections. This may be useful in future investigations if blood sampling is considered, particularly in resource depleted locations, however, for genome-wide studies larger amounts of DNA might be required.

Several of the researchers involved in the early study outlined above (Vieira, Orioli et al. 2003) have continued research in this area (Vieira, Avila et al. 2005). Their more recent study involved 91 CLP cases from the Philippines and 93 from Iowa together with 186 matched controls. The researchers found that point mutations in the candidate genes *FOXE1, GL12, MSX2, SKI, 7ATB2,* and *SPRY2* appeared in aggregate to contribute to as much as 6% of isolated CLP cases. The researchers indicated that a major challenge in their study was the frequent absence of the cleft phenotype in near relatives thus being suggestive of gene-environmental interactions (Vieira, Avila et al. 2005).

A further study (Zucchero, Cooper et al. 2004) that considered families who had ancestry in East Asia (including Japan, Vietnam, China, and the Philippines) concluded that *MSX1* and *IFR6* seemed to have a measureable role (up to 12%) in the causation of CL, CP or CLP. The researchers also indicated that mutations in other genes resulted in syndromes including *TBX22, P63* and *FGFR1*. While they concluded that DNA-sequence variants associated with *IFR6* were major contributors to the formation of CLP there was strong evidence of an over-transmission of the valine allele in the sample studied and that it may be related to the *IFR6* (CLP) outcome (Zucchero, Cooper et al. 2004). The researchers did not indicate the socioeconomic status of the participants or the environmental conditions in which they lived.

A Belgian study (Gahassibe, Bayet et al. 2005) supported the work of Zucchero, Cooper et al. (2004), however, they concluded that after implicating *IFR6* in the complex aetiology of CLP they were no closer to understanding which were the target genes or which pathways were implicated in the transcription of those genes. Research reported in 1999 suggested that there were independent aetiologies for the different types of

clefting (Brewer, Leek et al. 1999). This research team (Brewer, Leek et al. 1999) studied two unrelated children born with CP only, who both had strikingly similar clinical features. Both children had CPs, facial dysmorphism and mild learning disabilities. Chromosome painting studies suggested that a translocation had occurred in chromosome two, while further studies pointed to the site being *2q32* (Brewer, Leek et al. 1999).

Researchers (Ding, Wu et al. 2004) indicated that *PDGF-C* is a new and independent gene and the processing enzyme might be controlled by other CLP associated genes that could indirectly influence *PDGF-C* signalling. A section on Chromosome 4 where the *PDGF-C* gene is mapped showed a strong linkage association with CLP (Ding, Wu et al. 2004; Tang, Arjunan et al. 2010).

Table 1.3 (Krapels, Vermeij-Keers et al. 2006) presents an overview of the genes associated with the development of the primary and secondary human palate. While the table lists the genes that are active in facial differentiation determining why its function appears to be impaired causing a cleft still remains unclear.

Table 1.3 Genetic involvement in development of the human primary and secondary palate

Overview of genes involved in the development of the human primary and secondary palate		
	Early Embryonic Period	Late Embryonic Period
Primary Palate	Fusion Process Outgrowth of facial swellings, (*TGFA, TBX22?, RARA, SHH, IRF6?)* Opposition and adhesion, (*TGFA, TGFB3?, GABRB3?, IRF6?, SHH*) Epithelial Plate, EMT and apoptosis (*TGFA, TGFB3?, BCL3?, GABRB3?, MMP25, RARA?*)	Differentiation Mesenchyme Formation of lip (*TGFA*?) and alveolus Bone centres premaxilla (*MSX1*) and maxilla Musculature
Secondary Palate		Fusion Process Outgrowth and elevation swellings (*TGFA, MSX1, TBX22, RARA, SHH*) Opposition and adhesion (*TGFA, TGFB3, GABRB3, IRF6, SHH*) Epithelial Plate, EMT and apoptosis (*TGFA, TGFB3, GABRB3, BCL3?, MMP25, RARA?*) Outgrowth maxilla Bone centre or palatinum Musculature

Source: (Krapels, Vermeij-Keers et al. 2006)

Note: Question marks after certain genes indicate that the expression of these genes during the developmental process is still uncertain.

Key: *BCL3*: B-cell leukaemia/lymphoma-3; *EMT*: epithelial mesenchymal transition; *GABRB3*: Gaba (A) receptor B3 subunit gene; *IRF6*: interferon regulatory factor 6; *MSX1*: muscle segment homeobox 1; *MMP25*: matrix metalloproteinase 25; *RARA*: retinoic acid receptor alpha; *SHH*: sonic hedgehog; *TBX22*: T-BOX 22; TGFA: transforming growth factor alpha; *TGFB*3: transforming growth factor beta-3 (Krapels, Vermeij-Keers et al. 2006).

The genetic studies tend to show that there are possibly differing aetiologies for each of the three clefting anomalies and that while chromosome 17 is perhaps the major area of involvement for lip, and combined lip and palate defects, chromosome 2 may be an area for further study in relation to isolated palate anomalies (Murray 1995; Wyszynski, Beaty et al. 1996; Carinci, Pezzetti et al. 2000). Chromosome 4 appears also to be an area of interest for further study. The gene *TBX22* on the other hand seems to be a candidate linked to palate development (Zucchero, Cooper et al. 2004). Others (Braybrook, Doudney et al. 2001) maintained that *TBX22* is a major gene determinant crucial to human palatogenesis.

Genes, however, operate optimally within an environment where the appropriate nutritional elements are available, and can then be organised to form the various enzymes or proteins and co-factors required for replication and differentiation of cells and tissues (Waterland and Jirtle 2003). Research into gene – environment interactions is essential to understand why some individuals with risk alleles do and others do not experience a defect or disease. It seems evident that a genetic mutation may occur, but the question regarding its primary cause appears to be elusive. On the other hand it may be possible that a minor genetic variant pre-existing in an individual could be responsible for specific nutrients not being absorbed, and not being available to the replication (transcription) machinery when the face of the fetus is developing (Szabo de Edeleny, Goumidi et al. 2008). It appears that there is little cohesive research data that conclusively identify specific nutritional elements and the related genes involved in CL, CP or CLP.

1.4.4 Clefting in Relation to Other Birth Defects

Shaw, Carmichael et al. (2004) considered all congenital malformations in the USA and tried to establish links between them. They reviewed 3,548,991 live births and 23,239 stillbirths that occurred between the years 1983 and 1997 in Californian non-military hospitals. Shaw, Carmichael et al. (2004) determined that there were structural congenital malformations in 91,888 (2.57%) births which included 2,343 CP (0.0656%) and 4,072 CLP (0.114%).[6] No differentiation was made between syndromic and non-syndromic clefting. They looked for associations between CLP and other (chromosomal and non-chromosomal) birth abnormalities and found that clefting was more commonly linked to respiratory anomalies than to spina bifida. If chromosomal abnormalities (10,702) were excluded this did not change the relationship between CLP and other birth anomalies (Shaw, Carmichael et al. 2004).

Vallino-Napoli, Riley et al. (2004) investigated stillbirths in Victoria, Australia, between 1983 and 2000 and determined that CLP was higher among stillbirths and neonatal deaths than in the general live birth population. As these deaths would not be caused by CLP alone it must be assumed that other medical/developmental issues were the cause.

1.4.5 Familial Relationships with Clefting

There appear to have been few studies carried out to determine whether there is a familial relationship in clefting. A Polish group studied the clefting association between parent and child (Kot and Kruk-Jeromin 2003) in 540 children with CLP family histories. This sample was taken from 3,180 children who had been surgically treated at the Polish Lodz Medical Academy between the years 1972 and 2001. From this study two genetic groups were confirmed, each with a different risk of the recurrence of the clefting defect. One group contained those with CLP and the second with CP only. Kot, Kruk et al. (2003) found there was an association between the type of cleft the parent had and that of the child. Of children whose mother had a CL, 60% of boys had a similar cleft, but only 15% of girls. In mothers who had a CLP, the same type of defect was found in 70% of boys and 18% of girls. When fathers were taken into account similar male dominated results related to clefting were obtained. However, when the group where mothers and/or fathers had isolated CP were analysed, there were

[6] While these figures may seem small in percentage terms the total for CP and CLP is 1.8 children with clefts per 1,000 births which is generally consistent with other data quoted in this thesis.

significantly more girls than boys with the same defect as either parent (Kot and Kruk-Jeromin 2003).This research indicates some familial and sex chromosome links and suggests that girls may be more prone to CP while boys are more likely to have a CL or CLP. Perhaps the aetiology of CP is different to the other clefting forms.

Most gene association studies do not identify single gene variants that cause a particular disease. Indeed these studies usually identify risk alleles that may be present in some but not all of those afflicted, as well as those not afflicted. These gene variants are thought to increase disease risk in particular environments in which gene-environment interactions are said to occur. Peanchitlertkajorn et al. (2003) suggested that the cause of clefting is not merely genetic but a more complex mix involving environmental factors such as cigarette smoking, exposure to agricultural chemicals, and possibly antiepileptic drugs or alcohol consumption during pregnancy. These may interact with a gene modulating its expression while not altering a change in the DNA sequence, particularly in non-syndromic CLP. Alteration of gene expression in this manner is termed epigenetic change. Environmental factors were also suggested to be involved in these malformations by Wyszynski et al (1996) who also proposed that gene expression could be influenced by various external factors such as agricultural chemicals, alcohol consumption, medication use and cigarette smoke.

1.4.6 Influences During Pregnancy Related to Clefting

Felix-Schollart et al. (1992) in their study on the reproductive history of mothers with solitary, non-syndromic CLP, asked the question: “Are there three types of oral clefts with respect to the reproductive history of the mothers of the affected children?” They considered the occurrence of fetal loss, health and drug (medication) consumption of the mother during pregnancy, and gestation. Felix-Schollart et al. (1992) noted that vaginal bleeding in the first trimester of pregnancy was found to be related to CLP but not to CL or CP. They speculated that vaginal bleeding was a symptom of the same phenomenon as spontaneous abortions. They further observed that the taking of benzodiazepines and analgesics during the first trimester was associated with both CL and CLP, but much less significantly associated with CP further suggesting that the aetiology of the three cleft types may be quite different. Dixon, Marazita et al. (2011) in their paper understanding genetic and environmental issues also support the concept of there being differing aetiologies for CP and CLP.

1.4.7 Pharmacological Drug Use During Pregnancy

A Swedish study (Kallen 2003) considered the effect of various pharmacological drugs prescribed to women during pregnancy. This study reviewed the births listed in the Swedish Medical Birth Registry that records the medical data on 99% of births in that country. The study was restricted to births between July 1995 and December 2001 and compared the pharmacological drug use of women whose children were born with identified facial clefts to that of all other women in the register during that period. Pharmacological drug use during pregnancy was reported by 261 mothers of infants with clefts (25%) and 149,932 (26%) other women who had given birth during the study period (Kallen 2003).

Of the pharmacological drugs identified only naproxen and naproxen sodium achieved a statistical significance with a risk ratio of 2.72 (95% Cl 1.17-5.36) for a cleft (Kallen 2003) The researchers did not define the type of cleft and suggested some caution be taken with the results due to the small sample size. Naproxen[7] belongs to the non-steroidal anti-inflammatory drug pharmacological category (NSAIDS) (FDA 2004). These drugs are used for the management of mild to moderate pain, fever and inflammation. NSAIDS act by reducing the level of prostaglandins (Vane and Bottling 1998) (among others) within the body that are responsible for pain, fever and inflammation. Naproxen blocks cyclo-oxygenases (both COX 1 and COX 2) (Vane and Bottling 1998) that catalyse the formation of prostanoids including prostaglandins, resulting in a lower concentration and therefore lower levels of pain, inflammation and fever. One of the side-effects of Naproxen is that it may increase the blood levels of lithium by reducing its excretion via the kidneys, thus potentially leading to lithium toxicity (Waring 2006). There is no evidence that research has been undertaken to determine how this may affect a fetus. Likewise, there appears to have been no research conducted to establish the safety of Naproxen for use during pregnancy although a USA Food and Drug Administration information sheet (FDA 2004) does suggest that birth anomalies may occur if used during pregnancy. Other drugs found to have a high risk ratio for CLP were sulfasalazine and glucocorticoids (Kallen 2003). The conclusion drawn, however, was that in general in this Swedish population the consumption of pharmacological drugs during pregnancy did not directly relate to a higher incidence of

[7] In Chapter 10 of this thesis mention is made of billboard advertising of this drug attached to a civic building in Cebu, the Philippines, where clefting is anecdotally higher than in other countries.

CLP, but risk ratios were increased for these drugs: Naproxen, sulfasalazine and glucocorticoids (Kallen 2003).

A less comprehensive study (Felix-Schollart, Hoeksma et al. 1992) was conducted in the Netherlands involving 87 mothers of children with orofacial clefts. Using a standardised interview the prescribed pharmacological drugs taken by the mother during the first trimester of pregnancy were examined. This study, which considered pharmacological drug groups rather than individual drugs, suggested that there was a greater risk with benzodiazepines and analgesics (specific types not defined) and that these groups were associated with CL and CLP but not with CP.

The effects of oral retinoids on pregnancy have also been considered (Chan, Hanna et al. 1996). The Australian Drug Evaluation Committee (ADEC) lists only five drugs in *Medicines in Pregnancy* as category-X drugs (Therapeutic Goods Administration) (T.G.A. 2012). The TGA is the Australian government controlled body charged with the regulation of all therapeutic goods and devices sold in Australia. It is funded purely by taxes placed on industries operating in this market segment. These category-X drugs are considered to have a high risk of causing permanent damage to the embryo and therefore should not be used during pregnancy or when there is a possibility of pregnancy. The oral retinoids, isotretinoin (Roaccutane) and etrinate (Tigason) are two of the five category-X drugs listed. The drug acitretin (Neotigason) was also suggested to be included as a category-X drug (Chan, Hanna et al. 1996). A personal conversation with the TGA in 2013 confirmed that this is now a category-X drug, however, they could not advise when this occurred. Their website implies 2008 (T.G.A. 2010).

A low dose of vitamin A is essential for the maintenance of female reproductive function and for the differentiation of epithelial tissues (Clagett-Dame and Knutson 2011). Synthetic retinoids, which structurally resemble naturally occurring vitamin A, contain very high doses of the vitamin (compared to what is normally found in a supplement). The most serious side effects were observed when they were used in animal studies where the drugs appeared to interfere with neural crest cells during development and caused craniofacial defects. When the drugs were made available strong warnings were given against their use during pregnancy (Johnston, Morriss et al. 1977). CLP has been associated with the use of these pharmacological drugs during pregnancy and care must be taken by medical practitioners when prescribing them to women during the child bearing years. The TGA had previously produced booklets for

practitioners outlining this, however, the last publication was dated 1998 and since then they have directed medical practitioners and midwives to their website, (T.G.A. 2013).

1.4.8 Environmental Toxins

1.4.8.1 Tobacco smoking

A common environmental toxin is tobacco smoke. There have been many studies carried out over the past decades with most concluding that mothers who smoke during pregnancy give birth to babies with a lower birth weight (Kallender and Kallen 1971; Shivenck and Salafia 1999; Azulay Chertok, Luo et al. 2011).

Attempts have been made to connect orofacial deformities and cigarette smoking, showing mixed results because of the challenge to accurately determine the quantity of tobacco smoke inhaled. A study of 1834 CLP births between 1983 and 1992 from two birth registries in Sweden (the Swedish Registry of Congenital Malformations – SRCM – and the Medical Births Registry – MBR) investigated smoking habits in pregnant women (Kallen 1997). Since 1983 the MBR has contained information on maternal smoking habits in early pregnancy. As antenatal care is free in Sweden, most women first attend an antenatal clinic during pregnancy at 10-12 weeks' gestation where they are interviewed by a midwife and their smoking habits are recorded. The SRCM likewise covers all of Sweden and has a coding system for congenital deformities. In this study the CLP cases were divided into four groups, CL, CLP, CP and Pierre Robin Syndrome. Pierre Robin Syndrome is characterised by retrognathia or micrognathia, glossoptosis and airway obstruction (Sargent 2005). In almost 50% of the patients with this syndrome there is also a cleft palate present (Sargent 2005). All infants with a known chromosomal abnormality or with unknown smoking exposure in early pregnancy were excluded in subsequent analyses.

The subsequent statistical analysis showed a closer relationship between CP and smoking than for CL and CLP. This increased significantly for women who smoked more than ten cigarettes per day, compared with those who smoked less (Kallen 1997). One of the problems not addressed was passive smoking and whether or not those women who did not smoke themselves lived in a household where others smoked around them. Likewise, it did not investigate the women's work environments or social activities, where passive smoking might occur. The study showed no relationship between smoking and Pierre Robin Syndrome (Kallen 1997). No effort was made to

determine which elemental toxin in the cigarette smoke may be causing damage to the developing fetus.

1.4.8.2 Dichlorodiphenyltrichloroethane/dichlorodiphenyldichloroethylene

One of the most widely spread environmental toxins globally today is dichlorodiphenyltrichloroethane/dichlorodiphenyldichloroethylene (DDT/DDE) (Iwata, Tanabe et al. 1994), which has been detected in human adipose tissue in Poland (Tanabe, Falandysz et al. 1993), in fish in the Canadian Arctic area (Muir, Ford et al. 1992), in rice paddy soil in southern India (Ramash, Tanabe et al. 1991), in foodstuffs in Thailand (Tanabe, Kannan et al. 1991), and in association with the impairment of spawning in a variety of fish in coastal Southern California (Hose, Cross et al. 1989). Iwata, Tanabe et al. (1994) found that the levels of DDT and its derivatives were highest in developing countries. In the first Philippine study for this thesis Dr Sol Soloy from the Bureau of Agricultural Research within the Department of Agriculture in Manila indicated that chemicals including DDT were often available on the Philippine black market. A study conducted in the Gippsland Lakes in Victoria, Australia, found black bream with high levels of DDT/DDE and mercury (Fabris, Theodoropoulos et al. 1999). Yet these products had been deregistered for use in Australian agriculture in 1989 (Lee 1990). This shows that chemical residues remain a risk long after legislation has been enacted to remove them from the environment.

Research has been carried out into the effects of DDT exposure on women of child-bearing age and several links have been made (Cohn, Cirillo et al. 2003), particularly because DDT and its derivatives have estrogenic effects. Cohn, Cirillo et al. (2003) studied the effects of DDT exposure in 289 women in California born between 1960 and 1963 to determine their likely fertility. The women's mothers were all enrolled in the Kaiser Permanent Health Plan and the Child Health and Development studies and all had detectable blood levels of DDE and DDT. The researchers adjusted their results to eliminate any errors caused by ethnic origin, frequency of sexual intercourse, maternal age, education, body mass index, tobacco, or maternal use of alcohol. Cohn, Cirillo et al. (2003) found a direct relationship between an increased fertility of daughters whose mothers had high serum levels of p,p'-DDE, the analogue of DDT (p,p'-DDE is benzene, 1,1-(dichloroethenylidene)bis[4 chloro] and is also known by the synonym p.p' dichlorodiphenyldichloroethylene). p,p'-DDE is a recognised carcinogen and a suspected endocrine disruptor (Kelce, Stone et al. 1995). The researchers (Cohn, Cirillo

et al. 2003) indicated that increased fertility in daughters was an unexpected result as the opposite was found in the daughters of mothers who had high levels of DDT. They speculate that the anti-androgenic activity of DDE may mitigate harmful androgen effects on the ovary during early fetal life. The opposite effects of p.p'-DDE and p.p'-DDT might explain why large changes in human reproductive performance had not been noted after the introduction of DDT worldwide.

A collaborative study (Longnecker, Klebanoff et al. 2001) was carried out in the USA, involving a subset of 2,380 children[8] from 44,000 children born between 1959 and 1966, whose mothers' DDE serum levels were measured during their pregnancy. Of the 2,380 children, 361 were born preterm and 221 were small for gestational age. Longnecker, Klebanoff et al. (2001) found that the odds ratio for preterm birth and infant mortality increased steadily with increasing concentrations of DDE, and after excluding preterm births. It was discussed earlier in this review that there was a higher incidence of clefting amongst preterm births and while no direct association has yet been made between DDT and other organochlorides, the fact that they impact on increasing preterm births may implicate these toxins in this multi-factorial clefting problem. Studies on the mutagenicity of DDT (Smith 2000) have to date not yielded clear results, in spite of the fact that CLP has been listed as a possible outcome from its exposure. Smith (2000) proposed that further research is required to confirm or negate the relationship. Chong (2011) also cites DDT as being possibly linked to CLP.

1.4.9 Nutritional Associations with Clefting

In the section covering genetic associations it was suggested that for genes to operate optimally their expression and function is modulated by the micronutrient environment in the cells in which they are expressed (Hovdenak and Haram 2012). Nutrition therefore may play a role in the development and/or the prevention of birth defects. The role of nutrition in the aetiology of CLP was recognised early in the twentieth century when researchers suggested a possible link between diet without fresh red meat for pregnant jaguars and their cubs born with a cleft palate (Wyszynski and Beaty 1996). While the consumption of fresh red meat may not reduce or eliminate clefting in humans, diet and nutrition may be significant factors. No references have been found

[8] The ethnicity of the mother and child was not determined as the selection was made on the basis that they lived in urban areas and were selected by a centre specific item such as the last digit of a mother's medical record. The median socioeconomic status of the participants was 7% below the average USA worker's income.

linking a vegetarian diet or other specific diets to clefting to minimise the risk of a child having a cleft.

The human embryo is entirely dependent on maternal food intake and metabolism. From the moment of implantation nutrients from the fallopian tube and then the uterus are transferred to the conceptus via diffusion through the fetal membranes (Moore and Persaud 2008). During this time the embryonic nutrition is termed histiotrophic, being largely derived from the maternal uterine glands, and will gradually be replaced by hemotrophic nutrition, that is nutrients delivered via maternal blood to the placenta. At around twelve weeks' gestation the nutrition of the conceptus will be fully hemotrophic and by this time the facial lip and palate have formed (Moore and Persaud 2008). It can be seen that this period of development is critical not just because of the rapid change in the embryo but also because of the changing source of nutrition.

1.4.9.1 Folic acid (folate)

Maternal folate deficiency is associated with spina bifida (Shaw, Rozen et al. 1998; Prescott and Malcolm 2002; Ray, Meier et al. 2003), and therefore it is not surprising that researchers have considered whether the same deficiency may be a causal factor in CLP (Bienengraber, Malek et al. 2001). Folate is important in the synthesis of nucleic acids, the building blocks of DNA (Tamura and Picciano 2006). If this synthesis is disrupted in any way it could lead to aberrant DNA replication and subsequently defects in cell growth. Folate is also involved in the methylation of homocysteine to s-adenysyl methionine, an important source of methyl groups for cell metabolism (Wong, Eskes et al. 1999; Steegers-Theunissen, Twigt et al. 2013) and, importantly, DNA methylation. Because of this and the history of its association with neural tube defects (NTD), researchers first turned to folate in the search for a nutritional deficiency to link CLP (Prescott and Malcolm 2002).

Prescott and Malcolm's claims (2002), however, are largely unsupported by evidence, and therefore speculative. Their conclusion (Prescott and Malcolm 2002) was that low dose folate supplementation preconception may not have any great effect on CLP, but that where a predisposition to CLP is known, a dose of at least 4 mg/day prior to conception and continued until twelve weeks' gestation may be helpful in preventing CLP. This latter point, however, is also merely speculative, but well worth investigating further, as perhaps more than just folate may be involved (Steegers-Theunissen, Twigt et al. 2013). For example, research could consider whether deficiencies in vitamin B6,

B_{12}, antioxidants, particular minerals, or a combination of these elements also have an impact on CLP.

Bienengraber, Malek et al. (2001) used eleven pregnant rats to determine the effects of folic acid on the development of their fetus. The eleven rats, which carried a total of 75 fetuses, were injected with procarbazine post-conception to induce a cleft palate. Seven of the pregnant rats were then given 4 mg/kg of folic acid subcutaneously from the 14th to the 17th day post-conception. This level of folic acid supplementation appears to be a high dose based on the fact that the recommended dose for women approaching pregnancy is 400 µg per day (De Bree, Van Dusseldorp et al. 1997). As a control group three more pregnant rats carrying 24 fetuses were not treated in any way. All of the fetuses were born by caesarean section on the 20th day post-conception. Each fetal head was cut into 35 frontal sections and examined histologically. None of the controls exhibited clefting. Of the group that was not given folate 90% of the fetuses had a CP. While there were virtually no fewer cases of CP in the group given the folate, it was noticed that there were significantly fewer complete CPs. Bienengraber, Malek et al. (2001) concluded that while folate did not prevent the formation of the cleft, it reduced the severity so that the cleft was not as large. By inference, it was then suggested that considering the severity was decreased post-conception, had folate been given preconception the CPs may not have occurred. It could be questioned whether the folic acid may have been more efficacious if administered with other micronutrients or other elements if it was indeed helpful in limiting or preventing the formation of a CLP. The study itself was underpowered with uneven numbers in each study group and therefore the outcomes require further scientific support.

This research, while providing some information as to the effect of folate, could have been extended, as the researchers proved that by introducing procarbazine CPs ensued. Further studies are needed to consider what component of the procarbazine induced a CP. If this interaction is determined, it could lead to a closer understanding of the mechanism by which clefting occurs. Bienengraber, Malek et al. (2001) briefly explained that procarbazine may inhibit the inclusion of thymidine and leucine, resulting in impaired DNA and protein synthesis leading to altered gene expression or cell behaviour.

Bienengraber, Malek et al.'s (2001) research implies that if there is a deficiency in thymidine and/or leucine, CPs may result. Thymidine and leucine are important for all

cells, with leucine being required in growth factors and cell signalling functions. Finally, their work only considered CP, and in that sense assumed that there are differing aetiologies for the three forms of clefting, based purely on the fact that procarbazine normally only induces CP and not other defects. They indicated that other researchers have found that hydroxamic acid derivatives, when used in a similar manner, induced only CL (von Kreybig 1976) which supports their claim of differing aetiologies in clefting types.

While these studies were very early work they did encourage further investigations aiming to establish the link between folic acid and CLP. Researchers have considered folic acid from the point of view of detecting a direct link through to suggesting that high doses taken prior to pregnancy may avert clefting (Ray, Meier et al. 2003; Wilcox, Lie et al. 2007; Houston 2012; Li, Chao et al. 2012; Velazquez-Aragon, Alcantara-Ortigoza et al. 2012; Molina-Solana, Yanez-Vico et al. 2013; Shaw, Yang et al. 2013; Wehby, Felix et al. 2013). All researchers indicate supplementation with folate prior to conception may reduce either the incidence of a cleft or the magnitude of the cleft. Wilcox, Lie et al. (2007, P. 468) in their Norwegian study reported that the taking of a folic acid supplement antenatally, and into the first three months of pregnancy "seemed to"[9] reduce the incidence of clefting by up to one third. They also stated that other vitamins and dietary factors may provide additional benefit. The Australian statistics (Riley and Halliday 2008) show no real decline in CLP incidence in spite of the fact that folate has been recommended for all women approaching pregnancy and that many foods had folate added to them for almost 20 years.

1.4.9.2 Folinic acid

Paros and Beck (1999) investigated mice predisposed to having offspring with a CLP that were treated with folinic acid. They found that folinic acid administered during pregnancy reduced the incidence of clefting in these mice. This appears to be the only study relating CLP and folinic acid. Unfortunately, the researchers did not attempt to explain why folinic acid may be effective when others have shown that folic acid may only ameliorate the incidence of clefting (Ray, Meier et al. 2003; Wilcox, Lie et al. 2007). It could be that this strain of mice, and women who have a child with a cleft, may have some difficulty in utilising folic acid in the one carbon metabolic pathway. As

[9] The comment used by the researchers, "seemed to", appears less than scientific and therefore the results need further verification.

the researchers merely suggested that similar results may be found in human trials more studies need to be undertaken to corroborate their work.

1.4.9.3 Multivitamins

A Brazilian case controlled study was conducted (Loffredo, Souza et al. 2001) to determine whether there was an inverse relationship between the maternal intake of vitamins, both before and during pregnancy, and the occurrence of CLP or CP. Data were collected in structured interviews with the mothers of 450 cases of orofacial cleft children and 450 controls. The case study group were all parents of infants under the age of one year who presented to a hospital for corrective surgery for their children and who agreed to participate in the research. The controls were a group of 450 consecutively born children at one state hospital, excluding those with any congenital anomaly. Loffredo, Souza et al. (2001) attempted to determine which pharmacological drugs had been used during pregnancy, whether vitamin supplements had been taken prior to or continually through pregnancy, and whether the mother had smoked cigarettes. No attempt was made to quantify the supplements or pharmacological drugs taken during pregnancy, the woman's diet or socioeconomic status.

Loffredo, Souza et al. (2001) divided the case results into two groups, CP (n=96) and CLP (n=354). In the CP group, 59 mothers (61.5%) indicated taking supplements, as did 215 mothers in the CLP (60.7%). In the control group 327 of the 450 (72.67%) had taken supplementation. After further statistical analysis the researchers concluded that taking multivitamins during the first four months of pregnancy had a protective effect against CLP, but that there appeared not to be the same degree of protection where CP was the outcome. This research relies on the fact that the mothers were taking the supplements from day one of their pregnancies, which may not have been the case, as at that point many would not know that they were pregnant. The researchers were relying on the mothers' recall of events which in many cases was 18 months earlier and may have influenced the data, as it is very easy to forget the specifics of particular supplements over time and how compliant they were in taking them. Further, they considered all supplements as multivitamins and did not differentiate between them. Some mothers may have concluded that all supplements were classed as vitamins. The work was also confounded in the sense that only those mothers who presented with their children for corrective surgery were included. This may have been a group in a higher socioeconomic bracket than the general population as other mothers who could not

afford such corrections were excluded. To the credit of the researchers they undertook multivariate analyses, which did suggest that multivitamin supplementation appeared to reduce the incidence of CLP, but not CP. Further research, however, is needed to isolate which vitamins/minerals played the pivotal role, and the mechanism of the interaction with gene expression.

The role of vitamins in pregnancy more generally has also been considered. Bentley, Hermes et al. (2011) compared fortified food containing L-methyl folate and high dose vitamin B12 to what they defined as standard prenatal vitamins and minerals, particularly iron supplements, regarding the maintenance of haemoglobin levels and of anaemia in pregnancy. They concluded that while iron fortified food may result in a reduction of anaemia in pregnancy, a prospective controlled study was needed to confirm this and their other findings. Munger, Sauberlich et al. (2004), in a research study in the Philippines, found that a deficiency in vitamin B6 was associated with clefting, and as zinc is a cofactor to vitamin B6 a deficiency in this mineral may have also been implicated. Steegers-Theunissen, Twigt et al. (2013) investigated a possible association between disruptions in the one-carbon biochemical pathway and birth anomalies, concluding that poor nutrition was a contributing factor. Specifically they found that deficiencies in folate and vitamin B12 contributed to adverse pregnancy outcomes. However, while they indicated that significant epigenetic modifications to chromatin occur that correspond with normal development, subtle variations in one-carbon genes, and deficiencies in one-carbon substrates/cofactors, together with poor lifestyle such as smoking tobacco and the consumption of alcohol, disturb the one-carbon biochemical pathway and compromise fetal health. Steegers-Theunissen, Twigt et al. (2013) proposed that supplementation of a broad spectrum multivitamin which included folate and vitamin B12 prior to and through to week 10 of a woman's pregnancy could be of benefit in preventing birth anomalies. Correa, Gilboa et al. (2012) found that in mothers with type 1 diabetes there was a much greater risk of birth defects occurring in their children if they did not take vitamin supplementation containing folic acid prior to conception. Together the aforementioned studies provide evidence for the need for specific micronutrients before and during pregnancy to prevent congenital anomalies. Further studies into the protective effects of multivitamin supplementation during pregnancy are warranted.

1.4.9.4 Minerals

Zinc is one of the most important minerals in human metabolism as it is involved in more than 70 metalloenzymes and plays a critical role in normal growth and development, cellular integrity, and many biological functions, including protein synthesis and nucleic acid metabolism (Vallee and Falchuk 1993). Zinc is believed to be important for fetal growth, development, and immune function (Hurley 1981; Shah and Sachdev 2006; Mossey, Little et al. 2009; Uriu-Adams and Keen 2010; Chua, Cowley et al. 2012).

The function of zinc in both animal and human reproduction has been extensively studied (Shah and Sachdev 2006; Tian and Diaz 2013). Zinc status, which is commonly assessed by the plasma zinc concentration, appears to be lower in pregnant women than in non-pregnant women (Tamura and Goldenberg 1996). Tamura and Goldenberg (1996) noted that the decline appeared to commence in early pregnancy and continued throughout gestation. Indeed zinc is 35% lower in pregnant women when compared to non-pregnant women. This decline in zinc levels could be due to a number of factors, one of course being the hormonal changes that take place during pregnancy where the balance between oestrogen and progesterone changes (Mesiano, Chan et al. 2002) or maybe perhaps due to the increasing demands of the growing fetus. Other hormonal changes may also impact on this reduction in zinc levels. Likewise given that plasma volume expansion occurs in early gestation the reduction in zinc concentrations may be merely due to haemodilution (Shah and Sachdev 2001). Perhaps if a woman was already zinc deficient this reduction during pregnancy may impact on the fetus, but to date that has not been determined. A meta-analysis (Molina-Solana, Yanez-Vico et al. 2013) covering a wide range of factors considered related to clefting showed that low serum zinc had been investigated to determine its association with CLP. Three studies showed that low serum zinc was associated with CLP while one was not. It was concluded that maternal zinc may only be a risk factor when it is at extremely low levels. Chaffee and King (2012) in another meta-analysis concluded that the overall benefit to public health of zinc supplementation in pregnancy appears limited.

In many countries soils are deficient in important minerals and these are well documented. For example it is well known that in parts of Australia soils are deficient in selenium, and this is also the case in parts of New Zealand (Tinggi 2003). Considerable research has been conducted into the relationship between selenium deficiency and pre-

eclampsia (Vanderlelie, Venardos et al. 2004). While this has not been associated with clefting, research (Perkins 2006; Perkins 2011) suggests that oxidative stress which is a characteristic feature of preeclampsia may be due in part to reductions in the antioxidant activity of selenoproteins which result from selenium deficiency. As nutritional elements are often complementary to each other (Watts 2003) it could be questioned whether an increase in antioxidant status, perhaps through increasing selenium, may not reduce the risk of a child being born with a cleft. In the Philippines the staple diet is rice and the soils in which it is grown are deficient in nitrogen, phosphorus, zinc, sulphur and iron (Lantin, Quijano et al. 1990). Zinc, as mentioned above, is an essential nutrient for fetal growth. Iron deficiency anaemia in the Philippines is a serious public health problem (Angeles-Agdeppa, Capanzana et al. 2008) and iron fortified food is being pursued to improve overall health, especially of young women. It can be seen therefore that some pregnancy-complications have been directly related to nutritional deficiencies (Furness, Fenech et al. 2008), and by inference a similar pattern may be found with clefting.

It is obvious that while targeted research is required to determine the specific nutritional deficiencies that may impact on pregnancy resulting in an adverse outcome, there are people in the community at large who believe that if women have a good and balanced diet the child will be born free of birth defects. Wu, Imhoff-Kunsch et al. (2012) believed that while advanced medical therapies may support pregnant women in advanced economies, effective antenatal nutritional interventions in low-income countries are necessary for more anomaly-free birth outcomes. Other researchers also support the need for improved antenatal nutrition (Shrimpton 2012) but question how it can be improved and what mechanisms there are for providing it. This is made more difficult in areas where famine and/or food supplies are irregular, such as in Pakistan (Imdad and Bhutta 2012), where researchers proposed that balanced protein-energy supplementation could reduce small-for-gestational-age births, and other anomalies such as CLP, especially in undernourished women.

Lawrence, Keyte et al. (2011) explored the potential for using Sure Start Children's Centres in the United Kingdom to see whether through these centres information could be provided to socioeconomically disadvantaged women to improve their diets. One of their findings was that women tend to take advice better from those they trust, and while this may be their general practitioner, they were relatively unskilled in supporting

women to change their dietary habits. Lawrence, Keyte et al. (2011) suggested that Sure Start Centres provided the right environment for dietary changes to be initiated. The Sure Start model may have merit in other countries where health services are provided within relatively small villages with medical practitioners who are not permanently employed or readily available. One example of this is MECC (Make Every Child Count) which operates in Cambodia. Similar organisations operate in countries such as Pakistan and parts of Africa.

While clefting may appear to have many causal factors, indeed being multifactorial, it is clear that a more in-depth study may find closer links to CLP aetiology. Molina-Solana et al. (2013) in their meta-analysis found that the maternal factors most associated with CLP were tobacco (odds ratio 1.48), alcohol (OR 1.28), folic acid intake (OR 0.77), obesity (OR 1.26), stressful events (OR 1.41), low blood zinc levels (OR 1.82), and fever during pregnancy (OR 1.30). As discussed in the earlier parts of this chapter there are many additional areas that require further and more meticulous research before a conclusive statement can be made regarding the aetiology of CLP.

1.5 Theoretical Framework

The research for this thesis is based on an eclectic primary health theoretical framework. Both qualitative and quantitative approaches contributed to the evolving studies with a variety of methodologies used to fully explore and understand each research question as it arose. The specific methods considered by the researcher are discussed more fully in the next chapter and the specific methodology adopted for each particular study is outlined in their respective chapters.

1.6 Considerations

The literature reviewed has not found any clear or definitive cause for non-syndromic CLP. It is possibly multi-factorial, involving genetics, in some cases familial relationships, external influences concerning the environment, and/or lifestyle factors such as tobacco smoking. There does appear to be compelling evidence to suggest that the aetiologies of CL, CLP and CP are all different, with CP more associated with a gene variant in chromosome 2 and the other two defects being associated with chromosome 4 and/or 17. However, in all cases it has been suggested that multifactorial issues comprise elements of a gene-environment interaction.

That CLP anomalies, which can be artificially created in rats using either procarbazine or hydroxamic acid, could indicate the type of chemical interaction that changes gene expression, resulting in the cessation or change in cell replication, differentiation, and migration (Bienengraber, Malek et al. 2001). Little or no research appears to have been done in the last ten years along this line in CLP research, and certainly there is justification for further investigation.

Four drug types have been associated with clefting, these being sulfasalazines, benzodiazepines, glucocorticoids and retinoids (Kallen 2003). Unfortunately, the warnings provided with each do not extend to the possibility that birth defects may occur should they be taken prior to or during the early stages of pregnancy. The social drug nicotine (and perhaps other chemicals included in tobacco, irrespective of the method of use) associated with cigarette smoking has been associated with clefting (Kallender and Kallen 1971; Kallen 1997; Lammer, Shaw et al. 2005), but the research data are not conclusive and further work is needed, extending this to the amount of use and also the effect of passive smoking. Alcohol, another social drug, appears not to have been studied in terms of clefting (Shaw and Lammer 1999). If it is involved, it would be expected that high levels of clefting would then occur in those communities which have high alcohol intakes, but as Asian communities, where alcohol intake is low, appear to be more seriously affected by clefting, alcohol may only have an impact if other risk factors co-exist or are more significant. Maternal alcohol use is known to cause Fetal Alcohol Spectrum Disorder but its association with clefting is uncertain as some studies have shown an association while others have not (Mossey, Little et al. 2009).

Universal toxins such as DDT and organochlorides may be involved in clefting (Cohn, Cirillo et al. 2003). However, insufficient research has been undertaken to associate these toxins with clefting, in spite of the fact that other birth defects have been associated with high levels of these substances.

While some research has associated the use of multivitamin supplementation as a protective means against clefting it seems that folate deficiency on its own is not associated with CLP. Wilcox, Lie et al. (2007) suggest that while there may be disturbances in some of the biochemical pathways common to both NTD and CLP the two anomalies are not related. Normal gene expression requires nutritional co-factors and so deficiency, or over-supply, of certain nutrients and the timing of their availability in the development process should not be overlooked.

1.7 Studies and Settings

From the outset it was intended that the research for this thesis was to determine the factors associated with human fetal clefting. Early reading on the subject indicated that there were many issues surrounding CLP that also warranted investigation. In total seven discreet studies have been conducted involving varying methodologies. The studies are mentioned here in chronological order, however, they have been included in this thesis in an order that enables the reader to more easily see the relationship between each of the various studies.

The first study for this thesis in Chapter 6 was to determine whether the likely causal association for clefting was linked to a family trait, or whether it may be environmental or purely nutritional. This initial qualitative study was carried out in conjunction with an ORAL mission in the Philippines. It provided some insight into likely avenues for further research but there was no specificity for likely causal factor(s).

The second, a qualitative study in Chapter 7, was undertaken in Australia with the aim of adding to the Filipino data in Chapter 6. This study led to the consideration that traumatic stress at or around conception may be a causal factor for clefting.

Three further Australian studies in Chapters 3, 4 and 5 were undertaken using quantitative and qualitative methodologies. The aim of the two qualitative studies was to understand the experiences of young adults in growing up with a cleft, and the other to explore and understand the experiences of the parents of children born with a cleft. The aim of the third study was to determine the general public's awareness of clefting.

The sixth study in Chapter 8 was to compare the nutritional status of Australian women pregnant with a fetus diagnosed as having a cleft, to women whose fetus had no diagnosed anomalies.

The seventh study in Chapter 10 was conducted to investigate the hair zinc levels of women who had given birth to a child with a cleft, comparing these to women whose child was born without a birth anomaly. The larger part of this study was conducted in the Philippines over a spread of geographical regions to ensure that the participants were not just a small localised cluster group. The Australian component was conducted in the three eastern states, namely Victoria, New South Wales and Queensland.

The researcher also visited other countries and met with researchers to gain a greater understanding of the various issues they had studied involving clefting. The researchers were:

- Dr Kamil Hozyasz – Institute of Mother and Child, Warsaw, Poland, conducting research into CLP.
- Dr Terence Lao – Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Kowloon, Hong Kong, conducting research into adverse pregnancy outcomes, specifically birth defects.
- Dr Theavey Mok – Khmer Soviet Friendship Hospital, Phnom Penh, Cambodia, a surgeon with a special interest in facial anomalies.

1.8 Summary

Clefting has to date not been associated with a single causal factor and still remains best described as being multifactorial involving environmental, nutritional and genetic factors. For as much as has been learned about some critical factors for birth anomalies, clefting persists with much still to learn about it. Indeed it can best be illustrated as a rainbow of potential causal associations. The following chapters will explore some of these possible causations (as individual colours of the rainbow) in more detail while also investigating the psychosocial aspects of clefting.

Chapter 2 Research Methods

2.1 Introduction

This chapter presents the overall research design for this thesis, and the various methodologies and theoretical framework utilised to construct and conduct the studies. Figure 2.1 illustrates the evolutionary path followed for all of the research undertaken and identifies the content of the chapters that follow including the last which poses a hypothesis for clefting.

As with the rainbow analogy, previously stated, it was clear that there were as many colours as there were studies to be brought together to be considered both individually, and collectively, if the issues surrounding clefting are to be more clearly understood. This eclectic research is like a rainbow with each study being a colour, commencing with red the shortest of wavelengths in the visible spectrum (Rigden 1999). A mixed methods approach within a constructivist interpretive framework has been used to attempt to explore each aspect within a primary health paradigm. This approach allowed the researcher to explore public awareness, the personal experiences of those affected by clefting (both directly and indirectly) as well as taking a more constructivist approach to explore possible causal factors. As the dissemination of information is an important aspect of primary health, an innovative approach using music is also explored to increase public awareness.

The research design has taken an evolutionary path from the inception and accordingly there are differing methods and methodologies for each study in order to enable a responsive and comprehensive investigation. Each study incorporated rigour (Sandelowski 2000) to ensure integrity so that the conclusions could be justified. From the outset the researcher believed that to investigate clefting fully the psychosocial implications must also be explored to achieve comprehensive, deeper and richer findings.

In designing the research for this thesis, the objective was not just to study one facet of clefting from different points of view, but to investigate a number of factors impacting on the people involved, as well as attempting to determine why this anomaly had occurred in the first instance. Brewer and Hunter (1989) state that the

integration of more than one research method in a research design, as in methodological eclecticism, increases the feasibility of verifying and validating theories. This is because multiple methods can lead to multiple hypotheses and the definition of multiple operations (Brewer and Hunter 1989) – in this case it was hoped multiple causal associations for clefting could be identified as well as informed diverse insights into the longer term effects and aspirations of those who have personal connections with facial anomalies; such a mixed methods approach enabled clefting to be explored more inclusively (Sale, Lohfeld et al. 2002; Denscombe 2008)**.**

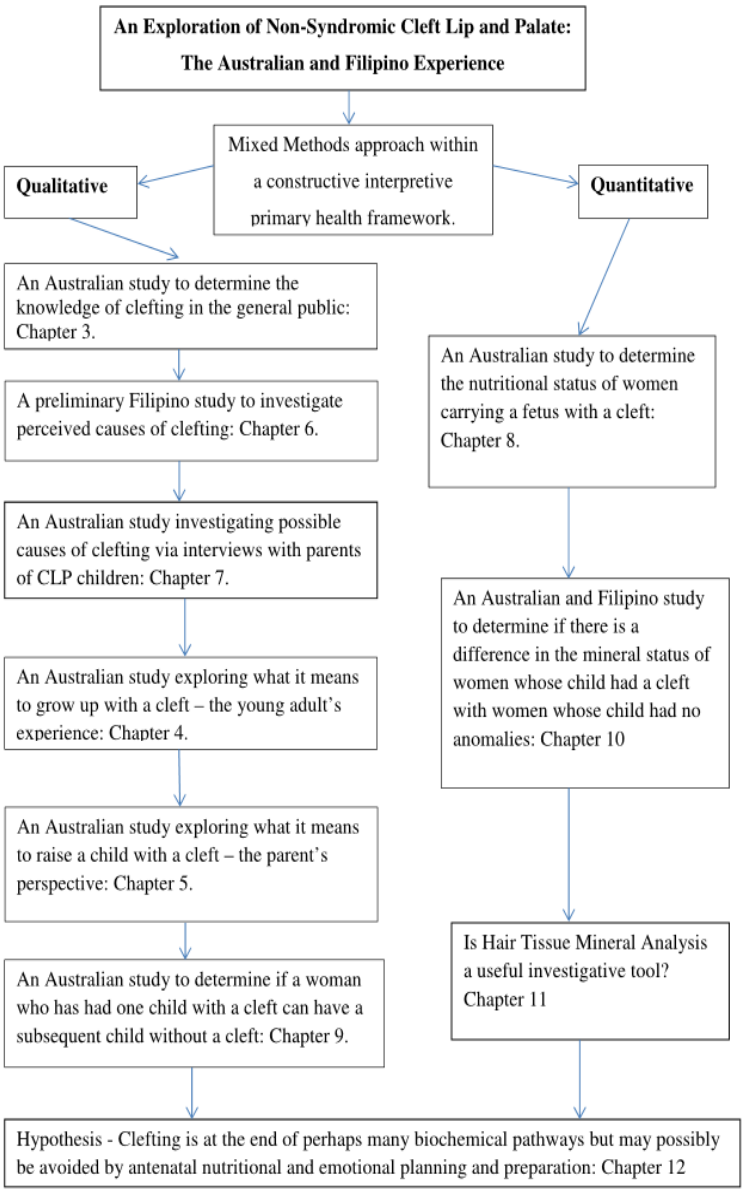


Figure 2.1 Evolutionary pathways for this research

2.2 Eclectic Research

The complete Oxford Dictionary (Dictionary 2013, Vol. 5, p. 586) defines the word eclectic as "to borrow or borrow from diverse sources, also of persons or personal attributes unfettered by a narrow system; broad not exclusive; made up of selections." This definition of the word then determines that research which is considered eclectic must not be bound by one philosophical ideology or paradigm. It must be inclusive rather than exclusive, and it must seek to unlock the truth of the subject matter by researching the subject in a number of differing ways to reveal the absolute nature of the subject under study.

Creswell (2009, p. 58) advises that eclectic research provides the opportunity to consider complex issues using six differing strategies. Of the six strategies the *concurrent transformative approach* is said to allow the researcher the most freedom as it is based on the researcher's own specific theoretical perspective to guide the study. The perspective can be based on any number of ideologies such as critical theory, advocacy, participatory research, or a conceptual or theoretical framework (Creswell 2009, p. 127-139). Both the qualitative and quantitative data are collected concurrently but each set of data stands apart until the researcher elects to bring them together, most generally in the discussion phase of the study. This allows in some areas the quantitative methods to take precedence and in others the qualitative. Methodological eclecticism ensures that all aspects relating to a particular study are considered (Sotirakopoulou and Breakwell 1992).

This thesis investigates not just a cause and effect situation within clefting but also attempts to understand the feelings of the individuals involved and how they coped with difficult periods in their lives. Sandelowski (2000) states that due to the complexity of human phenomena its study mandates more complex research designs to capture the individual aspects of each of the phenomena and to provide a deeper insight into how these relate to the individuals involved.

Knox (2004) states that researchers may miss out on potentially innovative or creative data collection methods if they feel tied or constrained by a particular philosophical stance. Knox (2004) indicates that much thought should be given to the hierarchy of research needs by initially identifying the variety of research methods available and then considering the relationship between theory and method. Johnson, Onwuegbuzie et al (2007) claim that mixed methods research will provide the most

informative, complete, balanced, and useful research results. Pursuing the mixed methods approach for this thesis, it was believed that clefting could be explored in a complete and balanced way just as this definition suggests.

The eclectic mix of methods used throughout the research for this thesis involved selecting the best method suited to each particular hypothesis and investigation. Brannen (2005) approaches the question of using an eclectic mix of methods in a very practical way by suggesting that the kind of research question not only leads to the choice of method but increasingly to a complex of methods. Traditionally research methods have been classified into two main avenues for enquiry, these being quantitative and qualitative. Schneider, Whitehead et al. (2007) insist that research which incorporates more than one discipline demonstrates an enlightened acknowledgement of their equal importance to advantage the research.

Hesse-Biber and Johnson (2012) emphasise that there is not a one size fits all approach to mixed methods research; its very nature allows the researcher to choose from an ever increasing number of internal paradigms which are evolving from the original concept of mixing methods.

2.2.1 Qualitative Research

Qualitative research has been shown to provide important insights into different perceptions of reality in the search for truth, especially in social research (Grbich 1999). Among its various forms it uses analytical categories to describe and explain social phenomena (Pope, Ziebland et al. 2000). Understanding a phenomenon from the point of view of the participants and their particular social and institutional contexts may be lost if sole reliance is placed on the quantification of numerical data (Kaplan and Maxwell 1994). This thesis was designed not just to quantify possible causal factors of clefting but to also explore and understand the anomaly from the point of view of both the family and the child born with a cleft. This fits well within the scope outlined by Kaplan and Maxwell (1994) where qualitative research methods are designed to help researchers understand people and the social and cultural contexts within which they live.

Hanson, Balmer et al. (2011) affirm that the goodness of qualitative research lies in what it promises to do well: build an understanding of how participants ‘make sense’ of things; appreciate context rather than control it; explore human potential to analyse and

interpret, and provide accurate, comprehensive, and descriptive foundations of the subject being explored (Hanson, Balmer et al. 2011, p. 375). In this thesis both the parents and young adults who were investigated put into words how they made sense of their situation and the experiences that they encountered on their journey of cleft repair thus providing real insight for this thesis and for others who may wish to investigate similar or associated health issues.

Caelli (2003) affirms that qualitative research may not be guided by just one method, but that the methodology chosen may involve several methods. Such a philosophy is also shared by other researchers (Sandelowski 2000). Kramer-Kile (2012) suggests that there is not a set way to engage in qualitative research and that those who participate in this should account for the choices they make throughout the research process and articulate their methodological decision-making along the way. Because of the complexity of the clefting subject, choices had to be made between qualitative and quantitative approaches, combining both in some studies, while for others a purely qualitative approach using a descriptive narrative method was used because this enabled the researcher to obtain much more intimate detail than otherwise could have been attained.

Bryman (2006) advises that qualitative research frequently produces surprises, changes of direction and new insights. Bryman (2006) also states that the imaginative application of techniques in quantitative research can also result in new understandings, and when combining qualitative and quantitative methods in a research study the likelihood of unanticipated outcomes is increased. The evolutionary pathway of this research study similarly generated surprises with new directions and insights as well as new understandings and unanticipated outcomes.

Sandelowski (2000) indicates that qualitative descriptive designs are typically eclectic but still have reasonable combinations of sampling, data collection and analysis. Creswell (2013, p. 185) summarises qualitative research in that it begins with an interpretive/theoretical framework, involves data collection in its natural setting which is sensitive to the participants or places involved, and analyses the data in an inductive and/or deductive manner, enabling the voices of the participants studied to be heard. In order to add rigour to qualitative studies, Caelli (2003) encourages researchers to revisit their study participants to confirm their data. This has been done in all the qualitative studies undertaken for this thesis.

A combination of narrative and qualitative descriptive studies incorporating thematic analysis has been used in some of the following chapters. Thematic analysis is used to discover themes emanating from the data where interviews with participants have been conducted. Boyatzis (1998) states that thematic analysis is a method for identifying, analysing, and reporting patterns (themes) within data. The method is said to minimally organise the data but can describe it in rich detail. Thematic analysis is used in the qualitative studies included in this thesis where individual participants have been asked to narrate their stories in relation to their clefting experiences.

2.2.2 Quantitative Research

Quantitative research, while concerned with measuring quantities or amounts, is based on a philosophy that aims to expand knowledge of the world empirically. Empiricism is founded on observation, measurement and comparison (Minichiello, Sullivan et al. 1999; Sale, Lohfeld et al. 2002). The science of quantitative research is interested in two laws or rules that govern the empirical aspects of the world, these being an unbiased observation of the world we live in, and the development of theories about these on which falsifiable hypotheses can be derived (Minichiello, Sullivan et al. 1999). Falsifiable hypotheses (Minichiello, Sullivan et al. 1999; Sale, Lohfeld et al. 2002) are clearly specified predictions that are presented in such a way that they can be either proved, or disproved. In this sense quantitative research assumes that there is a generally held truth or reality which if pursued can be measured or quantified in some form. The measurement is not based on what one thinks it is, but the researcher must be informed by determining the fact via some well-established theoretical framework. In essence, a quantitative study lays open the results for other researchers to conduct their own studies which will generally either support the original hypothesis or refute it (Minichiello, Sullivan et al. 1999; Sale, Lohfeld et al. 2002).

Quantitative research, however, does have some important characteristics which differentiate it from qualitative research (Sale, Lohfeld et al. 2002).

- The methods followed are inherently rigorous in that they are logical and statistically sound.
- There is internal validity in that the methods are able to explain the phenomena with independent and dependent variables.
- The findings can lead to generalisations being applied.

- The data were able to be replicated by others using the same techniques.

Quantitative methods deal with figures, graphs, and statistics without feelings or emotions of those studied being considered. This may be adequate for many research questions but where the participant's experiences are also to be considered quantitative analysis on its own is inadequate. A balance of the two approaches is needed to provide a complete understanding of the question being studied.

2.3 Theoretical Framework

This thesis considers the current scientific studies regarding clefting, investigates community understanding of the anomaly, the effect that it has on those affected or closely related to those affected, explores possible causal factors, and proposes suggestions for improved maternal health, health literacy, and health promotion. It therefore fits well within the definition of primary health care discussed in the next paragraphs. The researcher's involvement with ORAL and particularly the personal experience of travelling with the surgical missions in the Philippines identified an apparent need in that country for improved general community health, particularly in the very poor areas. Later, in Australia, being exposed to the work of CleftPals reinforced the consideration that improved general awareness of clefting and more targeted, innovative health promotion may assist in reducing the incidence both in Australia and internationally. From the outset it must be stated that no one, male or female would intentionally want a child to be born with an anomaly of any kind and it is from this premise that this thesis is predicated. The intention was to consider as many aspects of clefting as was possible within the scope of the research in order to determine what steps could be taken in the future to reduce the incidence of all anomalies and clefting in particular.

Greenhalgh (2007, p. 12) defines primary health care as follows:

> Primary health care is what happens when someone who is ill (or thinks he or she is ill or who wants to avoid getting ill) consults a health professional in a community setting for advice, tests, treatment or referral to specialist care. Such care should be holistic, balanced, personalised, rigorous and equitable and delivered by reflexive practitioners who recognize their own limitations and draw appropriately on the strengths of others.

This definition focuses on a personal relationship between the carer who has medical training at some level and the patient, however, the concept of primary health and primary health care goes beyond this. As this thesis found, support groups such as

CleftPals in Australia assist those in coping with the arrival of a child with a cleft and this organisation is only one of the many that provide similar assistance to those with health issues.

Other definitions which are more inclusive are:

Primary health care,

- incorporates personal care with health promotion, the prevention of illness, and community development;
- includes the interconnecting principles of equity, access, empowerment, community self-determination, and inter-sectoral collaboration;
- encompasses an understanding of the social, economic, cultural, and political determinants of health. (Keleher 2001).

and,

> Primary health care is socially appropriate, universally accessible, scientifically sound first level care provided by health services and systems with a suitably trained workforce comprised of multi-disciplinary teams supported by integrated referral systems in a way that: gives priority to those most in need and addresses health inequalities; maximises community and individual self-reliance, participation and control; and involves collaboration and partnership with other sectors to promote public health. Comprehensive primary health care includes health promotion, illness prevention, treatment and care of the sick, community development, advocacy and rehabilitation. (APHCRI 2009, p. 1)

While the Greenhalgh (2007) definition is closely linked to a medical model these other two definitions provide us with a much clearer understanding of what primary health care is and its scope of influence.

Although all aspects of the health care system have implications for public health, primary health care perhaps has the greatest potential impact. Greene Ross et al. (2007) state that attention to equity has to be at the heart of all health strategies whether they be public, private or in the form of primary health care. They also maintain that decisions made in this area must involve the community to ensure that the services are appropriate and that all health strategies incorporate a preventative approach alongside the curative, to ensure efficiency and appropriateness of approach.

The Australian government states that primary health care is at the centre of its overall health policy and in its National Primary Health Care Strategic Framework publication (Plibersek 2013) it states its vision as being to achieve the following:

- Improve health care for all Australians, particularly those who currently experience inequitable health outcomes.
- Keep people healthy.
- Prevent illness.
- Reduce the need for unnecessary hospital presentations.
- Improve the management of complex chronic conditions.

This vision is set within the framework of four strategic outcomes these being:

1. Building a consumer focussed and integrated primary health care system
2. Improving access and reducing inequity
3. Increasing the focus on health promotion and prevention, screening, and early intervention.
4. Improving quality, safety, performance and accountability.

This is a national strategy and as such it requires the endorsement of each state and territory for it to be put into effect to achieve positive outcomes. While the framework is an important step in the ongoing process of general health improvement it also relies heavily on the private sector and the individual consumer to be actively engaged as partners with the health care providers.

While the framework sets out the strategy, the reality of whether or not the health benefits reach all Australians is in some doubt. Indeed in its report, the National Health Performance Authority (NHPA) (Faulkner 2013, p. iv) indicates that the level of care is very dependent on where you live. The report further indicates that there is a variation in when care is available, a difference in waiting times, and the prices charged for services. However, across the country it was claimed that not one area did exceptionally well or poorly in service delivery. In relation to cost, the percentage of patients who delayed visiting a doctor due to the expense was up to five times greater in some areas than others. Data from the Indigenous areas and Torres Strait Islander communities appeared to be less comprehensive than that from other areas (Faulkner 2013). While the national health scheme, Medicare (Medicare 2013) and the Prescription Benefits Scheme, PBS (PBS 2014) provide much of the government health support required by

the community it seems from the NHPA report mentioned above that an imbalance is still present in the delivery.

The World Health Organization (WHO 2013) states that the ultimate goal of primary health care is better health for all and they set this goal out in five key elements:

1. To reduce exclusion and social disparities in health.
2. To organise health services around people's needs and expectations.
3. To integrate health into all sectors of public policy.
4. To pursue collaborative models of policy dialogue.
5. To increase stakeholder participation.

These goals very closely reflect the framework established for primary health care in Australia as described above, suggesting that irrespective of location, the approaches of all authorities in developed countries may be similar.

2.3.1 Exclusion and Social Disparities

Health equity means that people should not be denied access to life-saving and health-promoting interventions for unfair reasons, including those with economic or social causes (Marmot, Friel et al. 2008). Clearly, for primary health care to be improved, all areas of exclusion must be considered and polices put in place to correct the situation. The Australian government through its Medicare system (Medicare 2013) ensures that medical care is available to all, and in particular provides additional support for those born with a cleft. In addition, many prescribed medications are provided at nominal cost under the Pharmaceutical Benefits Scheme (PBS 2014).

Because the range of both need and services vary across the community and demographic groups, it is essential that all agencies, be they private or public, work to ensure that there is no duplication of effort, and that their services are organised in such a way to deliver the best possible outcomes for the greatest number of people (Marmot, Friel et al. 2008).

2.3.2 Health Integration in Public Policy

Governments should be the stewards of their national resources by maintaining them and improving them for the benefit of their population (Saltman and Ferroussier-Davis 2000) and it therefore follows that the careful management of their health systems should be a prime responsibility. The vision and direction of Australian health policy in placing a high priority on primary health care evidences this. However in Australia

health services are largely provided by state governments, but the regulation of drugs and complementary medicine and the provision of aged care and its associated health care is provided at a federal level. This division of responsibility provides the possibility for debate between state and federal authorities regarding responsibility and may at times prevent the seamless flow of both information and service provision from provider to patient. Integration of all health services into a seamless structure could ensure optimum performance and reach.

2.3.3 Collaborative Dialogue

While in the paragraph above it has been suggested that the state and federal governments may need to be better integrated in relation to health policy there are other organisations both within Australia and internationally where close collaboration will improve outcomes (Lasker 1997). Some of these inputs may lie outside the authority of both state and federal governments, which makes balancing the output even more difficult. Health research may to some extent lie within the universities which have government funding, but a large percentage of research will also be carried out by the private commercial sector or within private hospitals. The training of doctors, nurses and ancillary staff is of great importance and collaboration between each discipline must of necessity improve knowledge and therefore health outcomes.

2.3.4 Improve Stakeholder Participation

This thesis highlights the fact that in some areas the general population is ignorant of a number of health issues. This may not be because they do not want to know; it could be that the information they need has not been presented in a way that is accessible to them. Each person, however, must take some responsibility for their own health (Steinbrook 2006) by seeking out information either directly with their own health provider or with some responsible authority. Steinbrook (2006) suggests that many programs emphasising personal responsibility are often sketchy and often become unproductive as they may be seen as complicated or having unanticipated negative effects. An example quoted was that of giving up smoking which some may see as having the negative effect of weight gain and therefore they may resist the health message. Improving stakeholder participation then falls on the individual, the government, and the private sector collectively.

2.3.5 Health Promotion

Mahmud, Olander et al. (2013) suggest that the development of health communication for promoting health has largely taken place outside the health care services and when it does occur within this arena it lacks a broad socio-ecological approach needed to tackle lifestyle related and other ill health inequalities. They (Mahmud, Olander et al. 2013) believe that primary health care is the most suitable strategy for meeting the increasing need for health promotion and interventions in health consumers. They also suggest that information communication technology with the Internet at the forefront provides the ideal platform for achieving positive outcomes. Mahmud, Olander et al. (2013) propose that an ecological health promotion approach addresses socioeconomic and cultural factors that determine health criteria, as well as providing information and life skills to make appropriate health decisions. Fleming and Parker (2009, p. 280) agree that much health promotion lies outside the traditional health area citing fitness regimes, healthy eating, gym memberships, health insurance companies, workplace wellness programs and the marketing of supplements gaining more traction than the traditional health messages from government agencies. The authors indicate that there have been three stages in the development of health promotion since 1970 these being:

- Tackling preventable diseases and risk behaviour such as reducing heart disease, cancer, tobacco smoking and improving nutrition.
- Complementary intervention approaches such as the development of healthy public policy, personal skills, supportive environments, community action, and the provision of subsidised health services.
- Making contact with people through the settings in which they live and meet, such as schools and work places so that face to face information can be provided.

Nutbeam (2000) lists three health promotion outcomes (see table below) and how they can be measured to determine their effectiveness.

Table 2.1 Health promotion outcome and intervention impact measures

Promotional Outcome	Intervention impact measures
Health literacy	Health related knowledge, attitudes, motivation, behavioural intentions, personal skills and self-efficacy.
Social action and influence	Community participation, community empowerment, social norms and public opinion.
Healthy public policy and organisational practice	Policy statements, legislation, regulation, resource allocation and organisational practices.

Source: (Nutbeam 2000)

It is not enough to merely indicate: read this book, or, visit this web page (Nutbeam 2000); the challenge must be put in terms that incite the recipient to respond. The author also indicates that when dealing with the wide range of ages and educational levels it is difficult to have one message that covers all cohorts, and so targeting the information is critical. The health message must be simple, clear and concise; convoluted language makes understanding difficult and someone who may not have the ability to comprehend could be disadvantaged (Nutbeam 2000).

While it may not be the normal role of a researcher undertaking a thesis of this nature to suggest creative approaches to health promotion, the need for pre-pregnancy planning became apparent. It was decided to try and illustrate this in a novel way using music, hoping that other researchers may also consider engaging in new ways to influence those considering pregnancy or indeed other health promotion areas. The Royal Melbourne Philharmonic Orchestra and choir provided a short time to the researcher to produce a DVD[10] which is enclosed with this thesis. The aim of the DVD was to highlight the importance of pre-pregnancy planning both from an emotional and physical perspective.

2.3.5.1 Social action and influence

Today this aspect of health promotion is even more important. Communication systems have changed with social media becoming a dominant force, particularly among the young (Korp 2006). Peer to peer exchange of information is the norm and is divorced from mainstream communication channels. It has become a social norm to be on Facebook and to 'tweet' to one another, and to not be doing this is considered not

[10] The researcher had only 20 minutes to film this DVD and while it does cover the material intended more time could have resulted in better quality.

'cool'. Korp (2006) while discussing the empowering nature of this source of information, also warns that it has dangers in that it requires expert evaluation of the sources available and many consumers may not have the appropriate skills to determine what is appropriate to their need. Benigeri and Pluye (2003) share similar concerns, however, they do state that the Internet is a vehicle where knowledge is easily transferred from health professionals to the population to assist them in maintaining their health. If health promotion using the Internet is to be effective it must deal with how to present data accurately and professionally, to distinguish it from the hearsay that could come from unenlightened individuals (Benigeri and Pluye 2003). For those interested in reducing birth anomalies, social media may be essential as that could be the most effective channel to engage those in the age group approaching pregnancy as they are likely to be acutely aware of Internet resources.

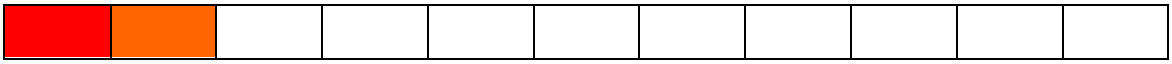
2.3.5.2 Healthy public policy

Public policy, while playing an important role in ensuring primary health, is limited by the public's acceptance and desire to be healthy (Grier and Bryant 2005). For example the Australian government has taken major steps to reduce smoking by increasing taxes, reducing advertising, and making suppliers have explicit health warnings on the packets (Government 2014). There has been some success in reducing the number of smokers but some persist, many may want to stop, but others see this as an imposition on their lifestyle (Guillaumier, Bonevski et al. 2014). Likewise, policies to reduce alcohol consumption (Agostinelli and Grube 2002) and to overcome obesity (Barry, Brescolol et al. 2009) are only successful if the person wishes to accept the policy initiative.

2.4 Summary

This thesis does not just concentrate on investigating the physical issues and possible causes surrounding clefting, but explores the social impacts on people's lives who are affected in some way by this birth anomaly and demonstrates how a community (CleftPals) which has no basic medical or psychological training continues to provide important care to those whose family is impacted by clefting.

Chapter 3 Knowledge of Clefting in the General Public



3.1 Introduction

This chapter presents the first of the eight studies that comprise the research for this thesis. This study involved members of the Australian community and investigated their knowledge of clefting. The family support group, CleftPals, which operates in all of the Australian states, undertakes much of the work assisting parents with CLP children in Australia to cope. This group does not have a high profile outside the major hospitals where corrective surgery is undertaken and hence the awareness and significance of its role and of the birth anomaly more generally may not be well recognised.

Chapter 1 identified that the incidence of a NTD was far less than CLP yet the Australian public awareness of a link between maternal folate deficiency and NTDs appears to be well known, being largely due to the publicity of folate supplementation by both government agencies and commercial suppliers of folic acid (Eicholzer, Tanz et al. 2006; Owen, Halliday et al. 2007). This knowledge has resulted in folate supplementation based on a NHMRC guideline (NHMRC 2013), recommended for women planning pregnancy, to prevent their child having a NTD. To date there are no similar recommendations in relation to CLP.

3.2 Research Question

The research question for this particular study arose from concerns regarding the ongoing challenge faced by CleftPals in trying to obtain funding for speech therapy for children born with a cleft palate, and support for research in CLP, both of which may be a consequence of limited knowledge of clefting in the general public. Accordingly the research question was:

> Does the general community in Australia know what a CLP is, and if so what level of knowledge does it have?

3.3 Background

The CleftPals organisation was established first in Victoria by the parents of a child born with a cleft. It was only through their lobbying to the federal government that initial funding was provided for CLP surgical procedures, and then after further

lobbying, dental care was extended to the CLP child until the age of 26 years (Medicare 2012; 2013; Solomon 2014)**.**

It is possible that people may consider clefting less of an issue than 60 years ago because they probably no longer see people with uncorrected facial anomalies. This is largely because of the surgical procedures and the legislation brought in by previous governments to fund rectification (Medicare 2012; 2013). The first surgery is normally carried out at three months of age for the CLP baby, predominantly involving the lip.

While CleftPals is a source of information for clefting there is the broader question of where the public obtain information on pregnancy health issues and whether the information which is available is being widely understood and absorbed by those who need it most. A Finnish study (Holappa, Ahonen et al. 2012) found that parents seeking advice on medications for their children primarily sought advice from physicians, followed to a lesser degree by nurses and pharmacists. Researchers in Northern Ireland (Lagan, Sinclair et al. 2010) investigated the extent to which the Internet was being used to gain pregnancy information. This study was web based and 97% of respondents used search engines such as Google to identify web pages that may provide them with the information they were seeking. The researchers concluded that the Internet played a significant part in the provision of information and also had a significant impact on the decision making of those accessing the web based sites. Of all the sites Wikipedia is the world's sixth most used website (Infeld and Adams 2013) and yet because of the way it has been established it lacks credibility. For example, Infeld and Adams (2013) found that in relation to gerontology of 10 important terms used in gerontology, 3 out of the 10 terms had articles on Wikipedia without content focusing on ageing. Only 4 out of 10 focused on ageing, however, of the articles that did focus on ageing the content rated comparatively highly. This indicates that invalidated data on the Internet may provide inadequate or, in some cases, misleading information. There are many websites where the general public may go for information on clefting using just the simple Google search engine, the following being examples:

- www.rch.org.au Kids Health Info
- www.humanservices.gov.au/.../cleft-lip-and-cleft-palate-scheme
- www.cleft.org.au
- www.health.gov.on.ca/en/public/publications/child/cleft.aspx
- www.wikipedia.org/wiki/Cleft_lip_and_palate

- www.entnet.org/content/cleft-lip-and-cleft-palate
- www.cleft.org.au/general-cleft-lip-palate-information
- www.leap-foundation.org/cleft-lip-and-palate-information
- www.cleftline.org
- www.nlm.nih.gov/medlineplus/cleftlipandpalate.html
- www.cleftsmile.org
- www.medicinenet.com › … › cleft palate and cleft lip index

The level of detail and the accuracy of the information contained on these websites is open to question. The majority of the sites listed above have no evidence of validation, nor are many of them evidence based, bringing into question how helpful they will be to a person seeking authoritative data relating to their enquiry. A question still remains as to whether the information provided is reaching the general public or merely to those who are already affected in some way by clefting. In this sense is the Internet the best means of promoting health or are other measures needed to increase awareness of positive health outcomes? Certainly it provides enormous exposure to the general public but it is essential if CLP is to be reduced, to have sites that attract those approaching pregnancy and to provide well researched information.

3.4 Literature Review

Only a small amount of literature could be found indicating that the general public had been investigated regarding its knowledge of clefting, or related issues. Statistical information is obtained by each Australian state and territory government (Riley and Halliday 2004; ABS 2005; Riley and Halliday 2005; ABS 2007; Riley and Halliday 2008) to generate birth data and information on various birth anomalies, as discussed earlier in Chapter 1. Literature reviews have been published relating to public understanding of other disabilities both physical and intellectual (Hall and Solehdin 1998; Scior 2011). Hall and Solehdin (1998) investigated the relationship between folic acid deficiency and neural tube defects and found that while public awareness in the USA had increased from 52% to 55% between the years 1995 and 1997 only 30% of women took a supplement containing folic acid prior to conception. Scior (2011) reviewed 75 studies covering public awareness, attitudes, and beliefs regarding intellectual disability and found that public knowledge was under-researched, but that age, educational attainment, and prior contact with someone with an intellectual

disability are more predictive of an attitude towards understanding this problem than the gender of the person surveyed.

Being aware of the public's depth of knowledge and attitude towards a health issue helps researchers to better understand the underlying problem, why recommended courses of actions are not followed, and propose solutions which may not only solve a current issue, but be more generally applied to other health issues (Ifeacho, Malhi et al. 2005). An early study gathered information regarding parents' and professionals' knowledge and awareness of clefting (Hill 1955) indicating that parents of children born with a cleft and many professionals had little information on possible causes and treatment regimes. Hill (1955) highlighted that it was critically important to involve family members, especially parents, in craniofacial team decision making and treatment planning so that they were well informed of the procedural path being taken. To this end it was important to assess their knowledge and ensure that they understood each step in the recovery process. Pannbacker (1977) investigated preoperative parental ideas of speech therapy after surgical management of the cleft and found that the parents had unrealistic expectations regarding outcomes. Pannbacker, Lass et al (1979) gathered information on their personal experience with cleft palate from medical students, parents and professionals within the medical arena and found that while parent groups served a useful purpose, students and professionals had more information and were in a better position to refer individual children with clefts to experienced specialists when needed.

The only study found comparable to the one undertaken for this thesis was by Middleton, Lass et al. (1986). This was a telephone survey of 1,200 people conducted over six cities in four states of the USA. They found that the general public had inadequate awareness of the clefting issue, 54.8% of respondents having never heard of a cleft. The study also attempted to determine whether participants knew who could treat a cleft, and whether or not there was a national body that represented the professionals working in this area. Only one respondent of the 1,200 interviewed mentioned that they were aware of the American Cleft Palate Association. The researchers (Middleton, Lass et al. 1986) made ten recommendations which they believed would increase general public awareness, all of a proactive nature with one being to develop "attention grabbing" methods to disseminate information.

No other public awareness studies have since been identified.

Professionals working in the corrective surgery arena have sought to determine the knowledge of their work, both within the medical profession in general and the public at large. Ameerally, Fordyce et al. (1994) sought to determine both public and professional awareness of oral and maxillofacial surgery (OMFS) and found that the public had little knowledge, and that the dentists who made up the direct care professionals in the survey were poorly informed of recent developments in the field. Hunter, Rubeiz et al. (1996) surveyed dental students, dental practitioners, medical students, medical practitioners, and the general public to determine their awareness of the scope of OMFS. They found a low level of recognition of their work across all respondents, and concluded that an increased level of education in all sectors was required. Twelve years later Rocha, Laureano Filho et al (2008) sought to determine the awareness of oral maxillofacial surgery among other health care professionals. They found that the general level of awareness was high, but the treatment of some problems, such as mandibular tumours, was quite low, with only 48% of respondents able to accurately refer a patient for specialist treatment. Ifeaacho, Malhi et al. (2005) included the public as well as health care professionals to determine whether the knowledge of oral and maxillofacial surgery had improved over a 10 year period. This study found that only 34% of the respondents knew what oral and maxillofacial surgery was, and none expected these surgeons to treat patients who were unhappy with their appearance. Two further studies undertaken in the USA determined that the general public awareness of oral and maxillofacial surgery was poor, with a similarly low level of the population having ever heard of oral maxillofacial surgery (Rangarajan, Kaltman et al. 2008; Russell, Strauss et al. 2008).

Other professionals working in different areas of the health environment have also found it useful to know what knowledge the public have of particular conditions in order to either promote awareness, or target treatment to specific groups. Highet, Hickie et al. (2002) undertook a telephone survey to determine the degree of recognition and understanding of depression and its treatment in the Australian community. In this survey only 50% of respondents could differentiate between depression and sadness. When asked who they would turn to if they experienced depression 45% reported family, 28% their general practitioner and 25% friends. The researchers concluded that future mental health campaigns would need considerable intensity and duration if they were to affect public awareness and understanding. A further survey was undertaken (Highet, Luscombe et al. 2006) following quite intensive marketing by the Australian government in relation to its national depression awareness campaign titled Beyond

Blue. This was also a telephone survey to determine the public awareness of depression. They found that 62% of respondents knew someone close to them with depression and that 19% of respondents suffered from depression. They found that 62% of respondents were aware of Beyond Blue and were therefore able to conclude that the active promotion of depression and related mental illness had contributed to the high levels of community awareness. The World Health Organization (Saraceno 2002) provided ten recommendations to be able to assist those with mental illness. A key element in the recommendations was to educate the public, as public awareness can correct misunderstandings about the causes and consequences of mental disorders, reduce stigma, and discrimination, and increase the use of mental health services. The Beyond Blue promotion illustrates how the publication of validated information can lead to a better public understanding of health issues thus confirming the WHO recommendation.

Community awareness studies have been carried out in many other areas of the health environment leading to a better understanding of how to promote health literacy about kidney disease (Jennette, Vupputuri et al. 2010), sickle cell disease (Hines, Mitchell et al. 2011), and cardiovascular disease (Yuqiu and Dreyer Wright 2008), for example. All have led to a current understanding of the public's knowledge relating to the topic and have indicated that further action is required if a reduction in the particular disease is to be achieved.

In undertaking this study it was believed that if the public's knowledge of clefting could be determined this may also lead to better outcomes for those who have children with clefts. It is also thought that if the public is more aware of birth anomalies in general, better preconception planning may result. This therefore, has been the first Australian study to investigate public perceptions and knowledge of clefting and it would appear to be one of very few since the early work of Middleton, Lass et al. (1986).

3.5 Ethics Approval

This study was approved by the Southern Cross University Ethics Committee (ECN-09-017).

3.6 Research Design

Triangulation was utilised for this study combining quantitative and qualitative approaches. A structured survey methodology comprising face-to-face individual interviews was conducted in public places involving consenting participants. The

inclusion criteria were defined as a member of the public who when approached was invited to participate and then gave verbal consent. The exclusion criteria comprised any person under the age of 18 years, as that would have required parental consent. The aim was to interview a random sample of people from the general public to determine their level of knowledge of clefting. The survey questions related to the participants' age, education, whether they had children or not, and more specifically their knowledge of clefting. The survey questions (See Appendix 1) were modified once interviewing was commenced because none of the participants knew how many operations a child may require to remedy a cleft, nor was there any knowledge of the cost of surgery. Subsequently participants were asked whether they knew if a child with a cleft needed speech therapy, and whether the government should provide that or not.

The purpose of the second part of question 1 in asking participants to describe a cleft was to ensure that participants did know, rather than pretending to know or guessing. All of the other questions, other than those indicated above, proved to be easily understood by the participants and concise answers were provided.

Participants did not provide any pen and paper responses for the survey but answered the questions presented verbally, with their answers recorded on a response sheet by the researcher. Personal details of participants were not recorded other than their residential post code, age and sex. Privacy and confidentiality were protected with anonymous responses. Each participant was recorded as a case number so that tracing back to a particular person was impossible. The data were analysed using descriptive statistics for the quantitative data and thematic analysis for the qualitative data.

3.7 Setting

The interviews were conducted by the researcher in the Australian states of Queensland, New South Wales and Victoria. These states were selected because they have the larger populations, and because travel costs were cheaper. Venues were selected in shopping centres, pedestrian malls and farmers' market sites, to access the best cross-section of the community, after obtaining permission from the authorities involved in those areas. In the case of shopping centres the management generally would not give permission to interview customers, but individual store owners consented, and were happy for their staff and customers to be interviewed, and the researcher complied with all aspects agreed with the respective owners. The participants either worked in the area where they

were interviewed or were visiting during the day. Most participants, apart from those in central Sydney, lived close to where they were interviewed.

The individual sites involved were:

Queensland:	Eumundi Market (semi-rural area)
	Brisbane City Mall
New South Wales:	Parramatta City Mall
	Soul Pattinson chemist shop – central Sydney
Victoria:	Doncaster Shoppingtown Mall (eastern suburban – Melbourne)
	Chadstone Shopping Centre (south east suburban – Melbourne)
	Airport West Shopping Centre (north-west suburban – Melbourne)

In all of the stores where interviews were conducted the owners provided a separate space so that the privacy of the person being interviewed could be protected. The researcher wore a badge identifying himself, and had a sign at the front of the interview space advising potential participants that a research study was taking place. In all cases the participant being interviewed sat beside the researcher. No attempt was made to audio record the interviews.

This study did not accommodate for ethnicity as this was not pragmatically possible to address.

3.8 Results

The qualitative data were analysed thematically and the quantitative data via descriptive statistics. Four hundred and six participants were involved, comprising 288 females and 119 males. A wide range of age groups, both male and female, was achieved as well as a broad cross-section of educational backgrounds providing a reasonably representational sample of the community at large.

A majority of the people interviewed wanted to talk about the study, particularly asking what was believed to be the cause of CLP. This presented a health promotion opportunity for the researcher after the interview to discuss the possible risk factors, and

to encourage the participants, with their consent, particularly those who were younger and thinking about a future pregnancy, to consider their emotional and nutritional status prior to making such a decision.

The following figures profile the participants and the results of this survey. The age of participants is skewed towards the younger end of the age spectrum; all interviews were conducted during the day and close to business districts. This skewed result is consistent with workforce statistics relating to people working in the vicinity of where the surveys were conducted. The average age of people working in the retail trade is 33.4 years of age, in accommodation, cafes, and restaurants it is 34.1 compared to education 44.1 and manufacturing 39.4 – Australian Government, Department of Employment (ABS 2013). The same statistics showed that among young people 25-29 years of age 25% of women had higher education qualifications compared to men 18% (ABS 2013). The participants who had children were largely in the 30+ age group which is also consistent with Australian statistics (ABS 2011).

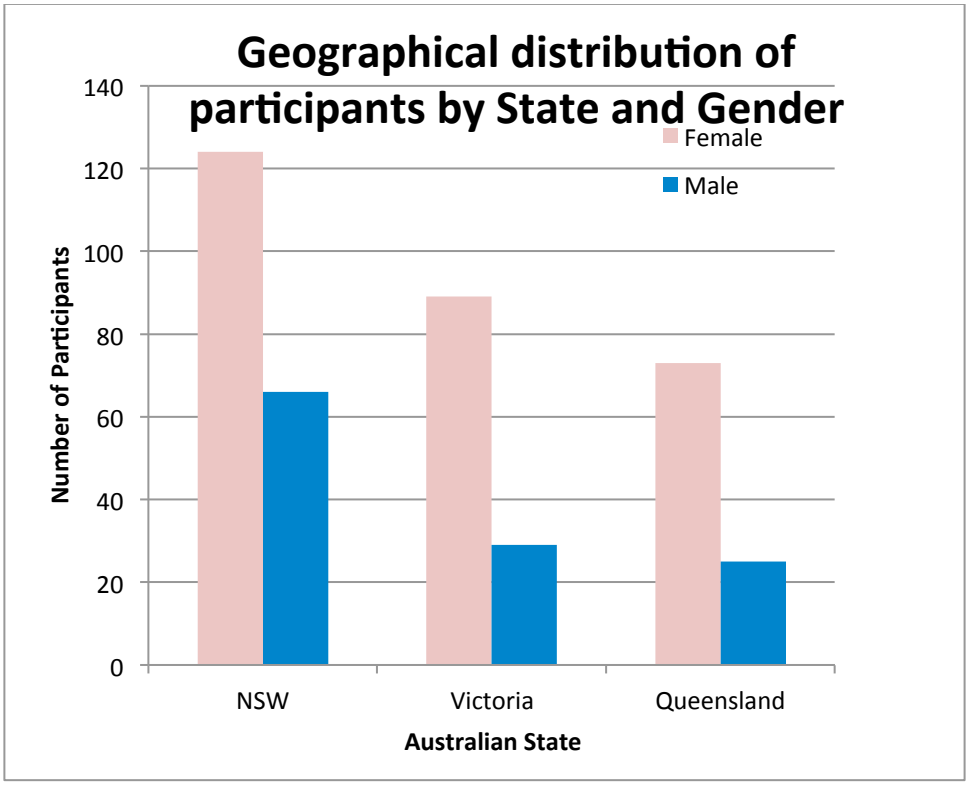


Figure 3.1 Geographical distribution of participants by state and gender

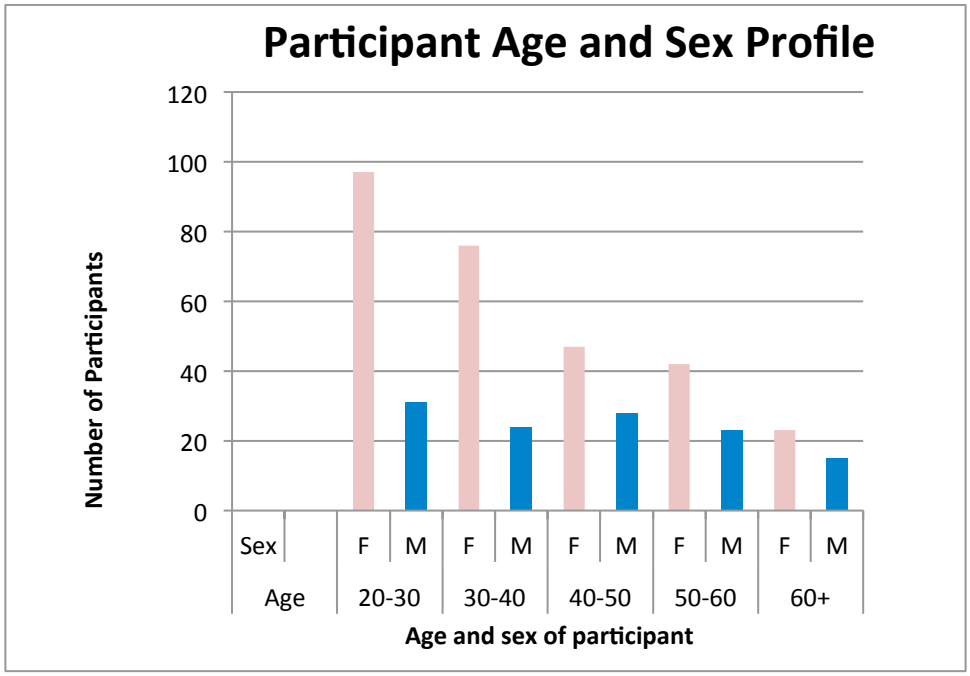


Figure 3.2 Age and sex profile of participants

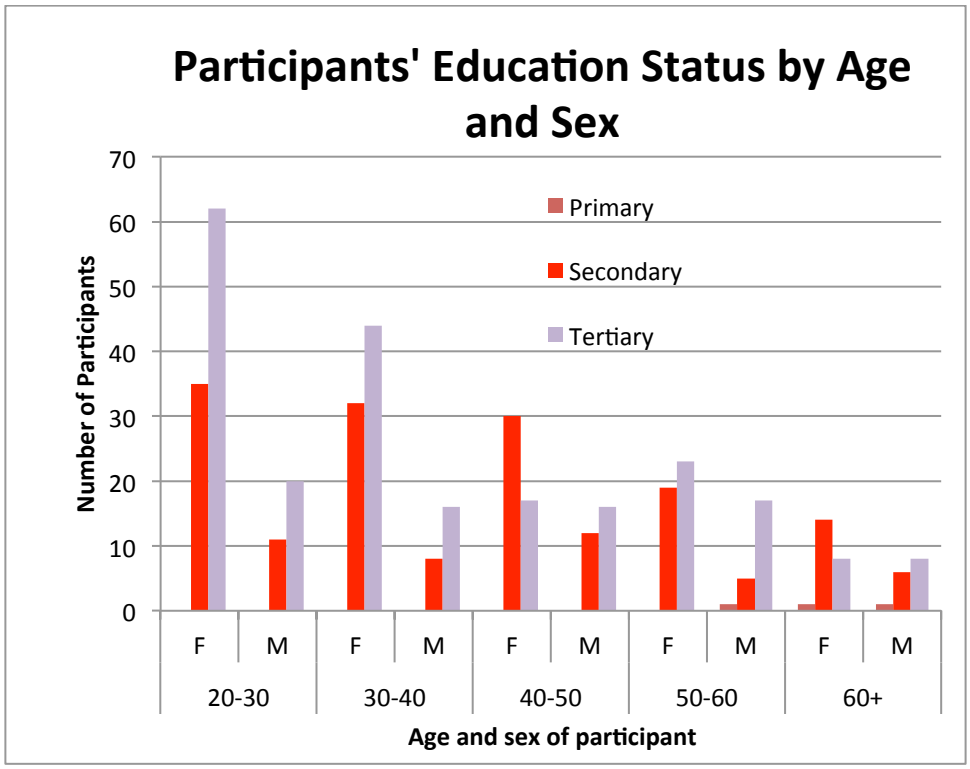


Figure 3.3. Educational status of participants by age and sex

Table 3.1 Participants who had children of their own

Age in years	Gender frequency		Parents who had children
20-30	F	97	9
	M	31	1
30-40	F	76	35
	M	24	9
40-50	F	47	38
	M	28	16
50-60	F	42	34
	M	23	18
60+	F	23	21
	M	15	10
Totals		**406**	**191**

Of the 191 participants who had children 21% did not know what a cleft was. In the group who did not have children 36% did not know what a cleft is. Every effort was made to be clear in the initial question when referring to the word cleft, even by referring the defect to the participants as a 'hare lip' as some participants may have known the condition by this name. This was not done to influence the participant but merely to be as certain as possible that the question was clearly understood.

Figure 3.4 compares the education level of those who knew what a cleft was with those who did not, suggesting that the education level may not contribute to the participants' knowledge of clefting. A Fisher Exact Test was then carried out which gave a p-value of 0.82 indicating that there was no significant association between education level and the knowledge of clefting.

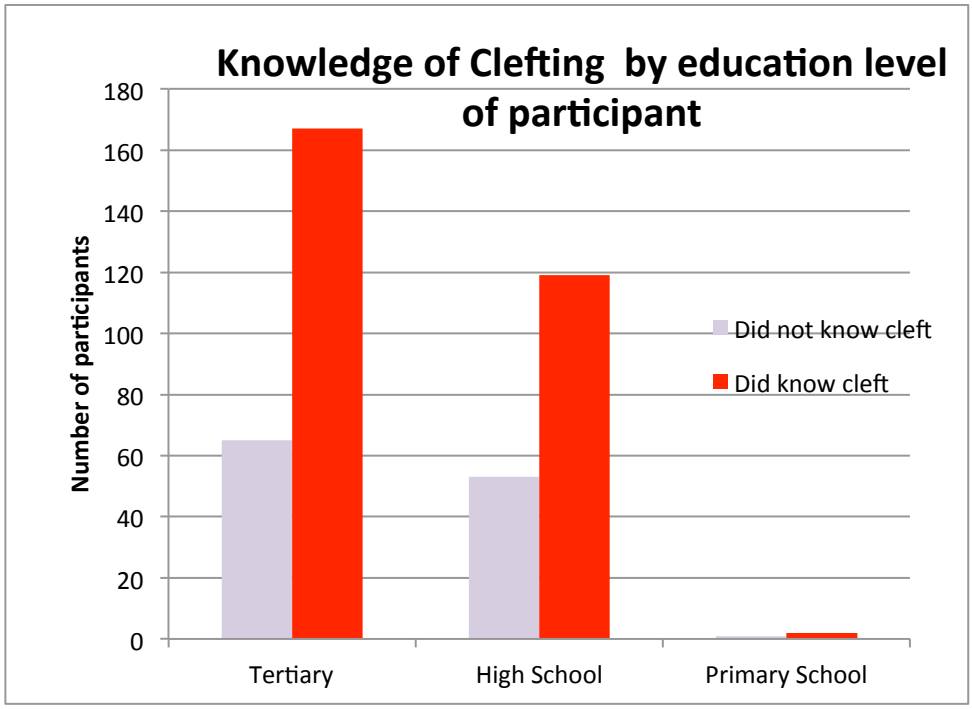


Figure 3.4 Knowledge of clefting by education level of participant

Table 3.2 Education of participants by age group

Education of participants by age group						
Highest education level attained	**Number of participants in each age group**					
	20-30	30-40	40-50	50-60	60+	Total
Primary school	0	0	0	1	2	3
High school	45	39	41	23	20	168
Tertiary	83	62	34	40	16	235
% High school	35.16	38.61	54.67	35.94	52.63	41.38
% Tertiary	64.84	61.39	45.33	62.50	42.11	57.88

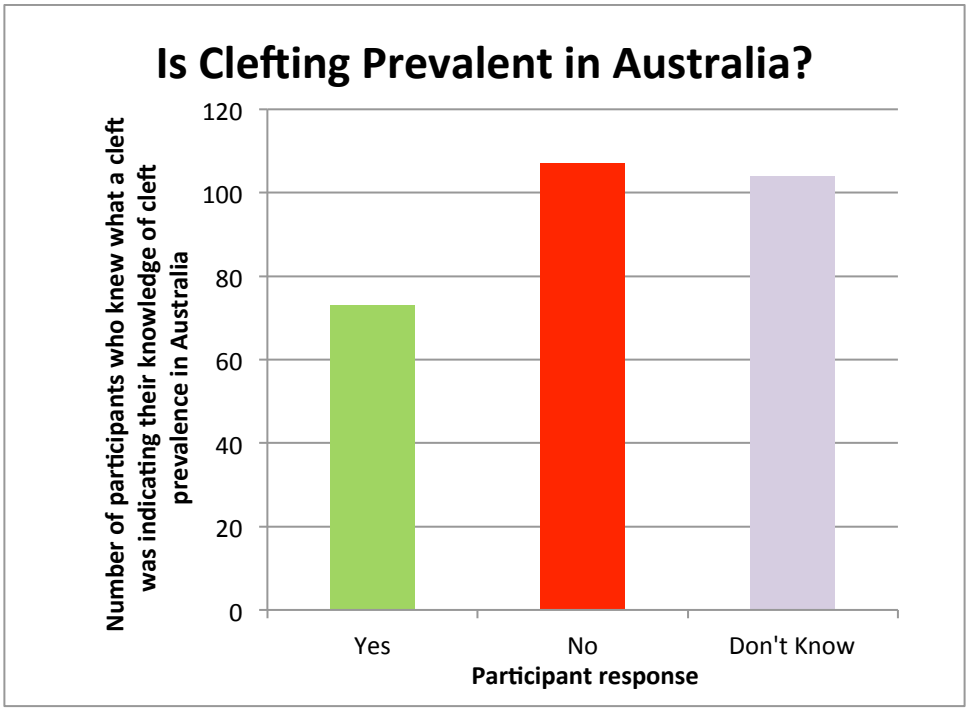


Figure 3.5 Is clefting prevalent in Australia?

(Responses by the participants who knew what a cleft was.)

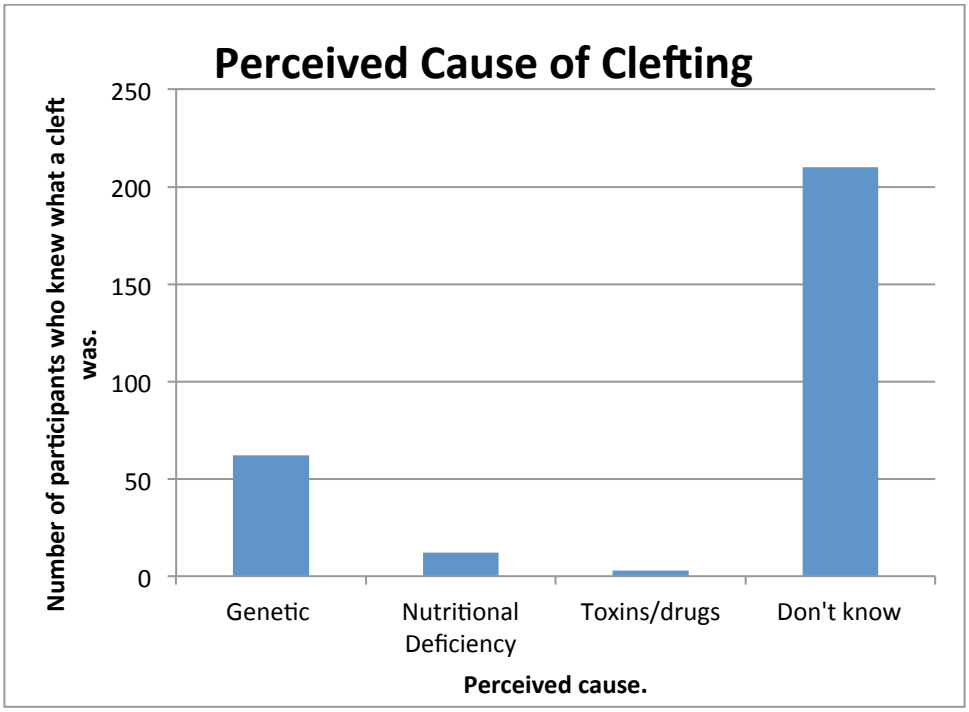


Figure 3.6 Perceived cause of clefting proposed by participants who knew what clefting was

Ninety per cent of participants who knew what a cleft was, knew that the cleft could be repaired and all of those knew that surgery was involved. The fact that 23 participants did not know whether it could be repaired and three said that a cleft could not be repaired raises some doubt regarding the actual knowledge of these participants and therefore the accuracy of their responses.

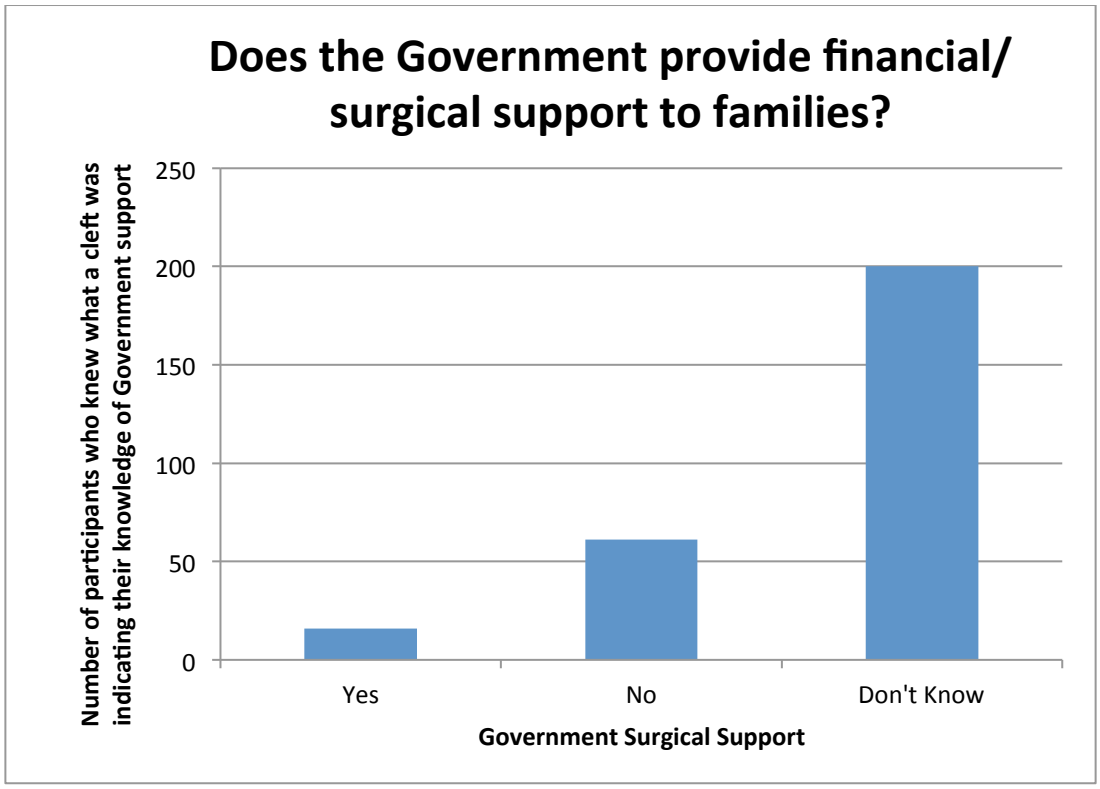


Figure 3.7 Participants' knowledge of the government's financial/surgical support to parents whose child was born with a cleft

Figure 3.8 Participants' knowledge of the requirement of speech therapy for cleft children

Ninety five per cent of all participants who knew that speech therapy was required believed that this should be provided by the government via the Medicare scheme.

Of those participants who knew what a cleft was 98% believed that the government should provide funding for research to investigate the cause. Neither of these questions were followed up to determine whether the participants believed this to be fiscally responsible, or whether differing forms of financial support could be offered, because that would have moved the discussion into the realms of politics, and well beyond the scope of this study.

3.9 Discussion

To fully explore clefting, it was important to determine the community's understanding of clefting, as without a baseline it would be impossible in the future to determine whether progress had been made in awareness. This aspect of the studies sat well within the theoretical framework for this thesis, and hence became the basis of the research question for this particular study – do people in the general community know what a cleft is?

3.9.1 The Face

As people often draw inferences from the facial appearance of others (Willis and Todorov 2006) it suggests that most would have heard of a cleft, or know of some aspects which might affect the visual appearance of someone's face. Willis and Todorov (2006) showed that judgements made about a person on seeing their face for just 100 milliseconds (ms) compared favourably with the same judgements made when there was no time constraint, indicating that we make a decision about a person almost immediately on seeing their face. Ambadar, Schooler et al. (2005) maintained that researchers who merely studied static displays of faces may have underestimated the importance of motion in deciphering the subtle expressions that permeate real life situations. Their study clearly showed that a deeper understanding of a facial expression is obtained when the expression is seen in motion. Seeing a static picture of a person then, does not give us an insight into that person's personality but a video taken of the same person may portray more traits. Currie and Little (2009) investigated how face and body image impacted the choice people made in relation to choosing their mate. The researchers found that for both sexes the face ratings were the best predictors of ratings. When both the face and body were shown to females the same result was achieved as when face and body images were shown separately. For males there was a different interpretation when the face and body images were shown separately indicating that the faces and body images may send different signals to males (Currie and Little 2009). These studies demonstrate that the face is important to people, and so it was surprising that more of the participants were not aware of issues relating to facial anomalies.

3.9.2 Lack of Awareness

Thematic analysis captures something important about the data in relation to the research question, and represents a patterned response or meaning within the data set (Braun and Clarke 2006). The theme in this study was the lack of awareness of clefting. If more fully explored it may mean that there is a general lack of awareness in the community relating to the broader question of conception and pregnancy information. The lack of awareness of clefting demands a response as to how this can be raised, and what more can be done to increase the understanding of the public at large for preconception health planning to try and minimise the number of birth anomalies. Participants with a higher level of education did not have any more awareness of clefting than those with low educational attainment. Education alone is not then the

answer but may relate more to how and where the information is provided. It was not surprising that the majority of participants who knew what a cleft was, were not aware of the incidence of clefting in Australia as the statistics on birth anomalies are only published in government bulletins (Riley and Halliday 2008), and only those seeking the specific information would take the time to find them due to the complexity of many government websites (West 2004). West (2004) in reviewing the way in which governments deliver information, particularly using the Internet, questions the effectiveness of this service delivery pathway, and suggests that due to the difficulty in obtaining the required information there is a danger of losing public trust. The researcher suggests that if finding information is difficult, or if it is not easily comprehended, the public may feel that it is intentionally being portrayed in this manner to avoid questioning.

Access to government information, particularly through government publications has been a fundamental component of the Australian democratic system (Missingham 2007). In 2006 the government proposed to review and consolidate its websites into a more streamlined presence in order to enhance awareness and use. In spite of this occurring, the use of the Internet in government sites to obtain data has only increased from 31% to 33% in the period from 2004 to 2007 (Missingham 2007). More recent statistics on the use of the Australian government website have not been published, however, its use may have increased as more and more emphasis is being placed on web access for general information.

Given that government and more general websites do not appear to be the primary source of information for clefting, it tends to confirm the position taken by Middleton, Lass et al. (1986) that "attention-grabbing" methods need to be adopted in order to more effectively communicate the knowledge about clefting. However, one form of technology that utilises the Internet and provides a more attention-grabbing environment is the social media arena of Facebook, Twitter and YouTube. Chalil and Sendhikumar (2012) state that all online social networks follow the fundamental principle of homophily; similarity breeds connection. People become connected to each other on the basis of socio-demographic, behavioural, and interpersonal characteristics that are the same. The researchers indicate that 23% of Facebook users check their account five or more times daily and that 76% of Twitter users are active tweeters. This shows that people of like mind are regularly communicating with each other using these

new technologies, and hence an opportunity exists to determine how to use this technology to provide information on important issues such as clefting. Chalil and Sendhikumar (2012) in their study proposed a mechanism using social networks to allow the identification of "same wavelength groups" (people with similar interests), and hence target information of specific interest to that cohort. The fact that a large percentage of the social network users are of parenting age would suggest that these channels may be important for disseminating information on clefting and pregnancy planning in general.

The lack of awareness of clefting may also reflect positively on the skill of the surgeons who repair clefts as techniques and access to these skills has greatly increased in the past two decades, this being partly due to the action taken by the Australian government in providing funding for the early repair of the children's clefts (Medicare 2013). The public loses sight of the problem if it is not brought to its attention by seeing children with clefts and so its attention must be drawn to the problem in other ways. The public does need to be aware that clefting still exists, and the extent to which it does, to further promote optimal preconception health care. It was not surprising that the majority of participants did not know what caused a cleft, as no one knows the answer to this. However, almost one quarter of the participants believed that the problem was genetic. This may reflect their lack of knowledge of genetic studies being carried out in the health arena. No attempt was made by the researcher to garner more information from participants as to why they thought that genes were involved because a much more detailed interview would have been required than had been planned.

Those participants who knew what a cleft was knew that clefts could be repaired by surgical procedures, however, they did not know that the funding in Australia for this was provided by the government. The majority of participants who knew what a cleft was knew that cleft children would need speech therapy at some stage in their lives, and again most believed that this should be provided by the government. This is one area that the government does not assist families. CleftPals, the family support group operating in every state in Australia, continues to run a program called Give our Kids a Voice, to bring this to the attention of the federal government.

Nearly all participants who knew what a cleft was, believed that the government should be supporting research in this area. Some caution should be taken with this response as the interviewer wore a name tag during the interview which had a Southern Cross

University logo. The participants could have thought that this was the answer that was required and or merely replied in the affirmative to satisfy the researcher. However, there appeared no hesitation on the participants' part to affirm that the government should invest in clefting research. Those who extended the discussion on this question believed that there should be research into all medical issues affecting young children.

3.10 Strengths and Limitations

The strength of this study was the geographical spread of the participants in terms of the east coast of Australia, the breadth of both their education and ages, and the revealing of knowledge of clefting within those surveyed. The limitations of the study included the small sample size, which prevents generalisation and the lack of representation from any rural and remote areas. Additionally the range of questions may have been problematical as more information could have been obtained had it been possible to conduct longer interviews, however, engaging participants for much longer would not have been practicable in the settings.

The study did not identify the Aboriginal or other cultural background of participants. Nor did it identify socioeconomic or economic background, language proficiency, literacy skills, or disabilities of participants and families; these matters were thought to be too much of an exploration into the more private areas of a person's life and were beyond the planned scope of this study.

3.11 Conclusions

This particular study showed that there is limited awareness of clefting within the Australian general public and more so amongst the younger age groups. This is an area where the government could promote an important public health message to optimise preconception health literacy and antenatal care. This study showed that the level of education does not relate to knowledge of clefting, and that even when participants have children of their own, it does not guarantee that they are aware of clefting and its ramifications.

This research shows that the participants involved supported the government in providing funding for speech therapy to cleft children, and for more general research into clefting.

This study, however, confirms that 'attention-grabbing' methods using new and innovative channels to promote preconception planning to avoid birth anomalies need to

be explored as it may be that people will not access websites dealing with this subject until a problem occurs.

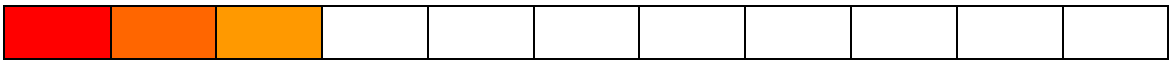
3.12 Recommendations for Further Research

Research needs to be undertaken to determine how improved health literacy relating to preconception care and education can be delivered to the general public with an emphasis on primary health prevention as a large percentage of pregnancies in Australia are unplanned (Richters, Grulich et al. 2003). This could be achieved to some extent by assisting the group CleftPals to raise their profile in the community. Contemporary methods for delivering health messages need to be explored using information media commonly being used by young people.

3.13 Summary

This study provided insight into the knowledge of the general public relating to clefting, but the breadth of that knowledge has been limited due to the small number of questions asked, and the time spent with each participant. It does, however, suggest that more publicity should be given to clefting, not so much from the point of view of the problem itself, but more towards better antenatal education, perhaps even at an early stage in a teenager's life. The use of contemporary media and formats that portray not just the problem but propose solutions need to be more fully explored. The next chapters investigate the experiences of both the child growing up with a cleft and that of their parents, in dealing with the issues.

Chapter 4 Growing up with a Cleft



4.1 Introduction

This chapter introduces young adults who were born with a cleft and narrates some of the issues that they experienced from early childhood into adult life. Neonatologists, paediatric physicians, and surgeons who specialise in treating and working with CLP babies and their families have some appreciation of the medical issues encountered by CLP babies with difficulty breathing, sucking, eating, and talking. Many babies born with CLP endure several rounds of corrective surgery and hospitalisation throughout infancy, childhood, and adolescence (Slator, Hammond et al. 2010). In addition, speech therapy and orthodontic treatments are commonly required and may extend into the late ’20s (Cher, Searle et al. 2008). Since the treatment of cleft lip and palate does not just restore function but has a major effect on facial appearance, as well as taking place during a vulnerable time in life when children/adolescents develop their identity and self-esteem (Allen, Hauser et al. 1994), it is important to understand the long-term effects of all of this on their wellbeing. As a consequence the researcher felt compelled to explore the issues faced by the CLP children.

4.2 Background

Through CleftPals the researcher had met a number of children and their parents with the experience of clefting. Most of the children were under 10 years old, and while they appeared lively and no different to other children except for a slight scar on their faces where surgery had been conducted, the researcher wondered if there may be issues that they had to face that are not of consequence to other children. Hence the aim of this particular study was to determine the perceptions and experiences of young adults and how they coped with a CLP and life in general as they matured.

4.3 Research Question

What are the issues faced by children who are born with a cleft as they grow into young adults?

4.4 Literature Review

While studies have been undertaken to determine the lived experience of people with, or caring for, those with disabilities (Cashin 2004; Berntsson, Berg et al. 2007; Brown, Goodman et al. 2009; Nordstrom, Skarsater et al. 2009; Van Huet, Innes et al. 2009) few have been found relating to cleft lip and palate.

Several early studies have been conducted considering the psychological adjustment of individuals with CLP. In particular some have reported a high degree of dissatisfaction and self-consciousness regarding appearance (Heller, Tidmarsh et al. 1981), another a low rating for body satisfaction (Clifford, Crocker et al. 1972), and evidence that individuals with CLP appear to be observers rather than participants in social interactions and feel neglected because of their handicap (VanDenmark and VanDenmark 1970). Heller, Tidmarsh et al. (1981) reported that many experienced problems in relation to the opposite sex, tended to marry at a later age than their siblings, and showed a longer dependence on their family with some needing counselling and supportive services outside the family group.

A retrospective study of 63 adults in Iceland involved a wide age range (25-50 years) (Bjornsson and Agustsdottir 1987). Many of the adults in the study had time to 'heal any wounds' (Bjornsson and Agustsdottir 1987) that had been present during earlier years. The individuals with facial clefts in the study were found to be relatively well adjusted and coped well with day to day life, and it was concluded that the subjects did not seem to perceive that the cleft consequences had influenced their life to any great extent. A further study (Chapados 2000) investigated the experiences of ten teenagers, between 15 and 17 years of age, using both questionnaire-based and semi-structured interviews. Many of these adolescent participants were involved in CLP surgery at the time and were not yet in a position to see how this would affect their career or lifestyle choices. The results of the study did indicate that they had experienced functional (not being able to communicate effectively) and interactional problems throughout their development. Chapados (2000) concluded that both those with a cleft and their families needed greater assistance and training to help them explain to others the implications of being born with a cleft.

A Swedish study (Marcusson 2001) investigated the quality of life, satisfaction with treatment and psychosocial distress in a group of 44 men and 24 women with a mean age of 24.2 years (range 19.5-29.2) with treated CLP and gender and age matched

controls with no clefts. They found that the cleft group rated some detached aspects such as life meaning (achievements/work, relationships/intimacy, religion/spirituality and self-transcendence/generativity) (Emmons 2003, p. 108) and family life significantly lower than the controls but not the more practical and tangible aspects of their daily living. A high degree of dissatisfaction was expressed within the cleft group with the surgical procedures that had been undertaken and this was endorsed by the surgical practitioner group. In this regard the surgical group recommended that further remedial surgery be undertaken on 38 of the 72 cleft participants. This dissatisfaction with the treatments that the participants had previously undergone may well have influenced the psychosocial aspects of the study.

A study involving 113 children and young adults in Northern Ireland (Hunt, Burden et al. 2006) involved self-reports of their psychosocial functioning after being born with a CLP. The age range was from 8 to 21 years, and so many of the participants were in the early stages of their surgical journey, and also had not yet faced some of the challenges that perhaps lay ahead. It was reported that the participants exhibited more behavioural problems and a higher degree of depression than controls who were recruited from a wide range of sources by circulating requests among parents who were members of hospital and university staff who had sons or daughters of a similar age willing to participate (Hunt, Burden et al. 2006). The study group were teased more often, were less happy with their appearance, and with their speech. With regard to anxiety and self-esteem there was no difference between the CLP group and the controls. This study may have had more significant outcomes had there been less disparity in the age group, and in their social development as the researchers found that age was a significant predictor of behavioural problems, happiness with facial appearance, and satisfaction with speech. Older subjects had more behavioural problems and were less happy with their appearance and their speech than those in the younger group.

Other studies (Stevens, Steele et al. 1996; Patel, Fisher et al. 2007) were reviewed to determine how self-esteem and confidence were expressed by young adults in their development when they had other non-life threatening disabilities. Mental health issues were said to be related to lower educational achievements, substance abuse, violence and poor reproductive and sexual health. On the other hand those with physical disabilities reported good self-esteem, strong family relationships and as many close

friends as those in the able bodied community (Stevens, Steele et al. 1996; Patel, Fisher et al. 2007).

The study outlined in this chapter tried to understand the perceptions of participants in growing up with a CLP and its treatment. It provides information for paediatric surgeons, physicians and other health professionals interested in long term outcomes for cleft children. It may also be of use for counselling parents or indeed for parents in assuring their child that the final outcome will be positive.

4.5 Ethics Approval

This particular study was approved by the Human Research Ethics Committee of Southern Cross University, Australia (ECN 9-018). Each participant provided informed consent.

4.6 Research Design

This study used a simple qualitative descriptive method to obtain information relating to the experiences of adults who had been born with a CLP. The objective was to choose a design that would give authenticity to participants' experiences and enable their voices to be heard. The study design was based on interviews around a semi-structured questionnaire which some claim is the most common technique for gathering data in qualitative health research (Draper and Swift 2010). The interviews were conducted in locations nominated by the participant. The participants volunteered to participate after hearing of the study through CleftPals. The participants lived in one of the three eastern states of Australia and did not know each other.

This study focussed on participants who had all been born with a facial cleft and who had all completed the surgical procedures to correct this birth anomaly. The design and analysis of this study closely followed that established by Fade and Swift (2010). Childhood oral biographies were obtained from adult participants aged 25 to 38 years old. All names have been changed to protect their identity. With the exception of Karen, Mary and Claire, all participants were associated in some way with CleftPals.[11] Karen was met incidentally and Mary was introduced to the researcher by a close friend.

[11] CleftPals is a family support group which provides physical and emotional support to parents who are either expecting a child with a cleft or who have recently had one come into their family.

4.7 Sample

The participants were located in the Australian states of Queensland, New South Wales and Victoria. Each gave consent to be interviewed and the interviews to be audio recorded. The participants had completed all the medical treatments associated with a non-syndromic cleft and were between the ages of 25 and 38 years. The only exclusion was if they volunteered but had a syndromic cleft.

4.8 Setting

The interviews were semi-structured and were conducted in the participants' places of residence or at their work place. All interviews were undertaken on a one-to-one basis in rooms where only the researcher and participant were present.

4.9 Data Analysis

Once the narratives had been collected thematic analysis was used to develop the themes within the data. Thematic analysis is a search for themes that emerge as being important to the description of the data (Daly, Kellehear et al. 1997). The process involves the identification of themes through careful reading and re-reading of the data (Rice and Ezzy 1999; Braun and Clarke 2006). It is a form of pattern recognition within the data, where emerging themes become the categories for analysis (Fereday 2006). Thematic analysis is a feature of qualitative research (Braun and Clarke 2006) and fits within a wide spectrum of qualitative methodologies including phenomenology (Smith, Larkin et al. 2009), conversation analysis (Hutchby and Wooffitt 2008), and discourse analysis (Wetherell, Taylor et al. 2001) among others. Ross and Green (2011), in studying anorexia nervosa, indicated that by using thematic analysis it enabled justice to be done to the women who spoke so intimately in the interviews. The researchers used a semi-structured questionnaire and audiotaped the responses before coding them and thematically analysing the data.

Others claim that thematic analysis is a qualitative method in its own right (Braun and Clarke 2006) claiming that it is not only extremely flexible in its use but is widely used, and is not included in other methodological nomenclature, such as discourse analysis or content analysis. The claim is that these other methods search for themes or patterns across an entire data set rather than within a data item such as an individual interview or interviews from a single person. Thematic analysis, due to its flexibility, covers both situations.

Thematic analysis can fall into two categories, the first being an inductive approach where the themes identified are strongly linked to the data themselves while a theoretical approach tends to be driven by the researcher's analytic interest (Braun and Clarke 2006). As in other qualitative methods several steps are needed within the analysis to ensure positive outcomes. Initially the researcher must familiarise him/herself with the data by reading through it over and over again and if interviews have been recorded to transcribe these so that this can be achieved. This allows initial codes to be generated. Codes identify a feature of the data that is of interest to the analyst or an element of the data that can be assessed in a meaningful way. (Braun and Clarke 2006). Coding identifies parts of the data set that can then be assembled into themes in a much broader sense to provide an overarching view of the particular part of the data corpus. The analyst can review the themes and go back through the coded items to ensure that the theme does in fact include all relevant material before providing a name for each theme. Finally the analyst must ask what each theme means, what are the assumptions underpinning it, what are the implications of this theme, and what is the overall story that the different themes reveal about the topic (Braun and Clarke 2006).

Each participant was interviewed by the researcher (GHW) in a semi-structured manner with a series of questions while allowing discussion to move beyond this. The meetings were audio-recorded, with additional consent, transcribed by the researcher, and sent back to the participant for audit review and for any changes they preferred. This was done to ensure that rigour was maintained for the study. The technique of referring the data back to a respondent is referred to as respondent (or member) validation (Pilnick and Swift 2010) where the data collected are referred back to the individuals who participated to ensure that what was recorded was completely accurate. The only variation to this was that the information provided by Claire after the first meeting was provided by email.

Privacy of the individual was protected by immediately changing the names in all documentation. Apart from the researcher no one has been party to the personal identification of the individuals involved.

Once the individual data had been read and approved by each of the participants it was coded using the computer program NVIVO to establish the nodes to confirm the themes from the data. Each transcript was uploaded to the program which was then run to

provide a cluster analysis of word similarity followed by word frequency. This was then reviewed to determine specific nodes. As each node was established, the sentence or sentences relating to it in each participant's data was copied to the node. On completion, if there was data from two or more participants within each node, the node was considered a theme and a descriptive heading was assigned to it. Braun and Clarke (2006) state that thematic analysis is not bound by rigid rules unlike other analytic methods where a large proportion of the data must display the evidence to be considered a theme. This means that judgement is required to determine what a theme is, as for some themes there may be little evidence to support it but the statement that is made may be integrally related to the overall topic.

4.10 Results

4.10.1 Participants

Jason lives in the Melbourne central business district. He is 30 years old and is a mechanical engineer. Jason was born with a unilateral CLP and no other medical problems. Jason appears to be a very confident young man, and seems very much in charge of his life. Jason was the fifth child in his family with none of his siblings having any birth anomalies.

David lives in a Sydney inner city suburb. He is 25 years old and was born with a bilateral CLP and no other medical issues. He is a university graduate and was working as a public policy consultant, but more recently is undertaking further studies at Oxford University in England. He is extremely articulate and appears to have a very outgoing personality. He has a younger sister and half-sister and neither was born with a cleft.

Mary is 37 years old and is currently undertaking further university studies. She is the oldest of three girls and was born with an isolated CP. Her two sisters did not have clefts. At 18 months' old she had her first operation, which she said went horribly wrong.

Karen is a 28-year-old who was born with an isolated CL. She is a university graduate now employed in the publishing industry as a national sales and marketing manager. She and her partner are currently renovating their home. She was the first born in the family and has two sisters who have no birth anomalies.

Rebecca is a 34-year-old who had a CLP. She works for a large engineering firm in charge of their document control. She is due to take maternity leave and this interview

was conducted just six weeks before her baby was due. She was the second child in the family and has a brother who does not have a cleft. She subsequently had a daughter with no clefts.

Ann is 38 years' old, married and has two boys. Neither boy had a cleft when they were born. Ann was born with a bilateral cleft lip and palate. She is a university graduate, has held senior positions during her working life and is currently employed part-time as a program coordinator for an interfaith organisation. She is a full time mother the rest of the time. She was the second child in the family and has an older brother and younger sister. The other siblings did not have clefts.

Claire is a 28-year-old and has an older brother and sister neither of whom had any birth anomalies. She is a customer service manager in a high profile industry having daily interaction with the general public. The fact that she had a bilateral cleft lip and palate is only noticeable to someone who has had experience in seeing the results of surgical procedures.

4.10.2 Findings

The headings for each theme were assigned to reflect the question discussed during the meeting and also to provide a descriptive synopsis of the responses.

The findings are presented as themes that were derived from the following nodes:

- First recollection/memory/difference
- Bullying
- Surgery/hospital/scared
- Why me/question why
- Friends
- Affect/life change
- Bad experience/worst time
- Family/support
- Career/work/job
- Other children/advice
- Parents/advice
- Doctors/surgeons/advice
- Community/caring
- Government/support

4.10.2.1 First recollection – I can't remember!

The question that formed this part of the discussion was: When did you realise that you had a cleft? The node from which this theme was developed was first recollection,

memory and difference. As all[12] of the participants had experienced major surgery in their early years it could be anticipated that this would be remembered with some clarity and pain.

Jason: My first memory is at five years of age and realizing that I was different in some way to other children. I looked around at school and everyone was different to me.

David: There was no exact time or date when I realised that I had a cleft. It was always a part of me. It was just like, this is how things are.

Mary: At six years old I realised my speech abnormality was caused by a cleft. I had always had speech abnormalities. I was always being driven to the doctor for something or other, but prior to that I just knew that it was really frustrating at not being understood. I knew that it was also quite frustrating for people to understand me.

Karen: I do not remember when I first realised that I had a cleft but I guess it was probably just from looking later at the baby photos. I can't remember the age but I was old enough to look at the photos and say: hey mum, look at that, what's wrong with my lip?

Rebecca: I knew that I had a cleft when I was very, very young because I was always in hospital, going to see doctors, so I knew there was something a bit different. This was probably when I was about four years old but I also remember at that time that I was just a regular kid.

Ann: I do not remember when I actually realised that I had a cleft, and it is probably more from stories that I have been told rather than my own recollection.

Claire: I'm not too sure when I first realised that I had a cleft, I do remember when I was probably about six or so being very conscious of my scar and experimenting with a few different creams to try and dull my scar. I'm sure I was aware of it before then as I would've been going to the dentist every few weeks and I had a plate from a very young age, so I'm sure I would've been asking my parents questions before then, but that is probably my first memory of being quite self-conscious of the scar on my lip.

4.10.2.2 Bullying – It's one of those things!

The question that formed this part of the discussion was: Were you ever bullied because of your cleft? The node from which this theme was developed was bullying.

[12] In this chapter the word 'all' refers to all those mentioned in the subsection as not all the participants commented on every theme

Jason: I was never really bullied as such but children are inquisitive. That's what you do when you are young, isn't it, and so at that time I realised that I had a cleft and that was why I was different. I had two older brothers and two older sisters and so I think that if there had been any bullying they would have sorted that out.

David: I was never a shy kid, by any means, so I never felt that it held me back in any way, in terms of being able to play or anything like that – I could also show off a bit that other children couldn't. I was able to make milk go through my nose, – that was pretty funny. At primary school I had a good group of friends and I used to knock around with them at ease.

At secondary school I was more part of a 'scene', but there was certainly some level of teasing at primary school but I think that that's more a part of childhood. Kids will find something to pick on. Even when you're at the top of the pile you still don't escape that. The cleft was certainly the focus of any teasing that took place but it wasn't particularly severe.

Rebecca: Children started to notice [the cleft] and would make comments. At around about seven the children were unkind, in fact very unkind. It's very hard but you have to get on with it. It hurts a lot. The teachers hear and they don't do anything. You just get on with it.

At the time the friends didn't really stick up for me. I think school yard politics is that you're on your own, most of the time.

The worst thing was the teasing, comments, and staring. I'm very self-conscious even now, extremely. It's the first thing I see when I look in the mirror, every day. There is a scar and even if it was smaller it is still a reminder of past events. It's not 'confronting' every day, but I'm very aware of it and very self-conscious. People still comment a little bit, not so much as before.

Claire: I don't really think that I felt any 'different' to other children, I was definitely bullied, but probably no more than any other children, and it was always directed towards my scar. I was lucky that my speech was quite good and I was fairly academic, so I always managed a good comeback! I think if anything it made me tougher. I wasn't one to 'dob' on anyone, I definitely fought my own battles.

4.10.2.3 The early surgery – It's not frightening!

The question that formed this part of the discussion was: What was the hospital/surgery like? The node from which this theme was developed was surgery, hospital and scared.

Jason: I had known that I went to hospital and it is funny that you forget a lot of things that happened at age five or around that time, but you don't forget about going to hospital. My most vivid memory was at age five going to the – Hospital with my mother. This was obviously not the first time in hospital but this time was for follow-up procedures and it sticks in my memory. The surgery was to rework both my lips and the palate.

David: I remember when I was little that the medical procedures were kind of fun … uncomfortable, but certainly not painful. I remember when braces were a big problem. In a lot of ways they were worse than the problems they were trying to fix at the time. And I kind of resented a lot of the medical treatment when I was twelve or 13, I wasn't really interested, there didn't seem to be much point to it. But then, 13-year-olds are pretty disagreeable anyway.

Claire: My first major operation was when I was about six months old so I don't remember that at all, I think I had another operation at 18 months as well. The first one I remember was when I was 10 years old and I had my first bone graft surgery. I was in hospital for about a week or so, my parents told me I was in a lot of pain, although I don't remember that. I know I was in a wheelchair for the week, as the surgery was on my hip.

4.10.2.4 Why me? I never really asked!

The question that formed this part of the discussion was: Did you ever ask your parents why it was you that was born with a cleft? The node from which this theme was developed was why me and question why.

Jason: My mother kept all the names of the surgery that I had but I never took much interest in the names but merely questioned along the way "Why do I have to go to a doctor? Why do I have to go to a speech therapist?" None of my friends have to do this and so the realization became more vivid that I had something a bit different to everyone else.

Mary: I just went along with what I was supposed to do. Some kids have a very strong mother (I did), they just do what they're supposed to do. You're going to have an operation, so I'm going to have an operation, and that's it.

Karen: I never questioned my parents as to why it happened and until recently never thought about it. In fact, it was a coincidence that the day we met was the first time I had spoken about it and on that occasion it was in the car with a friend travelling up to the markets. It never dawned on me to ask the question "why me?"

4.10.2.5 Friendships – No problems!

The question that formed this part of the discussion was: Did you find it difficult making friends? The node from which this theme was developed was friends and friendship.

Jason: The fact that my face looked different to others did not stop me making friends although I was a little self-conscious about it. During my childhood I can say that I never really had a negative experience. On the contrary, there were things I could do that other children could not. For example I had an under-bite and I could touch my nose with my tongue with absolute ease but none of the other children could come close to that. Even though I used this to my advantage I probably did not fully appreciate it, to be perfectly honest.

David: At High School the kids I hung out with weren't the cool kids anyway, we weren't good at sport. Realistically, I sort of feel it was as much a result of being a part of the group that wasn't any good at sport as much as anything else, and wasn't the coolest or the toughest group, certainly not the toughest group.

Socially I have no difficulties. I've had a girlfriend on and off, you know, different people for years; I've got a large circle of friends, and if those two things weren't true, it could be quite different. But because of those things, I think objectively I don't have a lot to complain about.

Mary: It is difficult in hindsight to understand how my speech anomaly impacted on my relationship with other children, but I remember in the first grade that I really only had the one best friend. I do remember that I took myself apart from the other children in class, I did sort of isolate myself from them. On the other hand, my mother was quite gregarious so she always had birthday parties with all the children and I never felt anything different then. I do remember for some reason in first grade my best friend, we were sitting in the girls' loo to have our lunch, perhaps it was just to have the space around us. I don't know why I chose that.

Karen: I was the oldest of three in my family. Neither of the younger children, both girls, had clefts. We lived in a small neighbourhood. One of my best friends had a sister who was older; there was a girl across the street who was older, so I had a bit of camaraderie with older kids that I knew who were sort of on my side.

Rebecca: I had friends so it wasn't a general thing. Probably at Grade 6 it had real impact but there were lots of other things going on in my life as well that when I look back I think they had an impact as well that wasn't helping the situation, so to speak. Yeah,

I had friends. The other things were not related to the cleft. And it wasn't because I was playing up or anything. It was just a difficult time.

Ann: I remember having a special friend, and having other friends as well. I'm still friends with some kids from school. It was quite a supportive environment.

Claire: I don't think I ever found it difficult to make friends. Even now I have an incredible network of people around me. I never really had any trouble interacting with other children, there was in fact another girl in my year at primary school who had a uni-lateral cleft lip and palate. I was a pretty outgoing kid and was involved in tennis and hockey both in and outside of school so I had quite a few different friends.

4.10.2.6 Confidence and resilience – I've got plenty!

The question that formed this part of the discussion was: What effect has this had on your lives? The node from which this theme was developed was effect and life change.

Jason: It was later that I realised how having a cleft had really helped me. It's just that you don't take things for granted. I mean, having solid food, having a shower, being able to laugh without your mouth hurting and having 400 stitches in your mouth that you're waiting to dissolve. I was talking to a guy the other day about one of the parents that I met, where before they dissolved the stitches you had to lie on your back for about two hours on end, while they took stitches out of your mouth. And I guess going through those things just gives you a good perspective, so when someone complains about having a sore toe or that the Medicare rebate should be higher, or maybe the baby bonus should be an extra $500, you think get a grip here. It gives you a very good perspective on life. For me too, if things go wrong at work or in my life, with a brother or sister or a girlfriend, it gives me the perspective that I wish it hadn't happened but it's not the end of the world. You know, I've been through these experiences and if I got through that I can definitely get through this. And not to be blasé about it but it gives you that confidence and intestinal fortitude that you can get through a tough experience. And I think it's later in life that it's helped me no end.

David: I guess there's probably a level of resilience that you do develop. But for me I struggle to draw that back to the cleft too directly. I'm loathed to say that it's because I had a cleft that I feel comfortable speaking in public, or something like that. I don't think it's that causal, without sounding too 'social-sciencey'. What I can say categorically is that there's nothing terrible, and I think that's the key point, that it's not a driving force.

Mary: I think it's made me speak better, because I've had speech therapy and drama, I think that I enunciate my words better, my mother was a primary school teacher and so my sentence structure is good, my vocabulary was always good, so sometimes the worst thing you have can be the best thing that way in the long run.

Rebecca: Because I can eat without food coming out my nose, the basic stuff that everyone accepts and takes for granted, it has been positive for me in a medical sense. I've never really looked at the positive side of having a cleft but I suppose it has made me a bit of a stronger person. In personality traits it brings out the best in you, makes you more accepting and less judgmental of others. In that way it's made me a better person.

Ann: I feel sure that having a cleft has assisted me greatly in being able to cope with life. Not only is the cleft a part of who I am, and part of what I needed to cope with growing up, but it taught me in life how to get up and do things, not to dwell on them, to be more resilient Children are honest and factual and I put it on the table, this is what I had, this is what I've been through in life. I've got resilience but I've had no choice but to have resilience.

Claire: If I had of been born in a country where these procedures weren't accessible/affordable I wouldn't have the quality of life that I have today. So even though at times it felt almost like a chore or an inconvenience to go to the clinic, I am grateful that I was fortunate enough to have access to the amazing practitioners that were in Perth at the time.

4.10.2.7 My worst experience – There was one!

The question that formed this part of the discussion was: What was the worst time for you? The node from which this theme was developed was bad experience/worst time.

Jason: The worst time for me was when I had my bone graft. I had my first rhinoplasty when I was 13. I've played sport my entire life, and that was the first time that it brought a halt to my life, and it was like: "Sorry, Jason but you can't play sport for a year." For the first time in my life it wasn't a one or two months' recovery, I had a 12 months recovery. That was probably the first time that when I thought that this is a bit unfair, and I wish I didn't have to go through this.

David: I did have speech therapy – which I really didn't enjoy. I found it almost demeaning and unnecessary, and I really resented it. It was very boring. But the speech therapy's very difficult, it's hard to retrain your tongue to speak in certain ways, so it's a frustrating process anyway. But that was around sort of 13. I had no time for that. I'd go, but I didn't enjoy it at all. In hindsight of course it was extremely

worthwhile. In a lot of ways you're comfortable and then someone tells you something's wrong and then it becomes a problem. I had become comfortable with life and I, in a headstrong way, thought I don't want to change anything, I'm happy. So I think I resented almost the 'problematising' process.

Mary: I think the cleft is a negative because it's caused other things to go wrong. I've had orthodontists and dentists and a whole host of interventions. The braces were awful, it was terrible, every time I went to the orthodontist my mouth would be full of blood because he would actually put the metal on my molars and it was awful.

Ann: The more recent operations began in my late high school years, when passing my HSC and going out with boys was far more important to me than what operation I was to have next.

I had an abbe flap operation which meant that I had a bit of my bottom lip cut and moved into my top lip, making my bottom lip smaller, (it was very big) and my top lip made bigger. I couldn't talk for two weeks and I had to eat through a straw. I did think that not being able to eat during this time I would lose some weight, but this was a real disappointment. I actually gained 5 kg. I was really pissed off about this.

Claire: I had another bone graft when I was 18 and I remember that very well. I was in a lot of pain as the bone was taken from my lower jaw and put into my upper jaw. I could hardly eat anything because my mouth was so swollen, even attempting to have painkillers was impossible because I could hardly swallow. I think I only remember that one so well because of my age. That bone graft was more to do with building up the bone so that I could have implants for my missing lateral incisors. I think I had the implants put in when I was 19.

4.10.2.8 Family support

The question that formed this part of the discussion was: Did you receive the full support from your family during your medical procedures and after they had concluded? The node from which this theme was developed was family/support.

Jason: I have an amazing mother and have had great support from all members of the family. We didn't have a lot of money but we did have health insurance, but I am sure there were times when my father wished that this [cleft] had not occurred. I am sure that he never intended that I should get that feeling but I have a feeling that it may have passed through his mind at times. It was probably more that this is an inconvenient thing to have happened.

David: The family issues that some may experience never existed in our family as my mother had had a cleft and she got through University in the 1970's and so she knew that this would in no way affect my future life or career. She also knew the processes involved for the correction and so I am sure that helped.

Mary: I am sure that my having a cleft impacted heavily on the family. I think it was a very negative thing because there was so much medical intervention. There was so much time spent correcting everything I said, taking me places, the money it must have cost, because the medical system is very expensive. I know my father must have been affected as he was starting his business, which I know was a dream of his, but I do think there was a money issue there.

Ann: My family set up the cleft lip and palate foundation in Victoria, now called CleftPals. To set up a support group now would be exhausting and overwhelming and hard work, so it must have been even harder for them, and they didn't have family in Victoria. They had moved from Sydney to Melbourne for work, and with two little children, let alone with someone with special needs like I had, and to navigate the medical system. It was a huge thing, and obviously it was unexpected, and they didn't know in those days the havoc it would cause for anybody. I won't say they revolved their life around it but they put a lot of work into putting the support group in place and getting themselves some emotional support.

Initially they would have been keen to get support for themselves, everyone does that for their own needs first, but now they do it much more for the common good of others, and for helping other people who are just starting on this journey. I'm proud of them for what they did for me, and what they continue to do for others.

Claire: The Children's Hospital in Perth is located in Subiaco, which is about 30 minutes from my parent's house, who live south of Perth. That was where I attended all of my dental and speech appointments, and most of my plastics appointments. I was a public patient for all of my procedures, when I was born they advised my parents to go through the public health system because the amount of appointments and procedures I would need would have ended up costing them hundreds of thousands of dollars had they chosen to go private. So I think for the time they had to give up to take me back and forward for my various appointments they were affected quite badly. Having to arrange the time off work would've been pretty inconvenient. I'm pretty sure the day I got my license and could take myself to the appointments was the happiest day of their lives! But realistically in the grand scheme of things they would probably say that this was a minimal effect on their lives.

4.10.2.9 Career choices – No problems!

The question that formed this part of the discussion was: Did the cleft affect your career choices or prospects in any way? The node from which this theme was developed was career/work and jobs.

Jason: Having had this experience has not been negative in achieving the career goals I set out to achieve and if anything I think that it has helped me. I think it has helped me to put life into the proper perspective. You need a few setbacks in life if you want to get where you want to go.

David: In terms of my career and choice of vocation I haven't shied away from work that is confrontational or is difficult or requires interaction with other people. It has not affected my willingness to speak publicly or to engage in potentially quite personal episodes within the scope of my work.

Karen: The cleft never had an impact on what I wanted to do with my life. I've always wanted to work in marketing or selling, I've been selling things since I was three or four years old.

Ann: My life settled with the completion of my first University Degree, this being a Bachelor of Arts and with summer coming up and no operations. That was enjoyable.

I went back to University to complete a second degree, this time in Social Work. After this I travelled overseas for eleven months, and then came home and started work as a social worker.

Claire: I don't feel having a cleft has affected my ability to do anything at all. I'm a pretty independent person and never really let anybody bring me down or tell me I can't do things. The only reason I would've thought I couldn't do something would've been because I literally can't do it, it would have nothing to do with my cleft.

4.10.2.10 Advice to other kids – Be positive!

The question that formed this part of the discussion was: what would you say to other children who have a cleft and what advice would you give them? The node from which this theme was developed was other children and advice.

Jason: It is difficult to advise other children but perhaps the best advice is that this does not stop you from doing anything as it has absolutely no bearing on your mental capacity.

Having a positive approach is just absolutely everything. So when you go for a surgery, if you're positive it will not be as hard to bear. It's like everything in life, if you approach it negatively the outcome's going to be negative, well, you know, I often think whatever attitude you have you get what you deserve, to a certain degree so always be positive.

David: For other children who may have a cleft I would like them to know that it's not a big deal. I think if things aren't going well it would be easy to use the excuse and pin it on the cleft but for me, and I hope for others, it hasn't been a big thing and I have just been able to get on with it. So you have a choice that you either get on with life or let life get on top of you.

Mary: If there are children with a cleft ever reading this, or their parents are reading this to them, I would definitely encourage them to do all the speech therapy that they can. Make sure that you really look into your diet, that's a really big thing, had I known more about diet I would have made sure that I didn't have processed food or sugar, you want your jaw development to be as good as it can be, and that's perfect timing for your teeth to come up straight and everything to work.

Rebecca: I would like to tell other children who might have a cleft or perhaps even another disability "Don't be silly like me, don't take it to heart so much. I've wasted a lot of time worrying about how I look, unnecessarily, and I've let it get to me at times and I shouldn't have.

Ann: If I was to talk to a child with a cleft I would say "be yourself". Everyone in life is different, everyone has strengths, challenges, and no one is better than another. The difference is that the cleft is a physical thing that people can see, because you can see it, it makes you look different to others, but it doesn't mean that some other kid doesn't have something that you actually don't know about. So it's about being yourself, being proud of who you are, and that's not easy for any kid or any person.

4.10.2.11 Advice to parents – Be supportive!

The question that formed this part of the discussion was: What would you say to parents whose child had a cleft and what advice would you give them? The node from which this theme was developed was parents and advice.

Jason: I don't think you can underestimate how important having family support is. They're the most important people in your life, and from my experience, once you've got their support, you've always got a safe place, and you've always got that support network.

David: To parents I would like to emphasise even more than to children, in terms of a sort of guiding way of looking at it, the position is that this isn't going to ruin things but the family support is an essential requirement.

Mary: To the parents I would say perhaps less intervention, speech therapy's fine but wait, let them grow into it, let their jaws reach the normal size, it's not going to trouble the children to not have things fixed straight away. Also really, concentrate on nutrition. If you know that it's a birth defect look at nutrition, that's a big thing.

Karen: I would really hope that any parent who has a child with a cleft will ensure that the child gets the best surgery possible. Looks are not the most important thing in life but it does help.

Rebecca: There is an important role for parents to play: they need to reinforce the fact that their child is beautiful and the most precious thing in all the world. I think sometimes it's nice to hear from your parents that you look ok as well.

4.10.2.12 The medical profession – Knowledge is everything.

The question that formed this part of the discussion was: How did you relate to the surgeons and medical staff? The node from which this theme was developed was doctors/surgeons and medical staff.

Jason: I think the touchy-feely side is lacking in the medical profession, the bedside manner needs to be improved. I think that it's not a big ask for the doctor to spend ten minutes talking once you are able to have a chat.

David: When I was little, at the Children's Hospital, you're very well case-managed, there's quite a cohesive team, and that was a lot of fun, it was very easy, and that wasn't hard at all. Then there's the period in between when I was no longer at the Children's, and I found it a lot harder to deal with the medical profession. I wasn't interested in it. When you turn 17 and 18 and hit the last phase, you get a really good relationship with all the surgeons. I'm not sure how they coordinate things but certainly I've got a great relationship with them all. I joke with my mother that the orthodontist has changed a lot over the years but that's clearly not the case – it's me, I've grown up.

Karen: Some within the wider medical profession need more knowledge of these issues. My mother says that when we went to see our first Doctor. He knew nothing about cleft lips or palates, it was all just "I've heard about it, I've read about it, I've got very little understanding." When we went back in he had a whole big pile of books marked with post-it notes, he had done research to find out what he needed to know.

Ann: I think the medical profession has changed in recent times but when I was going through the system their bedside manner was pretty appalling. I remember sitting in a room and ten of them were staring at me. I don't remember how old I was, I just remember them working out what to do, and staring at me and that was horrible.

4.10.2.13 The community – Does it know or care?

The question that formed this part of the discussion was: What does the general community know about clefting and do they care? The node from which this theme was developed was community and caring.

Jason: The community at large is blissfully unaware of the problem. I think if you have any form of cancer, everyone has an appreciation for what you're going through, or even if you have Attention Deficit Disorder, people know all about it, but if you have a cleft very few know what you have been through, or what a child and their parents may still have to go through.

Mary: I don't really know how much the community is aware of clefting. I think people are only concerned with things that happen within their immediate experience.

Karen: I think the majority of the community wouldn't know about it either, they've probably heard of the term 'cleft lip' or 'cleft palate' and that would be where it stops. I must admit I've had one and I don't know anything about it. If I don't know anything about it how can I expect the wider community or even the Government to know about it or be interested in it?

4.10.2.14 The government – You need to do more!

The question that formed this part of the discussion was: Does the government provide sufficient support for families of cleft children? The node from which this theme was developed was government and support.

David: I certainly believe that Medicare should cover all costs relating to children born with a cleft and this should definitely apply to speech therapy. Speech is probably one of the most essential parts of treatment. Because if you're not understood, people will act, anecdotally I would imagine, that people would act less generously, would be more standoffish, and would be less confident of your ability, if you can't express yourself. Speech then is far more important than the shape of your nose. Who anyway is ever born perfect?

Mary: Governments can play a much larger role by banning processed food, making sure mothers don't work in front of computers when they're pregnant, making sure that they don't dry up mother's milk and give children infants formula. Create a healthy

person rather than looking at things to remedy the problem once it has occurred. Perhaps that's a radical statement but my mother accepted the challenge after I was born and the result was two healthy girls followed [sic] me.

Ann: I know that some people are battling with the government about funding for speech therapy, which is worthwhile, but there are still other battles to fight too. I believe that at 38 I still have a cleft, and that I and all others who have been born with a cleft should be entitled to Medicare benefits as well as speech therapy when required.

4.11 Discussion

In this study participants speak for themselves and their narratives have provided an insightful understanding of what it is to grow up with a facial cleft. However, a major overall theme is the strength shown by the participants in demonstrating their positive attitude as they progressed through the reconstruction process. While none could remember much of what occurred before they were four years old it was soon after that they realised that they looked different to other children, and yet this did not seem to faze them, they were just different. It is obvious that all had endured physical pain from the surgery, and by inference emotional pain when faced with another bout of surgery. This impact on their lives appeared to be dismissed by them when they maintained that the surgery was not frightening, and they expressed their confidence and resilience.

The schoolyard has always been a place where bullying has occurred, and children who are even slightly different for one reason or another can be the target (Juvonen, Graham et al. 2003; Hunt, Burden et al. 2006; Christensen 2009). All had faced some teasing or bullying but this too, in the main, was dismissed as being a part of growing up. While this study did highlight a degree of bullying it was not to the extent that might be expected (Hunt, Burden et al. 2006), and in only one case did this go beyond the schoolyard and into adult life. Apart from this one case bullying does not seem to have had a lasting effect, and the participants appear to have shrugged it off as normal schoolyard behaviour that everyone goes through.

The surgery involved in repairing a cleft is complex and can continue over several years (Schendel, Montgomery et al. 2005). Going to hospital and having surgery at any age is not something that anyone looks forward to, and yet as children none of these participants found it frightening, nor did they find the surgeons intimidating. Certainly as they grew older and the surgery impacted more on their lives, different issues were encountered.

Perhaps it is possible to compartmentalise our memories so that the best things are remembered and push to the back the bad encounters in our lives. Researchers (Taylor and Brown 1988) suggest that overly positive self-evaluations, exaggerated perceptions of control, or mastery, and unrealistic optimism, are characteristic of human thought. It may well be possible that these adults have mastered that optimism, which has been able to impose filters on the past that distort their experiences in a positive direction. Time will obviously tell if the memories do return and how they affect their future lives. There are certainly examples of post-traumatic stress affecting lives for a very long time and perhaps none more so than troops returning from warzones. Post-traumatic stress disorder (PTSD) was officially categorised as a mental disorder in 1980 but still remains controversial (Muldoon and Lowe 2012). The researchers indicate that one of the paths to PTSD is where someone is intrinsically related to a traumatic event. In this study all of the participants knowingly or unknowingly have endured surgery of a traumatic nature and none gave the impression of PTSD. Impressions, however, can be deceptive and it is impossible to make such a conclusion having spent such a little time with the person.

Gassling et al. (2012), in their recent review, conclude that adults with CLP have significantly better stress-coping strategies than their healthy peers. Researchers (Glazebrook, Hollis et al. 2003) studying young people who were constant visitors to paediatric clinics found that they had a high level of emotional and behaviour problems. The researchers assigned this to the frequency of visits to the clinics. The participants in this thesis did not appear to have any long term emotional issues however no doubt from time to time they must have felt the urge to say “no – not again” (see quote below from a parent).

To anyone who has not been exposed to significant surgical procedures or been born with an anomaly of some kind it may be thought that the normal question a child could ask a parent would be “why me?” but in fact only one participant indicated he had asked the question: “why me?” If the thought was there with others, which it may have been at some time, it was never verbalised. The findings suggest that they accepted who they are and how they had been created. Stewart (2004) in describing this phenomenon of acceptance quotes the Buddha, “ as we think so we become”, which alludes to the idea that what occupies the space of the mind, and what is most practised will ultimately result in the perpetual reality of who we are. Ann, the participant, sums this up

succinctly when she states, “so it’s about being yourself, being proud of who you are, and that’s not easy for any kid or any person.”

Friendships are not always easy to make and even harder to maintain and the question was put to the participants whether they found difficulty in making friends. In any schoolyard situation where bullying of any kind is taking place so-called friends can quickly depart. Buysse (1993) found that children with disabilities tended to find at least one mutual friend and that this was generally based on similarities between the two. Other researchers (Pottie, Sumarah et al. 2004) describe the factors that foster or inhibit the development of friendships and the communal influences involved. For children who appear different it could be assumed that they might therefore have fewer friends due to their inability to speak clearly and to become a part of a community. This, however, was not the case and some found that the friendships established in those early days continued.

The most outstanding outcome from all the participants was their confidence, resilience, and strength of character. It could be considered that all these participants had every right to feel that they had drawn the ‘short straw’ in life and yet at no stage was this evident. The fact that some suggested that their lives were better because of the cleft was an incredible thought to comprehend. Here were children who had experienced severe pain and hardship and yet they did not see the cleft as an impediment but indicated that it had provided a solid grounding for their future. Linley and Joseph (2004) in studying positive changes in individuals following traumatic stress found that for many, the process of struggling with adversity could propel the individual to a higher level of functioning than that which existed prior to the event. In the case of these young adults there was no prior existence to the event but certainly they appeared to see the positive side to their hardship.

In all our lives we experience events that are difficult to cope with and so the question relating to the worst experience these adults had encountered could have been directed at life in general but in this case it was directed particularly at their development during the years when treatment was being received for the cleft. The indication was that age 10-15 were the hardest years, both from the point of view of any bullying that took place, and any surgical procedures that occurred at that time. This suggests that this is the critical time for both parental and schoolyard support. In recent time CleftPals has created a youth group for children aged from 6-18 called Cleft Stars. They meet socially

on a regular basis with adults who have also had the cleft experience. This group aims to provide the additional support required.

All participants coped well with their cleft and appeared to come through their experience as positive, resilient and perhaps stronger because of the anomaly but there were bad times for each. For some it was the surgery, for others the dental work, and yet others the speech therapy that was part of the treatment. After the event the raw emotion was not present but it became more a reflection on the experience, as in their words, they "just got on with life". The females in the study were more aware of any scarring. A study investigating the ability to come to terms with facial surgery (Furness, Garrud et al. 2006) found that there were mixed emotions regarding appearance. One participant who had a facial cancer removed stated that she did not give a 'toss' about what she looked like because she was alive and that the cancer was a far bigger issue than how she looked. Reactions are therefore relative to the perceived disadvantage.

The most important theme was that the participants saw the need for family support. The support provided was stated to be pivotal in the ability of each participant to cope, not only with the surgical procedures that occurred, but also in the daily issues that they had to deal with. It is interesting to see how this support welded the family into a more cohesive unit as well as seeing the participants' gratitude to the rest of the family. Obviously the parents had made it their duty to be there for them and no doubt the parent-child relationship became strong perhaps merely due to the fact of sharing extended time during these periods. Waylen, Stallard et al. (2008) studying the effect of parenting on future child health found that 83% of parents exhibited sub-optimal parenting during the first four years of a child's life and that the relationship established in those early formative years influenced both the parent-child relationship and the child's social and emotional development in later life. The participants in this study obviously had parents who represented the 17% who were not only optimal but exceeded all expectations.

As can be seen in the next chapter this relationship does not change the exuberance of youth nor the challenges of adolescence when one mother describes herself, in the next chapter, as wanting to dissolve into the floor when her son was being rude to an orthodontist.

> David was becoming rather uncooperative about the whole thing. I could have throttled him, at least going privately you knew he was going to see the same orthodontist each time, even if he was unspeakably rude to him.

Waylen and Wolke (2004) describe how adolescents are particularly concerned with their identity and yet are expected as maturing adults to synchronise their beliefs with the important people around them. This duplicity of demands presents the challenge to the individual in those teenage years. Teenagers are often disinterested in family involvement in activities, nor do they appreciate that involvement (Molinari, Everri et al. 2010). For these participants it was quite different. It was both needed and highly valued.

A further strong theme was the level of achievement that these adults had attained. All had succeeded in their education and careers and did not feel that the cleft had been an impediment – these adults were 'achievers'. This may well have been their nature irrespective of the CLP, however, considering the fact that some of the difficult surgery was carried out during their developing years they were able to put this aside and concentrate on their studies and excel. Researchers in the USA (Rule and Ambady 2011) indicated that by looking at photographs of faces they could judge the strength and power of an individual and this judgement related to the success of the individual. They believed that faces affect individuals throughout their lifespan and in particular employment opportunities. Based on this it could be expected that having a cleft, which perhaps left a scar on one's face, or a speech impediment, may impact heavily on the career that one would like to pursue. None of these participants found the cleft to be an impediment, and most seemed to have gained strength from this early adversity.

Asked what they would say to children who were born with a cleft, the participants showed great maturity and insight. Reading books or hearing stories of others is just not the same as living through an event, be that pleasurable or in this case seemingly traumatic, and so the personal experiences of those who have lived through such events are important and all participants were willing to share this with others. The participants again reinforce that the children should concentrate on being positive. All communications whether verbal or written can sometimes be difficult to take and what one wishes to express in a certain manner can be misinterpreted by the receiver. The real question not covered here is how do you communicate effectively with young people and will they be receptive. This thesis does not cover that aspect. Young, Dixon-

Woods et al. (2003) explain the difficulties that we as adults face in communicating with young people. The young are acutely aware that there is a difference in social positioning between adult and child and are also aware of their own vulnerability and lack of maturity. This puts them at the disadvantage of being a hearer and acceptor rather than an equal partner in any discussion. In the study (Young, Dixon-Woods et al. 2003) one young person was reported to say "I probably wouldn't ask what something meant … just cos I might look stupid." Perhaps this suggests that they may listen to someone who is closer to their own age group who has had the same experience.

When questioned on what advice they would offer to parents of a cleft child it was clear that the participants knew that their own parents had suffered due to their cleft but that the support they received was appreciated. Parents in this situation will experience an emotional roller coaster of ups and downs (Kearney and Griffin 2001), and yet they have the responsibility to support not only the child, but each other. Kearney and Griffin (2001) found that while the parents experienced much anguish and sorrow they also expressed feelings of hope, love, strength and joy. These participants again concentrated on suggesting a positive attitude and, in particular, that irrespective of the cleft the parent should emphasise that their child is beautiful, loved, and an equal member of the family. Young, Dixon-Woods et al. (2003) found that parents find the task of communicating with their children enormously complex, which is influenced by their need to construct a parenting identity on the one hand while trying to protect their children's wellbeing and promote an optimistic version of reality on the other. The participants in this thesis overlooked the potential difficulties and purely focused on the parents being positive and supportive.

The initial experiences of these participants with the medical profession were lost in their memory but each had surgical procedures over a long period of time and so the comments relate to the period post that age. In most, the experiences were positive, helped by the personality of the surgical staff and where not they provide important feedback for others to consider. The relationship with the medical profession was interesting in that overall there appears to be a need for more of what one describes as that 'touchy-feely' attitude rather than the more clinical approach. Obviously as the children grew older they appear to have at least understood, if not accepted, the more direct relationship with the profession. Young, Dixon-Woods et al. (2003) in their study cite one participant as saying, "I think sometimes they talk to both of us but sometimes

they just talk to mum and I'm just – hello I'm sitting here – especially the consultants just talking to mum. You know, um hello."

Perhaps in the early part of their treatment the participants were looking for a more 'motherly' approach from the medicos. Perhaps a larger role needs to be played by the nursing and support staff in these situations as they may have more time to devote to the less clinical aspects. Redsell et al. (2006) relates that patients thought that nurses had more time for them and were more compassionate. In a further paper (Redsell, Jackson et al. 2006) the researchers indicate that where nurses are involved the patient may have lessor expectations and therefore report higher satisfaction after the event when those expectations are exceeded. Certainly the role of nursing in relation to the care of cleft children should not be overlooked or underestimated.

Participants were asked from their experience whether they thought the community was aware of clefting and its impact on family life. With one in every seven hundred children in Australia being born with a cleft (Riley and Halliday 2008) it should be expected that the general community would be well aware that children are born with such an anomaly, however Chapter 3 of this thesis indicates that this is not so. The fact that the surgeons now operate on the CL at three months means that children may not be seen with gaps in their faces. The participants' perceptions indicate the lack of knowledge at a personal and community level and were quite convinced that the general public was not really aware of the level of clefting in the community or how those with a cleft were affected. This may lead in some cases to people in the public arena being unable to positively relate to a person who has a cleft, and certainly their (lack of) knowledge of the surgical experiences the cleft adult has had could make having a discussion about the issue difficult. They tended to reflect that the lack of publicity given to clefting was merely a consequence of good surgical practice and the financial intervention of the government in ensuring a timely repair of clefts.

Any question regarding government support always brings into question where the money can be best spent, and there is also a tendency for governments to not look for areas to increase financial support unless it is for existing programs that were promised when they formed government. The family support group, CleftPals, appears to have been the driving force over the past 45 years lobbying the federal government to provide services for children born with a CLP. This has enabled each child born with a cleft to gain a Medicare health card to cover surgical procedures (Medicare 2012). This

does not, however, cover speech therapy. More recent pressure brought on the government by CleftPals has seen the Medicare program cover extended to the age of 26. Most of the participants had had speech therapy and were aware of its role in their development even though they appear not to have enjoyed that part of the journey. They all realised that this was not sponsored by the government and therefore either their parents or an insurance company met these costs. All believed that the cost of this should fall within the Medicare program.

4.12 Conclusion

It was clear from the study that all of these young people achieved their goals to this point in time and believed that the cleft had in fact seemingly helped them to gain a true perspective on life.

Their courage in sharing their experiences for this study is to be applauded. They appear to have a 'strength of purpose/character' that many would consider well above the ordinary. For health professionals involved in the treatment of children with cleft lip and palate this study provides reassurance as the children put the surgical experiences to the back of their minds as they proceeded to live normal lives following the completion of the procedures. For parents it provides more than just hope as these children have all overcome the adversity of having a cleft at birth, the associated surgery, and have not only gone on with a normal life but have gone on to achieve success in their identified careers. Perhaps the best conclusion was the comment by Mary:

> Throughout all these journeys, although my cleft has been a bit of a pain at times, it has never stopped me from doing anything that I wanted. If anything, I believe it has strengthened my character and ability to achieve goals. Although my cleft has had, and will continue to have, a financial and emotional impact on my life I have made it an accepted part of me and have striven to achieve the best for me within this.

4.13 Strengths and Limitations

A strength of this study was the willingness of participants to volunteer once they heard about the study. They approached the interviews in a relaxed manner and were open and honest in their responses. They willingly reviewed the transcripts and discussed them openly.

A limitation would be that a shy or quiet potential participant may not have volunteered. Obviously the small number of participants was a limitation, as was the fact that with

the exception of one all had been born in Australia and were of white, English literate, Anglo-Saxon heritage. While the geographic spread was extremely wide there was only one who had been born in a more remote country area as the parents of the others lived in or close to major cities.

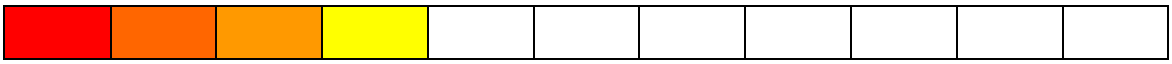
4.14 Recommendations for Future Research

Research focused on determining how cleft children can be given further physical and emotional support would seem to be appropriate. This study identified that attitudes between the medical profession, the participant and parent could be improved. How can this be achieved? Is this the responsibility of the hospital or the individual providing the service? Larger studies focusing on self, esteem, and coping strategies of young people with disabilities are needed to add to the body of knowledge so that they have the necessary tools to lead a normal life.

4.15 Summary

This study has given an overview of the manner in which young adults have coped in their developing years after being born with a cleft. All appear to have been able to put the difficulties behind them and accept the normal challenges of life. In doing this they appear to have a good perspective on life's real values and by being a part of this study have offered to help others who may experience similar challenges. They, no doubt, continue to feel for their parents who walked the same pathway but looked at it from a different perspective.

Chapter 5 Raising a Child with a Cleft



5.1 Introduction

One could imagine from an outsider's point of view that being challenged by a traumatic experience could be a harrowing experience for the individual. Having a child who is born with a cleft could be seen as such a challenging experience causing anxiety and concern to parents in relation to how to raise their child, and how to protect them from what could be frightening and/or demanding experiences. Often we as outsiders can be less than tactful in our discussions with the person or the family involved because of lack of understanding. An insight into how parents of children with a cleft cope may enable a better understanding for everyone.

5.2 Background

Through CleftPals the researcher was introduced to a number of parents whose children were born with a cleft. In all cases these were the parents of the adults who were participants in the previous chapter. While the researcher has interviewed many parents of cleft children only those who had adult children were considered for this study in order to try and cover the whole of life experience to date.

5.3 Research Question

The objective of this study was to try and understand the experiences the parents had been through in caring for a child born with a cleft through to adulthood; in particular to enable exploration of the specific issues parents encountered in raising a child with a cleft and therefore the research question was:

What are the issues faced by parents whose child is born with a cleft?

5.4 Sample

The parents who were introduced by CleftPals were contacted and the outline of the study was presented to them. Only those who agreed to participate and signed a consent form were included in the sample. If at the initial discussion it was found that their child had multiple anomalies they were excluded from the study.

5.5 Literature Review

While studies have been undertaken to determine the lived experience of people caring for those with disabilities, many of these studies have centred on looking after children with intellectual disabilities (Broberg, Blacher et al. 2009; Dukmak 2009; Gerstein, Crnic et al. 2009). All these studies (Broberg, Blacher et al. 2009; Dukmak 2009; Gerstein, Crnic et al. 2009) conclude that parents caring for children with such disabilities are exposed to greater levels of stress than normal families. They also suggest that the parents appear to risk greater exposure to other adversities such as continually having to change their own lifestyle to cope with the altered requirements of the child throughout the caring process. On the other hand the literature (Broberg, Blacher et al. 2009; Dukmak 2009; Gerstein, Crnic et al. 2009) tends to show that parents develop a resilience that allows them to cope with the stresses as they arise.

Nicolaou et al. (2009) investigated maternal experiences of interacting with premature babies in the hospital neonatal unit. They found that the mother was unsure and anxious about interacting with their infants, and while they felt supported in the neonatal unit the health professionals in the general community by comparison were perceived to lack the knowledge and expertise to assist them. Other researchers (Jackson, Britt-Marie et al. 2003) investigating both maternal and paternal experiences with premature babies found that the mothers reported more stress and poorer adjustment than the fathers. Holditch-Davis and Shandor-Miles (2000) found that health care providers and especially nurses have a major role to play in reducing parental distress by maintaining ongoing communication with the parents while providing competent care for their infants. This same feeling of helplessness and/or stress is easily understood and similar feelings may be experienced by other parents whose children suffer from birth anomalies and in this particular case a cleft.

At the time of undertaking this study no literature was found that had considered the issues surrounding the parenting of a child born with a CLP. Since completing the study research has been published in the United Kingdom (Nelson, Kirk et al. 2012) which supports many of the findings of this study undertaken some 18 months prior. The researchers found that the parents had conflicting emotions, ranging from grief to elation on the completion of the child's cleft surgery. Parents were concerned at the uncertainty of the future for their child and the long-term treatment. Parents also had at times a feeling of social exclusion as they faced negative reactions from family and

friends. In an earlier paper Nelson et al. (2011) reviewed the existing qualitative and quantitative literature regarding parents' experiences. The researchers stated that much of the literature related to social and service aspects such as child feeding support, especially in relation to the early stages of the children's lives. The research relating to parents was said to be variable (Nelson, Glenny et al. 2011) with a narrow emphasis on cross sectional deficit-oriented psychological approaches focussed mainly on the mother. They concluded that research is needed to investigate how both mothers and fathers might experience the long term and complex treatment journey as children become older.

5.6 Ethics Approval

This study was approved by the Human Research Ethics Committee of Southern Cross University (ECN-09-016).

5.7 Research Design

This particular study used a simple qualitative approach with a survey descriptive methodology. Thematic analysis was used to derive the themes from the data collected. An interview method was used to gain oral audiotaped responses of the parent or parents of their child who was born with a cleft. All parents consented to being interviewed and to having the interview tape recorded. All names of participants were changed to protect their identity.

In each case a one-on-one meeting was held with the participants and the researcher and the interview was conducted in a semi-structured form based on a series of questions with flexibility for participants to extend the discussion as they saw fit. Once the interviews were completed the data was transcribed and sent back to the participants to make any changes they wished. This was done to ensure rigour for the study and to ensure that the participants were not only comfortable with the presentation but that the accuracy of the data could be confirmed.

Qualitative research methods are designed to help researchers understand people and the social and cultural contexts within which they live (Sale, Lohfeld et al. 2002). In this sense then, this study investigated the experiences of the parents in raising and supporting their children through to adulthood, with the research centred on gaining a better understanding of the problems that these parents had to cope with.

5.8 Setting

The parents interviewed lived in the Australian states of Queensland, New South Wales and Victoria. All of the interviews were conducted in the homes of the parents. This was not set as a requirement as the mother was asked prior to the interview where she would like the interview to take place. Where a partner was not present a female friend attended with the woman providing the information. The ethics committee had requested that someone other than the researcher be present when conducting the interview as they thought that this could be a very emotional experience for the person being interviewed. In only one case was the father able to attend the interview. The reason for his absence was not raised at any time either by the mother or by the researcher.

5.9 Data Analysis

Once the individual data had been read and approved by each of the participants it was coded using the computer program NVIVO to establish the nodes to confirm the themes from the data. Each transcript was uploaded to the program which was then run to provide a cluster analysis of word similarity followed by word frequency. This was then reviewed to determine specific nodes. As each node was established the sentence or sentences relating to it identified in each participant's data was copied to the node. On completion, if there was data from two or more participants within each node, the node was considered a theme and a descriptive heading was assigned to it.

5.10 Results

5.10.1 Participants

Wanda and Charles live in an outer Melbourne suburb and are the parents of Jason who is now 30 years old. He is their fifth and youngest child. Jason has two older sisters and two older brothers. Wanda is a primary school teacher and Charles is a mechanical engineer.

Judy is the mother of David and lives in an outer suburb of Sydney. Judy was a teacher and was born with a cleft palate. Since having David, Judy has had a daughter who does not have a cleft.

Helen is the mother of Kirsty who was born with a unilateral cleft lip. Helen lived and worked on the family sugar cane farm but was also taught in a primary school in a small town in northern New South Wales.

Coral is the mother of Mary and lives in a regional town in New South Wales. Coral was born in Australia and Mary's father was born in South Africa. He is no longer living with Coral.

5.10.2 Findings

The data are presented in terms of the themes that were derived from the following nodes:

- Cleft identification/timing
- Distress
- Hospital experience
- Photos
- Family Support
- CleftPals
- Occurrence
- Procedures
- Assist others

The headings for each theme were assigned to reflect the question discussed during the meeting and also to provide a synopsis of the responses.

5.10.2.1 When did you know? – We didn't.

The question that formed this part of the discussion was: When did you know that your child would have, or had a cleft? The node from which this theme was developed was cleft identification/timing.

Wendy and Charles: The first we knew that Jason had a cleft was shortly after he was born. When Jason was conceived women did not have scans and so we had no inkling that there could be an issue. Of course the realization that in my rush to have a baby so quickly after a miscarriage I may have caused the cleft, was painful. I certainly have thought it but have never spoken it out aloud.

Judy: I had no idea that David would be born with a cleft and really only knew when there was deathly silence in the room immediately after he came into the world.

Helen: Kirsty was born in a rural NSW Hospital but because I had complications with the birth I was sent to a regional Base Hospital. I didn't know she had the partial cleft lip until I was brought back from there, which was the day after her birth. When she was

born, I didn't recognise the cleft, the doctor didn't say anything and so it was a surprise when I was made aware that she had this.

I did not even focus on the cleft. It was the euphoria of having my first child, and just the whole birth process, and to me she was a new born and certain colourings take a while to settle, and I didn't notice anything. I had had scans but nothing had been picked up.

Coral: I didn't know before she was born that Mary would be born with a cleft and so it was quite a shock to realise that this had occurred. I had an inclination that something was not quite right as I had a very ominous feeling throughout the pregnancy, and I don't know whether it was because the pregnancy didn't go well at the beginning, or whether I felt it was going wrong.

5.10.2.2 Distress – Yes there was.

This theme emerged from the question: What were the difficult times after being told that your child had a cleft? The node from which the theme was developed was distress. The sentences chosen from the node reflect in different ways and at different times how this distress was experienced.

Wendy and Charles: [After the birth] Charles and I were in shock. We were looking at each other exhausted – during the delivery I had felt concerned for my own health, for the health of our baby and was threatened by the whole situation. Charles looked pale and I assured him that Jason would be our last [child].

Our Doctor came to speak to us. He prefaced his comments with "I think we have a problem." We did not pick up the gravity of the situation and answered "We don't care, it's over." It was then he explained Jason's condition. We could not take in what he was saying and it wasn't until the midwives brought Jason over to us that we understood.

I felt that the first week after the birth must have been very different and difficult [having to relay the news to others] for Charles. We have a large extended family and many friends. While I stayed protected and supported in the hospital Charles was the one who had to explain the condition to everyone and field all the questions that caring family and friends asked.

Coral: I felt very hurt when the paediatrician told me that Mary had a syndrome. I felt like a failure. It then made me feel like rejecting my baby.

I was so distressed because they put me in a room with a lady who had a healthy baby and she was phoning everyone and she was really happy and I was really sad,

and every time she phoned someone to say "I've got this Jo Ellen and she's really beautiful", and there's her baby and mine wasn't there; I was just devastated, and so in a way I rejected her. It wasn't a bonding at all.

Helen: There is no doubt that there will be tough times when your emotions are completely strained. Obviously when she was operated on was really difficult because I nursed her until they took her into the operating theatre, and at nine months she's aware, and she started to scream at the top of her lungs and they took her in and doors were shut.

The next time I saw her was hours later in intensive care, and she looked very peaceful, beautifully cleaned up and she had all this metal hardware on her. They explained what that all was and why. As she breathed she had heavy deep shudders that went through her body and I asked about that and they were quite truthful and said that would be trauma.

5.10.2.3 The hospital experience

This theme emerged from the question: What was your hospital experience like? This was developed from the node: hospital experience. This brought out some surprising thoughts from the parents which are reflected in the sentences chosen from the node.

Wendy and Charles: I [W] remained in the delivery suite to rest and it was here that I met the man who was to be Jason's plastic surgeon. He was amazing and it was a pity that Charles was not able to be there. We had no idea that he would come in. The hospital or our Doctor must have called him in. He assured me that the condition was not life threatening and that Jason was, apart from the cleft, a normal healthy baby. Throughout he was positive and explained with excitement the inroads being made by Doctors in this area. He arranged a time for our first consultation before leaving.

Judy: I am not sure whether the Doctor who delivered David had sufficient knowledge to help me but because you've got a cleft yourself you know far more than any Doctor is going to know about it. I suspect in that case, the Doctor almost defers to your greater knowledge. His response was more "um, I'll get you to see a paediatrician and we'll get this on the road quickly."

Helen: My doctor handled it very well and I had a good relationship with him – he was this gentle lovely competent man who made me feel very comfortable. He answered all my questions, and yes, it was all very straight forward from what he said to me. Yes, I realise there are always risks with surgery, going under aesthetic and all that, but everything was handled so professionally. He knew which specialist to contact and I left that all to him. He knew exactly the person, and he was ideal.

Coral: I felt very hurt when the paediatrician told me that my baby had a syndrome. I felt like a failure. It then made me feel like rejecting my baby. The nurse said this is a syndrome that happens in the sixth week [of pregnancy] and she might be blind or deaf and she might not be able to speak and I thought of Helen Keller and she just said I want to tell you the worst scenario – and this is when you've just had a baby, one day old and that caused me to reject her more – did they think about my feelings or is it that they just don't think? I don't understand, really.

5.10.2.4 Before and after photos

The question from which the photos node was established was: Do you think before and after photos would be helpful to women who had just had a cleft child?

Judy: Some people might not find seeing the photos as useful as I did but I think once you've had a day or so to look at your own child it could be helpful. It's such a confronting sight anyway, that once you've mentally accepted the fact that your child is OK in every other respect and can look at the child, then you can look at the photos of the stitches and you think it's wonderful. But if you're still at the point where you can't even bear to look at your own child and the gum, and how fleshy the whole thing is, then I think you would find it difficult.

5.10.2.5 Family support – It's essential.

This theme emerged from the question: How important was family support to you? The node that was developed was family support, which was created from the words family, support, and family support.

Judy: I know that my husband felt really hemmed in just by the act of having a baby -. He was very accepting and was very good. His parents were fantastic; honestly, I had so much support from all the family.

Helen: My husband's mother was probably a ten minute drive away, grandparents about a twenty minute drive away. We were all very close and they were all supportive.

Coral: My husband was very good, very helpful and more accepting than me. I thought he's so accepting and I'm not. He did a lot of positive reinforcement, and [later] he looked after her when I went to work.

5.10.2.6 CleftPals – They were very supportive.

This theme evolved from the question: Did you receive outside help particularly from CleftPals and was this positive or intrusive? The node from which this was developed

was CleftPals. CleftPals is a family support group made up of families who have already had a child with a cleft.

Wendy and Charles: The following day [after the birth] a representative from CleftPals visited me. She gave me a pack which included a scoop feeder, plastic bottle and literature about the condition and their association. We joined but let our membership drop after two years as we found some in the group to be quite negative and looking for sympathy. I didn't want to look for problems where there were none.

Judy: The doctor had told me that I had to make a bonnet for David. I had no idea [how] to make this bonnet or even where to start. It was overwhelming. I asked him how do you do it and he said the mothers know. I said which mothers and he said the mothers at CleftPals. One of the mothers came in and made the bonnet for me while she was visiting.

Almost immediately when we got home from hospital CleftPals were having a conference and so we went along which was really too soon. It was a one day conference and people were talking about 'abbe flaps' and that sort of thing and I found it really distressing. That was too much information and too soon.

5.10.2.7 Why did this occur? It's a mystery.

This node emerged from the word occurrence in answer to the question posed to parents: Why do you think that this occurred? Or was this pregnancy different to the others that you have had?

Wendy and Charles: This pregnancy was no different to the others. Of course I was much busier. We had two children at school and two at home. As far as I can recall I was healthy throughout. I had had a miscarriage two to three months prior to becoming pregnant with Jason. Today doctors would probably advise women to wait a while before trying again but we were given no such advice.

Judy: This was my first pregnancy and the only thing that happened when I look back was that I was a vegetarian when I became pregnant. I took folic acid and I did eat legumes, nuts, eggs, cheese and fish to get sufficient protein. I think that I was very careful to try and maintain my protein level. I had a fair bit of morning sickness and as a result of that I couldn't bear the thought of a sloppy vegetarian meal any longer. All I wanted was a steak, a nice dry steak, and at that point I thought that's the end of the vegetarianism, I need a steak. I was about 10 weeks pregnant when this happened and I suppose my body was telling me "you need protein".

After making this change to my diet the rest of the pregnancy proceeded without any further problems. I went to my GP once a month and more frequently when I approached full term.

Helen: Prior to the pregnancy life was really good. I lived on a cane farm, I was teaching at a Primary School at the time. We had a property on the coast, and I was 32 when I fell pregnant. It was planned; I was absolutely delighted. I had no issues with the pregnancy, it was a really healthy pregnancy but because of my age I knew there could be problems.

We went through all sorts of questions when we looked at things later, and the only thing that may have had any connection was (I didn't know I was pregnant at the time), we had just bought a property, there was an old shed that was to be taken down. We cleared out the contents before it was destroyed and it had been like that for decades and all kinds of things in there, so the only thing that came to me was the dust and dirt and what may have been there even in the way of chemicals that I may have inhaled, because we spent quite some time sorting through things.

Coral: I was married for two years and then decided I wanted a baby, so I went off the birth control pill, and then I didn't get my first period. I went straight in and got pregnant. Straight away I went "oh no", I wanted to get my body back. And that night was a night of drinking.

While I wanted to have a child I really thought that it was important to allow my body to stabilise after being on the pill – and that just set off alarm bells for some reason. I just thought "oh no".

I was also concerned that I was doing computer work – I was studying, – and I was in front of these computers all the time, learning how to do the [air] trafficking. So for six weeks in front of these huge computers – and I was plugged in and when I got pregnant I had computers here, around me, and I mean all around.

The problem as I saw it was not the stress so much but the radiation that concerned me, it wasn't like they have the safety computers now, this was the 70s, and so the computers were huge things, and there were probably a room of 60 people all plugged in together, and you didn't get a break, you were in there eight hours with a short break, so it was not really the stress in the job, it was stressful just sitting there in front of a computer all day.

I daresay that I did feel that I was under stress, because they [my employers] were very efficient – and after that – I'm about six weeks pregnant, and – I was in front of the computers.

5.10.2.8 The procedures – Ongoing

The node in this section was based on two words 'procedures' and 'coping' however the question asked was: Can you explain to me some of the procedures and the way that you and your family coped with these.

Wendy and Charles: Our only problem all the way through was the knowledge that there would be surgery, after surgery, and although the Doctors kept us in the loop, they were always throwing a curve ball in when least expected. We would go along to an appointment understanding what the next stage would hold, only to be told something different. Procedures were being improved all the time, new techniques were being developed, and on top of all of this, Jason was growing. As he got older Jason found this very hard. While he may not appear so, he is a very gentle character with a soft centre. The Doctor would talk to him in what seemed the 3rd person with scant regard for his feelings. He never complained, he never said "Why me?" and I have asked him a few times over the years but this approach was hurtful.

Jason has an aversion to hospitals now and for that you cannot blame him. His initial surgeries were performed at the Children's Hospital but all the rest took place in private hospitals where the condition and the aftercare needs were not really understood.

Judy: We were privately insured and I wanted a particular Doctor who had been my plastic surgeon, and in fact about 15 minutes after David was born my doctor said "Do you have a preference in plastic surgeons?" and I said "I only want the one who treated me." So because of that, I saw him privately, but I saw another Doctor publicly. Speech therapy I did privately. When David was about 13 maybe I started going completely private. I don't really know why. I think it was to do with the orthodontistry. I think that David was moving into a new phase of orthodontistry with bands and that sort of thing, and for some reason, why I can't remember, we started going privately, which was a mistake really.

Part was to do with cost but by this stage David was becoming rather uncooperative about the whole thing. I could have throttled him, at least going privately you knew he was going to see the same orthodontist each time, even if he was unspeakably rude to him. I think possibly if he was seeing a different one each time it would have exacerbated David's resistance but the outcome would have been the same. He was so rude to him, and David's not a rude person. He was dreadful! I was mortified. Just wishing that the earth would open up and take me, and him, because he was so rude, but, they're quite good friends now.

Certainly the last lot of surgery that he's had, from 18 onwards, or everything from 17 or 18 onwards, it meant that we'd have more control over when he'd have the surgery, to make it fit in with university, so that was important.

Helen: There is no doubt that there will be tough times when your emotions are completely strained. Obviously when she (K) was operated on was really difficult.

The next time I saw her was hours later in intensive care, and she looked very peaceful, beautifully cleaned up and she had all this metal hardware on her. They explained what that all was and why. As she breathed she had heavy deep shudders that went through her body and I asked about that and they were quite truthful and said that would be trauma.

I understood that. Once she came out of the aesthetic and for the next 8-9 days I was there. That was traumatic, mainly because I had a pullout bed in her room, she had a couple of really stressful nights with obviously pain etc.

Coral: By six and a half, her breathing [M] was bad, her voice was nasal and the words were very indistinguishable. I could understand her more than others. Mothers can work out what babies want. So by age six and a half I knew that other children were not able to understand her. We chose our own specialist this time and we found someone who had just started a new experiment called a phalangeal flap, and he had actually invented it. We'd never heard of it, but he said he would take the tissues from the back of the throat to make a flap, but to never let her have a tonsil operation, so that she could block the air to her nose. I remember looking in her mouth after the surgery, I could still see the soft palate was still split, and I asked what happened, and he said you don't actually sew the soft palate, it's too soft or something, and it would come together itself. Then she had speech therapy.

She had a lot of difficulty, because my husband was South African with a very pronounced British accent, I had the Australian accent and she was growing up in California. We would say 'aeroplane', and it would be 'air-o-plane', and the pronunciation was [banana] banAAna, bAAnana, and it was so, she had to learn that different accent as well. So it was a little hard for her but she did really well.

She did not seem frustrated by this as she seemed to manage really well.

We had the orthodontal work when she was 12 or 13. The orthodontist was an elderly man, and then again, me being completely me I had not checked him out, or asking who is the best? I just took whoever we first met. And I'm not sure if he did a good job because he pulled all her teeth out and she should have had more teeth left, and it was very painful for her each time she went. I felt that's what she had to have,

she had a very crowded mouth with lots of teeth, and you have to have teeth pulled out, but I'm not sure whether it would have been better to leave the teeth and force the jaw to grow more, or wait until she was older to do it. So, I suppose I just went along with this. Had I known what I know now life could have been easier for us all.

5.10.2.9 Could we help another family? Perhaps.

The node 'assist others' emerged from the question: If you were aware of another family who knew that their child would have a cleft how could you help them or what advice would you give them?

Wendy and Charles: What would we do for a family that has just been told that their child has a cleft? My husband said first and foremost he would introduce them to Jason. "A picture is worth a thousand words" as far as I'm concerned. The best thing you could do for someone in this position would be to show them a 'before' and 'after' photo. Certainly tell them that many surgeries may be involved but this is what your child will look like.

Judy: If I was asked to advise a woman who had been diagnosed as carrying a fetus where the scans had shown that the child would have a cleft I'd be wary of giving advice of any sort. This is because I know some of them may have been considering terminating. This was not an option in my day as you wouldn't know at 12 weeks that you're going to have a child with a cleft. So it's a whole place of consideration that I have no experience of. I would, however, not have considered termination even if I had known as I knew that I was a worthwhile person and was confident that my child would also be one. Being alive is worthwhile. But the whole area of prenatal diagnosis, at a stage where termination is possible, is something that I have no experience of.

If a woman has just given birth to a child with a cleft I would probably tell them that in the whole scheme of things, having a cleft doesn't affect your ability to fall in love with people, for people to fall in love with you, it doesn't affect your intelligence, or your integrity as a person. But I wouldn't tell them it's only a small thing because it's not. I'd be really wary of giving advice because it's still an awful shock when you have the child, and they look so confronting. Even if you've got a cleft yourself you weren't aware of what you looked like as a baby. I'd be wanting to say that I believe that I am a grown-up, integrated, well-adjusted, normal, intelligent person, and hold that up as an example, or introduce them to my son who is also a University graduate and say that you come out alright at the end.

Helen: In looking at my experience if I was to be ever asked what advice I would give to another family faced with similar issues I would say I that while my story took place 28 years ago I would tell them modern science and medicine is constantly improving. I had a very positive experience in life with something that was an issue and could have been very distressing so I had a really positive outcome. I had really good people supporting me. I would tell them that my journey which was so positive could also be their story as well. The fact that so much more is known about these things these days, there are support groups, there is information and knowledge, there are procedures that are probably far better than they were 28 years ago.

Also, that when I was in hospital with Kirsty there were children in there who had very severe clefts. One little one had no top lip whatsoever and she was in for her 3rd or 4th surgery and I saw her and they'd constructed a complete top lip for her. She was a beautiful looking 4-year-old, and the fact that we all have things that aren't quite perfect, so to speak, and there are people to help us, there is knowledge and expertise to help us, we all go on, it's not the end of anything, it's an opportunity, it's really an opportunity.

Coral: If a woman today is diagnosed as carrying a fetus with a cleft I'd say carry on. You have all the medical systems in the world to repair the cleft. The child's brain is fine and the child will be fine. I don't agree with termination for any child really, they've all got a chance.

Irrespective of whether the mother has a child with a cleft or any other anomaly I think that mothers need to have nurturing because they feel they've failed, I felt first of all when the paediatrician came in and said you have a defective baby. I felt like a failure, like it's all my fault. I think mothers blame themselves when their baby's not healthy. This is not the case, how can someone be blamed for an occurrence when they did not know what the cause was in the first place? Had the cause been known and then the person took a risk blame may be apportioned but not in the case of a cleft.

5.10.3 Letter from a Participant

The following letter covers most of the aspects that we sought to investigate but more importantly it shows the emotions that were experienced at each stage in the child's development and how these feelings are still somewhat raw to this day.

Dear Graeme,

Re-our conversation last night.

Jason's condition is tied up with so many emotions that it was difficult for me to put those feelings into words last night. Of course the realization that in my rush to have a baby so quickly after a miscarriage I may have caused the cleft, was painful. I certainly have thought it but have never spoken it out aloud or had it brought up by a total stranger before.

I like my mum's explanation best. A lovely woman and mother of five, she just looked at me and said "Your mouth mechanism has failed – no more children!" Simple and to the point. In addition to this Jason is Jason because of what he has lived through. The bond we have is deep and strong and I am incredibly proud of the man he has grown into.

What would I say to a parent facing the same situation? That's a question I find difficult to answer in one sentence. I think we were very fortunate. We were secure in our relationship. We had four wonderful children already and an incredibly supportive extended family. We were supported by a most caring family doctor, a very positive, open and, as we were to learn, a highly skilled plastic surgeon. Added to this Jason was a most beautiful healthy baby. He was well loved from the first minute and accepted by all.

This is not to say that we had no problems but nothing so great as to make us despair.

If we had known prior to the birth I'm sure my pregnancy would have been much more difficult. There would have been fears, questions would have been asked, answers sought, well-meaning people would probably have said awkward things and offered all sorts of advice. Parents do need to know above all that although their child will face many operations the journey is well worthwhile. Taking a healthy child into hospital is a daunting, emotion filled experience. I shed many tears as Jason was wheeled into operating theatres and while he slept recovering in hospital beds. I cried because my child was scared, in pain, and I as his parent could do nothing about it but be there.

We are fortunate that Jason is resilient and has a very good self-image. These qualities have enabled him, although scared, to undergo and recover from his many procedures. As he grew older Jason took charge of his treatment. He never went to consultations alone but over time he led the discussions, asked the questions and demanded the answers. He was profoundly affected by a few of the practitioners he dealt with. Mr Bruce Levant his Oral and Maxillofacial Surgeon until his untimely death told Jason when he was about 13 to take charge of his treatment. To question why procedures were

necessary and to call a stop if and when he needed it. I am indebted to him and to a doctor at the Children's [Hospital] who did an endoscopy on him when he was about 18. He restated these same things to Jason and just knowing that others understood made a great impact.

Thank goodness doctors strive for perfection but reality must be taken into account. Who amongst us has the perfect look, the totally symmetrical face the perfectly modulated voice?

You asked if there was anything the government could/should do. The condition should be regarded as congenital [disorder] and all necessary treatments should be appropriately funded. Speech therapy, dental work such as implants, jaw alignments along with all reconstructive work up into adulthood should carry a reasonable Medicare and private rebate. Nursing staff should be regularly updated by specialist doctors or hospital educators on the appropriate aftercare for cleft patients.

We are very proud that Jason wants to make a difference for cleft children and their families into the future. So anything we can do to help just ask.

Yours Sincerely

Wendy

By the way, at that first meeting with the Plastic Surgeon he told me that Jason may find life difficult here in this world but that in South America many years ago he would have been heralded as a God. A fertility god no less!

5.11 Discussion

Today because of the use of antenatal ultrasound, parents who have a fetus diagnosed with a cleft, can if they wish, plan ahead and even see surgeons before the birth occurs. They have the opportunity, if they so desire, via groups, such as CleftPals, to talk to other parents who have been through these experiences. From the data obtained for this study only one woman had antenatal scans, and because there was no prior diagnosis in any of the cases their pregnancy planning was limited.

The thematic sentences chosen above not only show in each case that the cleft was a surprise to the mother, but they give an insight into some of the feelings associated with gaining that information. In these sentences we can feel pain, fear from the deathly silence in the room, trepidation on the part of a doctor who was unsure what to say to the parent, and shock that triggered the mother to look back over her pregnancy to try and find a reason why this had occurred. We also see that even though one of these

women had had an antenatal scan it had failed to detect the cleft and so there was an added element of surprise and a feeling that she had been let down by the technology. For those operating in primary health care, particularly in the neonatal area it is important to be aware of the mixed feelings and emotional responses to childbirth.

The responses of shock, exhaustion, hurt, distress and strain are all words synonymous in some way with distress and are part of the emotional response to having just been delivered news that a much sought after new family member was going to need extended medical treatment. Mixed with these emotions was also a sense of failure that it was the mother's fault, and yet there was no basis for this. In the case Wendy was concerned for her husband as she felt that she had support, while Charles had, in her opinion, the more difficult job of telling the other family members. This showed that even when a person has difficulties of their own they can show compassion for those around them. The distressing times were not just at the birth, as Helen describes the distress she felt when her child was operated on. As discussed previously we see in all these events situations where the primary health care could have been handled better. In Wendy's case perhaps the situation may have been better if the doctor had said that there were some issues with the baby which would need attention rather than indicating that there was a problem which suggested that he may not have an answer. In Coral's case placing her in a private single room would have saved her the feeling of failure, and in Helen's case advice on what might be expected prior to and following the operation may have prepared her better. In all cases it appears that some of the distress could have been ameliorated.

In three of the cases we see positive thoughts which are entirely due to the way the medical staff conducted themselves in their relationship with the parents. The doctors in these situations presented the facts as they were and provided assurance. In Judy's case where the doctor felt unsure of his own skill he was able to immediately defer to the woman but also agree to get a paediatrician involved. By contrast, in the last case, presenting a mother with pure speculation before a proper diagnosis could be carried out was not only foolish but caused great distress. Such was the experience of these women.

In other parts of this overall project women had spoken about the value of before and after photographs of other children. Some thought it to be important to demonstrate that good outcomes were possible while others did not. In this group only one of the parents commented on the value of photographs. This meant that a theme could not be

developed. The sentence was included in the data because Judy's comment demonstrates the difficulty a person may have in looking at before and after photographs when you still have difficulty looking at your own child who has either an unrepaired or recently operated on cleft. This again highlights the delicacy that must be pursued by those coming in contact with women experiencing these traumatic/emotional issues when their child has been born with an anomaly.

In relation to family support the sentences chosen in the data reflect the importance of the husband/partner at this time, and beyond that they also reflect the importance of the extended family. There are important words in these sentences apart from the word 'support'; they include, 'accepting', 'very close', 'positive reinforcement' and 'looked after'. These words indicate empathy on the part of the person providing the support and selflessness that goes beyond mere friendship. Within the sentences the mother expresses her need to receive this support and to not be left alone to endure a hardship on her own. There was no hesitation by the mothers in praising the support that was provided to them and the immediate family. There is a wider implication from this theme that we should always be ready to provide support to others in need, irrespective of whether or not they are family. The results that emanate from offering such support may be surprising.

The aim of CleftPals (CleftPals 2013) is to help new families from the moment when the fetus is diagnosed as having a cleft through to the child's teenage years. The organisation is fully funded by parents, without government support. Not all the mothers had contact with CleftPals but the sentences included in the data reflect the reactions of those who did. One of the important points that come from these comments is that the timing of information, and the level provided is critical. The women received initial support from CleftPals but once the information become too technical, and/or intrusive a negative outcome followed. It is also important to note that within peer support groups such as CleftPals there can be those who are seeking attention and this can impact negatively on those who are looking at issues more positively.

It was not expected that the parents would be able to say why their child was born with a cleft as even today there is as yet no definitive reason for a child being born with a cleft. However the question was asked to try and get an opinion. Obviously there are many reasons why this may have occurred but perhaps, like many other birthing issues, clefting still remains largely unexplained. The sentences selected describe these

pregnancies and it can clearly be seen that there is no common ground, as each woman is describing her personal journey and each has differing experiences and also differing thoughts regarding the cause.

In the section above that covers the surgical procedures involved, the sentences selected are much longer because of the descriptive nature of the answers, but they were selected to show the variety of procedures, the way the parents dealt with each issue as it arose, 'the curve ball', and to some extent how their child dealt with it. In this section we again see how important communications are – not just with the parent but also with the patient. As time goes on the child is developing through the teenage years into a young adult who has had more than his, or her fair share of adult exposure, perhaps much more than children without anomalies, and so their maturity needs to be taken into account by the practitioners they are coming into contact with. The parents were dealing with an ever evolving set of circumstances and to some extent the child was a participant, but just on the periphery. The section again shows the parent's stress in dealing with the problems and their frustration at not being in control.

In relation to these parents helping others who may find themselves in similar positions it is interesting that every response was that they would give positive reinforcement to the parents. The language may in many cases be too direct if used in the way stated and as shown above may be inappropriate at different times throughout the journey. This positive attitude has been shown practically by the majority of these parents continuing in differing ways to be involved with CleftPals,

Raising a child with any disability must be challenging, and indeed that was the experience of these families. From the outset the parents faced uncertainty for their child's future and the journey for each parent was different. One participant who herself had had a cleft was aware of the future and took some control of the situation very early in the process. The others relied heavily on the medical community for the selection of professionals and for positive reinforcement and yet some of these staff appeared not to fully appreciate the emotional state of the parents at the time. This same situation appears to be the case with the family support group CleftPals, which appeared to have a 'one solution fits all cases' approach and yet showed enormous support at times. It was clear from the parents that a gentle, caring, and positive attitude on the part of the doctor was much more helpful in planning a path for the future of the child's medical treatment than merely a declaration that problems existed but could be rectified. This

study makes it very clear that every individual case is different and that each person involved must be treated in a very personal manner. Each had differing emotions and these appear to have changed over the course of the treatment period. The children matured and the relationship with both parent and practitioner changed with time bringing new challenges. The procedures were difficult for parents at times as they felt for their child as they entered surgery and did not see an end to these ongoing operations.

The letter which followed one interview demonstrates the difficulty one of these parents had in talking about their experiences. This highlighted to the researcher the need to be aware of the body language of the person being interviewed as that indicated whether or not the questions being posed were pushing beyond the set boundaries. These changes in bodily response were noticed in some interviews but not in others. This suggests that some parents had put the bad times behind them while others were still dealing with the trauma of the journey. From the outset had the researcher been more aware of this it may have altered the direction of some discussions. Where the parents spoke without the emotions showing it may have been possible to drill down into their experience and get a deeper understanding of how they were able to deal with the issues that seemed so challenging to others.

5.12 Conclusion

Being a parent is challenging but when your child is born with an anomaly the problems that must be faced become exponential. These parents showed the emotion of raising their child and dedicated themselves to access the best treatment available. These parents were not aware that their child would have a cleft and so there was, in some cases, shock and in another a sense of urgency to get the best treatment for the child. In others the decision-making was largely left to the practitioners. Parents experienced distress in coming to terms with the reality that their child would need ongoing treatment and felt for the child as he or she was operated on. The hospital experiences differed but it became clear that at times the words used by staff were inappropriate. This too occurred when the family support group CleftPals was involved. The early support was helpful but much less so in later contact. It was not expected that the parents could define the cause of the anomaly but each parent offered opinions which indicated in many cases that they looked for blame within their own activities.

While the hospital procedures were ongoing each family found great inner strength to deal with each situation as it arose. The parents felt that the young adults at times were frustrated with being ignored or sidelined by the practitioners and one thought that this resulted in the child's bad behaviour. The parents had distress, joy and sadness, fear and relief, and finally elation and pride in knowing that their child had achieved success in their life. They were all prepared to help others in similar need but each presented different options on how to provide such assistance indicating that it must be tailored to suit each particular situation.

5.13 Strengths and Limitations

The major strength in this study was the willingness of the parents to participate and their dedication to helping other parents who may experience similar challenges. A further strength was the spread of participants across the east coast states of Australia. The limitations were the small number of participants and the fact that in many cases the interviews were one-off face-to-face followed by email or phone communication. Follow up face-to-face interviews would have been helpful.

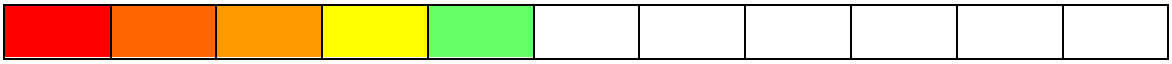
5.14 Recommendations for Future Research

This study presented a small but important group of parents and some of their experiences but further research is obviously necessary to more fully explore ways in which parents with a cleft child can be supported. It seems clear that hospital training programs must include how emotional support can be provided in conjunction with medical procedures. From a community perspective more information needs to be provided about clefting so that when a family needs support the appropriate responses can be provided that do not leave the parents feeling alienated.

5.15 Summary

The parents in this study demonstrated the devotion that is directed towards children. They suffered when their children were undergoing surgery at least as much and possibly more emotionally than the child. They persevered and saw their children succeed, and now delight as other parents might with what they see as a well-adjusted individual. The question remains – what causes this birth anomaly?

Chapter 6 Preliminary Investigation in the Philippines



6.1 Research Question

To determine whether a path could be found for a targeted future study into the aetiology of CLP in the Philippines, this study aimed to collect qualitative data by interviewing people closely related to the health issues surrounding CLP. A questionnaire was developed to discuss clefting in a broad sense with a range of individuals including mothers in relation to the following topics:

- Familial relationships
- Nutritional status
- Environmental toxins in air, soil, water, and the food chain

6.2 Introduction

ORAL planned to send a surgical team to the Philippines (two missions are normally organised each year with up to 100 procedures being conducted over a 10 day period) to correct clefts in the faces of children born with a CLP. The author was invited to accompany them in order to carry out a concurrent study. The surgical mission consisted of surgeons, anaesthetists and nurses and was carried out at the General Malvar Memorial Hospital in Quezon City, a suburb of Manila. All necessary registrations had previously been submitted and approved by the Philippine authorities.

The problem of CLP in the Philippines has long been recognised, not necessarily because of its severity (as that has not really been documented across the country) but because plastic surgery to correct clefts in the faces of children born with this affliction was not generally available and therefore the problem was more evident (ORAL 2006). Surgical correction was not available for two reasons: firstly there are insufficient trained surgeons in the Philippines, as many relocate to other countries where higher salaries can be obtained, and secondly because only the ‘rich’ can afford to have their children operated on.

Several charitable groups have recognised this problem and have formed missions to provide surgery for children in the Philippines whose parents cannot afford even the

most basic medical attention. Prior to a surgical team arriving in the Philippines, a number of organisations broadcast that a team of surgeons from Australia, or perhaps another country such as the USA, will be operating on children with CLP, at no cost to the parents, on a certain date, and that the parents can register their child for the operation.

While several groups undertake these missions, ORAL, an Australian volunteer organisation, has been using the General Miguel Malvar Memorial Foundation Hospital for the past ten years, and so the staff at this hospital has been exposed to a large number of children and parents affected by this birth anomaly. Therefore the staff at Malvar and the surgeons who operate there would be primary sources for practical information. In addition, because the breadth of the study was wide, it was decided to interview staff within the Philippine Departments of Health, and Agriculture, and also staff at the University of the Philippines for their collateral input.

6.3 Background

In order to obtain an appropriate input it was decided to visit a location where the prevalence of CLP is anecdotally higher (ORAL 2006) than in other parts of the world, and to interview people closely associated with these health issues. The country selected was the Philippines.

Because the literature suggests that CLP is a multi-factorial problem (as previously discussed in Chapters 1 and 2), it was decided to also interview people who were familiar with nutritional and environmental issues that may impact on the problem.

Therefore the aim of this study was to undertake a wide ranging investigation exploring a number of possible scenarios which may in some way be associated with the problem of clefting, so that more targeted research could be undertaken in the future.

In parallel to the main question of trying to determine causal links, a side objective was to determine whether there were certain geographical areas or groups of mothers in the Philippines where a higher incidence of clefting occurred suggesting that a local problem existed. As the Philippines is a poor country, the question of whether a lack of adequate nutrition is implicated in some form seemed to be a valid path to explore. Finally, as some previous work had questioned whether environmental toxins could cause or contribute to CLP, an exploration regarding whether toxin exposure may be an issue also seemed a relevant course of study.

6.4 Literature Review

The literature directing the course of this study was that outlined in Chapter 1.

6.5 Ethics Approval

The exploratory study proposal was submitted to the Southern Cross University Human Research Ethics Committee (HREC) which approved the program (ECN-05-163).

6.6 Research Design

This research centred on an enquiry of one ethnic group that had experienced a common phenomenon, that is, a knowledge of clefting. The methodology usedwas a simple qualitative descriptive approach.

6.6.1 Ethnology

An ethnological study is the study of people whose beliefs, material aspects, actions, and artefacts are influenced by their culture's implicit or explicit ways of being (Minichiello, Sullivan et al. 1999). The aim of the researcher is to get as close as possible to the participants' ways of believing, and to report these as truthfully and as faithfully as possible. Ethnology involves an ongoing attempt to place specific events and understanding into context by combining field experiences with other areas of knowledge to provide a fuller and more meaningful outcome (Tedlock 2000). This can involve pure observation, structured questionnaires or merely intuitive responses following the immersion of the researcher within the target of the research. It has been said that the ethnographer should be free to 'muddle about' (Wolcott 1979). It is also proposed (Grbich 1999) that it is better to enter the field with foreshadowed problems rather than preconceived ideas, which may limit one's views.

6.6.2 Phenomenology

This method tries to understand a phenomenon. In the human sciences it concerns itself with the study of things within human existence (Roberts and Taylor 2002) because it acknowledges, and ascribes values and meanings that people link to their own existence. The process requires that a general question is developed which is then explored in terms of the target group's experience and feelings. The researcher aims to reflect as closely as possible the essence of the experience (Grbich 1999).

The understanding of these two methods provided a background for the researcher to develop themes which could be tested against other existing work, via a further

literature review to finally establish a list of possible avenues, for a more targeted research program.

6.6.3 Structure

A questionnaire was designed as the basis for discussions with the mothers of children with CLP. A further questionnaire was developed to open up discussions on various issues when talking with people outside the health domain, such as the agricultural and pesticide authorities. The topics, therefore, that could be discussed were not capped in any way as it was thought that semi-structured interviews would allow the participants to add their own ideas regarding cause and incidence should they wish to. All the documents relating to this study are attached in Appendices 2-5.

It was a fortunate coincidence that at the same time three students from the University of the Philippines who were undertaking an undergraduate degree in nutrition were also working at the hospital. They were gaining experience in interviewing patients and in this case the parents of children with clefts, and they invited the researcher to join them; this extended the investigation and provided a more quantitative outcome. The students interviewed approximately 80 families but due to time constraints the researcher sat in on only 41 of these. Their assistance allowed the information to be obtained which is now recorded in the results section. Specifically they collected the food intake of the participants over a one week period thus allowing an average diet of those presenting to be prepared. These data were made available to the researcher.

The interviews conducted with staff and also with people outside the hospital precinct were much broader, allowing for the participants to put forward their own ideas and to suggest areas where further research may be needed. The questions asked during these interviews, but not exclusively, are set out in the questionnaire located in Appendix 5. These questions were designed to open up discussion regarding possible pollution from obvious sources and also to include other substances, which may be in use for legitimate reasons, but due to inadequate controls may have ended up unwittingly as pollutants.

6.7 Setting

The major site where the interviews were conducted was the General Malvar Memorial Hospital located in Quezon City, a suburb of Manila in the Philippines. This is a private hospital owned and operated by the Malvar family. Consent had been obtained to undertake the research at that facility. Other interviews were conducted at the

University of the Philippines, Diliman Campus, the Quezon City Hall, the Agricultural Department in Quezon City, and the Department of Health in Manila.

6.8 Data Analysis

Where numerical data resulted from the interviews these were entered on an Excel spread sheet for closer examination. The major points identified by individuals were recorded, and have been summarised below. Ideas that flowed from interviews were tested where possible by discussions with other participants to add rigour to the study and later to be the subject of a further literature review.

The dietary information was collated and analysed using the Foodworks and Nutricheck[13] computer analytical programs. Data relating to the 'average Filipino diet' were obtained from staff at the Department of Food Science and Nutrition, University of the Philippines, Diliman Campus. Other data were analysed with the assistance of an independent statistician from the University of Adelaide Medical School using the Pearson and Cochran-Mantel-Haenszel statistical methods, which were considered most appropriate for the data collected.

6.9 Results

Interviews of a semi structured nature were conducted with the visiting surgeons and nurses who form part of ORAL, the USA team who accompanied them, the nurses and doctors at the hospital (particularly those involved in gynaecology and obstetrics), professors in nutrition from the University of the Philippines, the Departments of Agriculture and Health at Quezon City and Manila. The results of these interviews are recorded below, however, for a summary see Table 6.1. The information gained from the students is shown in Table 6.2 and Figure 6.1, which follow later.

[13] Foodworks is software designed to allow the analysis of dietary intake and may be purchased online at: http://www.xyris.com.au/. Nutricheck uses the same input data however provides an analysis of other nutritional elements and complements the Foodworks program. http://www.nutritionmedicine.org/index.php?id=7

Table 6.1 Interview summary

Interview	Expressed as having a likely causal relationship					
	Genetics	Poverty	Nutrition	Pollution	Attempted Abortion	Fertilizer/ Pesticide
Surgeon 1	Possibly	Perhaps?	Perhaps?	Perhaps?		
Surgeon 2		Perhaps?	Perhaps?	Perhaps?		
ORAL Director	Possibly					
Philippine Doctor 1		Possibly				
Philippine Doctor 2		Possibly				
Obstetrician					Possibly	
Head Nurse		Possibly	Possibly	Possibly		
Health Department		Possibly	Possibly	Perhaps?		
Fertilizer and Pesticide Authority		Possibly		Perhaps?		Perhaps?
University of Philippines Prof. 1		Possibly		Perhaps?		
University of Philippines Prof. 2		Possibly	Possibly	Perhaps?		

Perhaps? – Refers to a qualified comment that there may be a connection.

Possibly – Refers to the fact that the respondent believed it a credible cause.

6.9.1 Medical Staff

6.9.1.1 Surgeons

Both Australian surgeons who operated at the time and who had conducted many reconstructive operations previously were asked what they thought was the major cause of CLP. One believed that there was a genetic implication. The other surgeon was not sure but indicated that it was strange that the affliction appeared to be more common amongst the poor. When shown the Philippine government nutrition statistics, both suggested that nutrition may be a factor but could not explain how this might be involved. However, it is probable that the purchasing power of the poor is much lower and they opt to buy calorie rich but nutrient scarce foods eg. foods high in sugar content (Appelhans, Milliron et al. 2012).

It has been proposed that reduced protein intake by impoverished females prior to or during pregnancy may reduce the level of essential amino acids to lower than optimal. This may affect the efficiency of liver detoxification, for example, the sulphur rich amino acid such as cysteine is a component of the endogenously synthesised peptide glutathione, which is required for conjugation of xenobiotic chemicals (Iyaniwura

1990). Other researchers (Fowles, Stang et al. 2012) have also concluded that poor diet especially during the first trimester is negatively related to stress, depression, and meal preparation.

The surgeons could not comment on other aspects of the questionnaire, as neither had had much contact with the general public nor with organisations in the Philippines, apart from the people at the hospital.

6.9.1.2 Operation Rainbow Australia Limited – Operations Director

The Operations Director of ORAL thought that the prevalence of CLP in all countries was similar, but because the poor could not have the problem rectified, it was more evident in poorer countries such as the Philippines. Her own belief was that it was a genetic problem and that, apart from this, there were probably no external influences involved. She accepted that it was possible for a genetic alteration to occur due to the effects of an external environment, but would not comment beyond that.

6.9.1.3 Resident Philippine doctors

The Philippine doctors at the hospital believed that CLP was an affliction of the poor, but were unsure as to whether it was nutrition or toxins that were implicated. They were unable to comment whether the incidence of CLP in the Philippines was high or low as they had nothing to compare with, and given that records were not maintained at a national level, could not even give an estimate of the incidence nor relative risk in relation to causative factors other than poverty.

6.9.1.4 Resident obstetrician

The only exception to the comments made by the local doctors was proposed by the obstetrician who believed that self-attempted abortions within the poorer communities were far greater than people generally accepted, and that deformities of this nature should be expected. In fact, she had seen terrible deformities resulting from attempted abortions. She indicated that the drug misoprostol (Cytotec^{TM}) (uterine contraction, cervical dilation) appeared to be readily available on the black market and it was used for this purpose. She also indicated that mahogany seeds (i.e. possible toxicity of natural limonoids) (Fowles, Mootoo et al. 2012) were also used and could be obtained at the local market.

Cytotec (Oqbru and Marks 2012) is a commercial name for misoprostol, a prostaglandin (PGE1) analogue used in conjunction with non-steroidal anti-inflammatory drugs

(NSAIDs). Its main use was to reduce the risk of NSAID-induced gastric ulcers. The side effects of this drug, in relation to pregnant women, are well documented by the companies which have promoted the product indicating that if taken during pregnancy, birth defects may occur. Misoprostol is extensively absorbed and undergoes rapid de-esterification to its free acid form which is responsible for its clinical activity, and unlike the parent compound, is detectable in plasma. The alpha side chain undergoes β-oxidation and the β-side chain undergoes Ω-oxidation, followed by reduction of the ketone to give prostaglandin F analogues. In Australia it was approved in 2012 as a medical abortifacient. It is also used following a miscarriage and at the end of a pregnancy to ripen the cervix for labour induction.

The drug Cytotec is now no longer available in the Philippines, although it appears that there is old stock available through what are termed by the Filipino doctors as 'quack doctors' and other unscrupulous individuals. The other drugs available were said to be misoprostol and Arthrotec. As mentioned above, misoprostol, either sold under a commercial name or under its own chemical name, has been shown to cause problems if taken during a normal pregnancy. Arthrotec also contains misoprostol as well as diclofenac sodium, an NSAID, and so has similar problems to the other products mentioned. The obstetrician stated that she had seen many children born following attempted abortions where the mother had used Cytotec and if a child only had a CLP it would be a miracle, as most of those children had far more major deformities, including of the genitalia, as well as CLP.

Mahogany seeds, as mentioned above, were said to be readily available from street vendors who sell them under the guise of 'aids to menstruation'. The obstetrician indicated that quinine was also used in attempted abortions as an alternate to the seeds, although the therapeutic dosage used for malaria appears harmless (McGready, Thwai et al. 2002).

6.9.1.5 Resident head nurse

The head nurse at the hospital was in charge of assessing all children brought for surgery to ensure, firstly, that they were in a fit state to be operated on, and to assess the type of treatment required. She also obtained the authority from the parent or caregiver and in this context has more to do with the parents than any other person at the hospital. The doctors at the hospital undertake a complete physical assessment of each child, with blood being taken for laboratory analysis. As with the medical doctors the head nurse

could not make an assessment regarding the incidence of clefting but expressed the opinion that it was a more common occurrence in poorer communities.

She was not able to provide an unbiased and conclusive opinion as to whether there was a genetic implication as, in general, the hospital does not question the mother regarding familial associations, nor does genetic screening for possible polymorphisms associated with clefting (i.e. MTHFD1 1958G) (Murphy, Gurramkonda et al. 2014). However, she did indicate that it is rare for a mother to present with two children both having CLP. Her opinion was that CLP was very much related to poverty and was quite certain that nutrition, or its inadequacy, was a major contributor.

6.9.1.5.1 Belief systems

The head nurse explained that in her discussions with the mothers over a number of years, many of the parents had unscientific biases or superstitious ideas/belief systems concerning the cause of the problem. For example:

- Some mothers had fallen during their pregnancy and believed that this had caused the baby to move, pushing the child's fingers into its face.
- One reported that it was due to 'karma'; she had teased a neighbour's child who had a cleft lip when he was young and so this now was the 'payback'.
- Another had said that her sister told her that a classmate had a cleft and because she thought about this quite often, it had occurred to her child through 'mental affirmation'.
- Another mother believed it was because she kept on looking at a neighbour who had a cleft.

This belief system is, according to other doctors at the hospital, quite widespread. It is known in the Tigalo language as 'pamahiin', which can either mean 'a set of beliefs' or 'superstition'. The doctors reported that women have also indicated that the problem occurred because they ate popcorn during pregnancy, looked at a monkey, looked at a pig or at some other animal or plant. These ideas are founded in part by illiteracy and also by socially accepted concepts in poorer communities. There appears to be a need to place blame on a natural phenomenon, which is incomprehensible, and related merely to folklore. This is also seen in other conditions such as epilepsy (Radhakrishnan 2009).

6.9.1.5.2 Diet

Due to the close relationship the head nurse developed with mothers of CLP children and the doctors, she was able to provide, in conjunction with the medical students, an approximate daily food intake of the parents (i.e. food frequency questionnaire/7-day dietary recall). She indicated that some mothers had claimed that at least one day per month they had insufficient money for any food (i.e. starvation/stress). The daily intake of the diets of the poor were analysed using the Foodworks and Nutricheck programs and compared to a similar analysis of the average Filipino diet of which details were provided by Professor Florencio the head of the Department of Home Economics at the University of the Philippines, Diliman Campus.

The computer analysis of these diets was based on an assumption that the parent would be a 32-year-old female, weighing 50 kg and 150 cm tall. The energy requirement was based on a moderate activity level. This was said by the nurse to be a reasonable assumption. Some caution should be placed on the data as the computer programs were not designed for the Filipino population (i.e. RDI may vary). The analysis of the data is summarised below. It should be noted that the computer program used showed the RDI for total folate at 200µg whereas in Australia the recommended daily intake for women approaching pregnancy is 400µg and more recently this has been extended to 600µg. (Eicholzer, Tanz et al. 2006). Lastly, several other nutritional element levels were not considered by the computer programs e.g., vitamin B12, as only major elements were covered.

Typical Diet of Philippine Poor

FOODS

Breakfast	
Rice,White,Boiled	1 cup (cooked)
Coffee,black,NFS	1 coffee cup(180ml)
Sugar,NFS	2 indiv packet (1 teaspoon)
Lunch	
Rice,White,Boiled	1 cup (cooked)
Fish,NS Type,Dried	20 g
Green Leaf Vegetable,Ns Type,Cooked,Fat Not Added In Ckg	20 g
Bean,Green,Raw	20 g
Squash,Button,Cooked,Fat Not Added In Cooking	30 g
Dinner	
Rice,White,Boiled	2 cup (cooked)
Pork,Stewing Cuts,Stewed/Braised	20 g
Bean,Green,Raw	20 g
Squash,Button,Cooked,Fat Not Added In Cooking	20 g
Drinks	
Water,Bottled,As A Beverage	2 bottle(200ml)

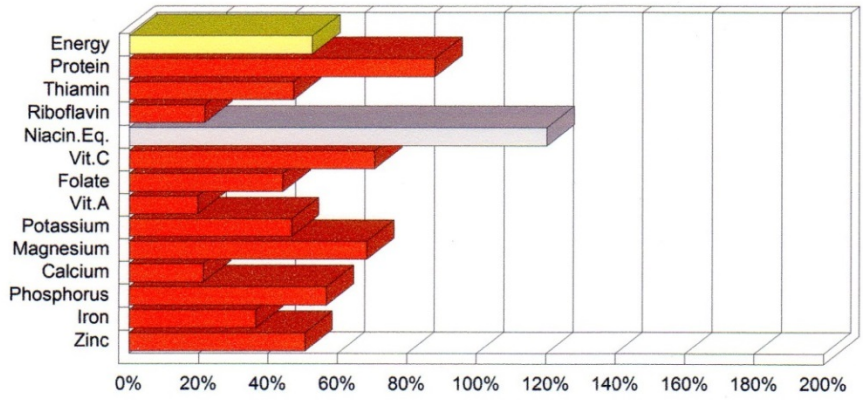
ANALYSES

	Avg/Day	RDI	RDI(%)	Status
Weight	1498.00g			
Energy	4696.36kJ	8905kJ#	(53%)#	
Protein*	39.59g	45g	88%	Less than RDI
Total Fat	6.24g			
- Saturated Fat	1.20g			
- Poly-unsaturated Fat	1.56g			
- Monounsaturated Fat	1.46g			
Cholesterol	48.80mg			
Carbohydrate	223.71g			
Sugars	10.84g			
Starch	212.86g			
Water	1218.69g			
Alcohol	0.00g			
Dietary Fibre	7.40g			
Thiamin*	0.42mg	0.89mg	47%	Less than RDI
Riboflavin*	0.29mg	1.34mg	22%	Less than RDI
Niacin	8.26mg			
Niacin Equivalents	17.13mg	14.25mg	120%	
Vitamin C*	21.20mg	30.00mg	71%	Less than RDI
Total Folate*	88.50ug	200.00ug	44%	Less than RDI
Total Vitamin A Equivalents*	147.80ug	750.00ug	20%	Less than RDI
Retinol	10.20ug			

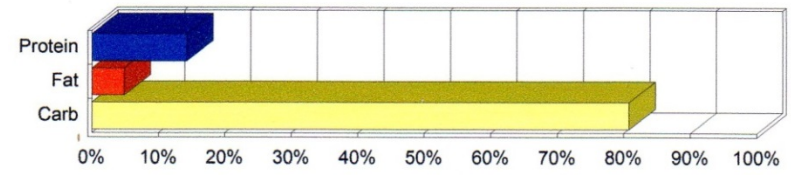
	Avg/Day	RDI	RDI(%)	Status
B-Carotene Equivalents	822.60ug			
Potassium*	910.98mg	1950.00mg	47%	Less than RDI
Magnesium*	184.66mg	270.00mg	68%	Less than RDI
Calcium*	170.80mg	800.00mg	21%	Less than RDI
Phosphorus*	568.40mg	1000.00mg	57%	Less than RDI
Iron*	4.38mg	12.00mg	36%	Less than RDI
Zinc*	6.08mg	12.00mg	51%	Less than RDI
Kj from Protein	14.30%			
Kj from Fat	4.90%			
Kj from Carbohydrate	80.80%			
Kj from Alcohol	0.00%			
Kj from Others	?%			
Fat as Mono	34.60%			
Fat as Poly	36.97%			
Fat as Saturated	28.44%			

- Estimated energy requirement
* - Less than RDI or minimum goal, or greater than maximum goal
RDIs based on: Female, 32years, 50kg, 150cm, Moderate Activity

RECOMMENDED DIETARY INTAKES



RATIO ENERGY FROM PROTEIN, FAT, CARBOHYDRATE AND ALCOHOL



Philippine National Average

FOODS

Rice,White,Boiled	282 g
Fish,NS Type,Dried	99 g
Pork,Stewing Cuts,Stewed/Braised	48 g
Cabbage,Bok Choy,Raw	30 g
Squash,Button,Cooked,Fat Not Added In Cooking	38 g
Bean,Green,Raw	38 g
Banana,Common,Raw	77 g
Milk,Fluid,Skim/Nonfat(Fat<0.16%)	44 g
Sweet Potato,NS Colour,Boiled,Fat Not Added In Ckg	17 g
Sugar,NFS	19 g
Oil,Canola(Include Rape Seed Oil)	12 g
Mixed Nut&Seed Mix	10 g

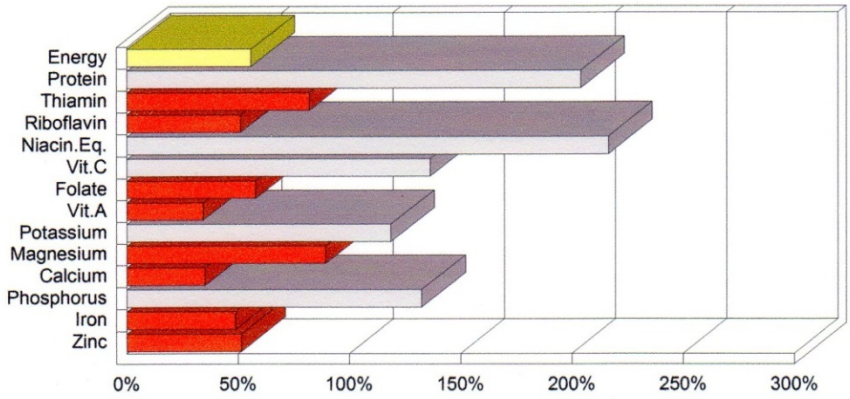
ANALYSES

	Avg/Day	RDI	RDI(%)	Status
Weight	714.00g			
Energy	4970.37kJ	8905kJ#	(56%)#	
Protein	91.84g	45g	204%	
Total Fat	37.19g			
- Saturated Fat	6.46g			
- Poly-unsaturated Fat	12.27g			
- Monounsaturated Fat	15.74g			
Cholesterol	195.48mg			
Carbohydrate	121.45g			
Sugars	37.60g			
Starch	83.86g			
Water	449.74g			
Alcohol	0.00g			
Dietary Fibre	6.69g			
Thiamin*	0.73mg	0.89mg	82%	Less than RDI
Riboflavin*	0.68mg	1.34mg	51%	Less than RDI
Niacin	13.02mg			
Niacin Equivalents	30.84mg	14.25mg	216%	
Vitamin C	40.90mg	30.00mg	136%	
Total Folate*	115.86ug	200.00ug	58%	Less than RDI
Total Vitamin A Equivalents*	256.12ug	750.00ug	34%	Less than RDI
Retinol	42.06ug			
B-Carotene Equivalents	1285.30ug			
Potassium	2312.76mg	1950.00mg	119%	
Magnesium*	241.16mg	270.00mg	89%	Less than RDI
Calcium*	281.47mg	800.00mg	35%	Less than RDI
Phosphorus	1325.60mg	1000.00mg	133%	
Iron*	5.87mg	12.00mg	49%	Less than RDI

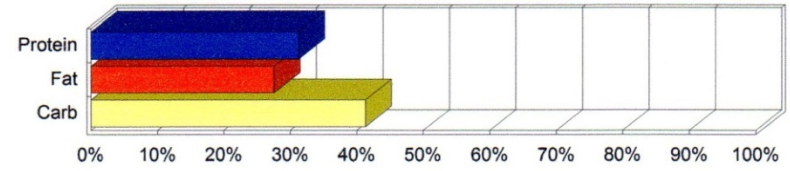
	Avg/Day	RDI	RDI(%)	Status
Zinc*	6.17mg	12.00mg	51%	Less than RDI
Kj from Protein	31.21%			
Kj from Fat	27.51%			
Kj from Carbohydrate	41.28%			
Kj from Alcohol	0.00%			
Kj from Others	?%			
Fat as Mono	45.66%			
Fat as Poly	35.59%			
Fat as Saturated	18.75%			

- Estimated energy requirement
* - Less than RDI or minimum goal, or greater than maximum goal
RDIs based on: Female, 32years, 50kg, 150cm, Moderate Activity

RECOMMENDED DIETARY INTAKES



RATIO ENERGY FROM PROTEIN, FAT, CARBOHYDRATE AND ALCOHOL



6.9.1.5.3 Food fortification

The nurse indicated that the government had made some attempts to both improve the nutritional value of food (Philippine Republic Act No. 8976), and had also provided some supplementation such as iron and folate, although she could not elaborate on this. (The university students who were present at the hospital referred to Professor Florencio the head of the Home Economics Department at the University of the Philippines, Diliman Campus, as being an academic authority in this area.)

Professor Florencio in a later interview confirmed that the Filipino government has recognised the problems connecting malnutrition with various health issues although not directly with CLP and has instituted programs to try and resolve some of these issues, (Florencio 2004). In particular, the government provides iron supplementation (ferrous sulphate 200 mg sometimes with 400 mg of folate) to vulnerable groups, including pregnant and lactating mothers. Because of the government's desire to correct perceived deficiencies, salt is being iodized, presumably for metabolic abnormalities associated with hypothyroidism in children. However, this has low penetration in the market and even lower in the poor areas due to the high price of iodized salt. Rice is now being fortified with iron but the product is more expensive and so again market penetration is lower in poorer communities. Farmed fish are fortified with vitamin A, although this appears to be a fairly recent innovation (Florencio 2004).

6.9.1.5.4 Food supplement supply

Multivitamins and other supplements, the nurse said, are offered in some areas through the local health centres, where there is said to be inadequate training of the individuals either prescribing or dispensing the products. The most common supplement offered is ferrous sulphate (200 mg).

6.9.1.5.5 Water purity

The nurse stated that many of the families that came to the hospital seeking surgery for children with clefts lived in areas where the water supply would be drawn from 'deep wells'. However, it was also stated that the term 'deep' was somewhat misleading as many wells would be no more than a few metres deep and could easily be contaminated with groundwater or nearby polluted rivers and lakes. Families whose source of water was from such wells often did not boil the water as this would be an additional cost for them. Thus, teratogens such as organic forms of arsenic and their bacterial metabolites

may present yet another possible cause for CLP, as bacteria found in ground water may metabolise numerous compounds (Rodriguez-Freire, Sun et al. 2012)

6.9.1.5.6 Pollution

In relation to pollution, the nurse felt that this was unacceptably high in many areas of the Philippines and particularly in the large cities such as Manila and surrounding satellite cities. This view was confirmed by everyone interviewed during the research process. Indeed, the area around the hospital was shrouded in vehicle exhausts and the shanty housing area adjacent to it showed every sign of being extremely polluted, which is a result of unregulated waste management practices by shanty village inhabitants (Bernado 2008) – again another education related issue.

6.9.1.6 The students

The student study added to the breadth of our own questionnaire with the data gathered being tabulated below (Table 6.2). The information was gathered from 41 mothers who between them had produced 143 children. Of the 143, 45 had clefts (prevalence of 31%). In only four of the families surveyed was there more than one child with a cleft, and no mother had more than two children with a cleft. In 16 cases the child was a first child and in 17 cases the child was the mother's last child. In two of these cases both the first and last child had a cleft. Of the cases where the child was a first child, eight of the children were not only the first but the only child of the mother. These data were analysed with the help of an independent statistician from the University of Adelaide using the Pearson and Cochran-Mantel-Haenszel statistical tests. Overall, no statistical significance was seen with either the age of the mother or the position of the child within the family in relation to recurrence of clefting.

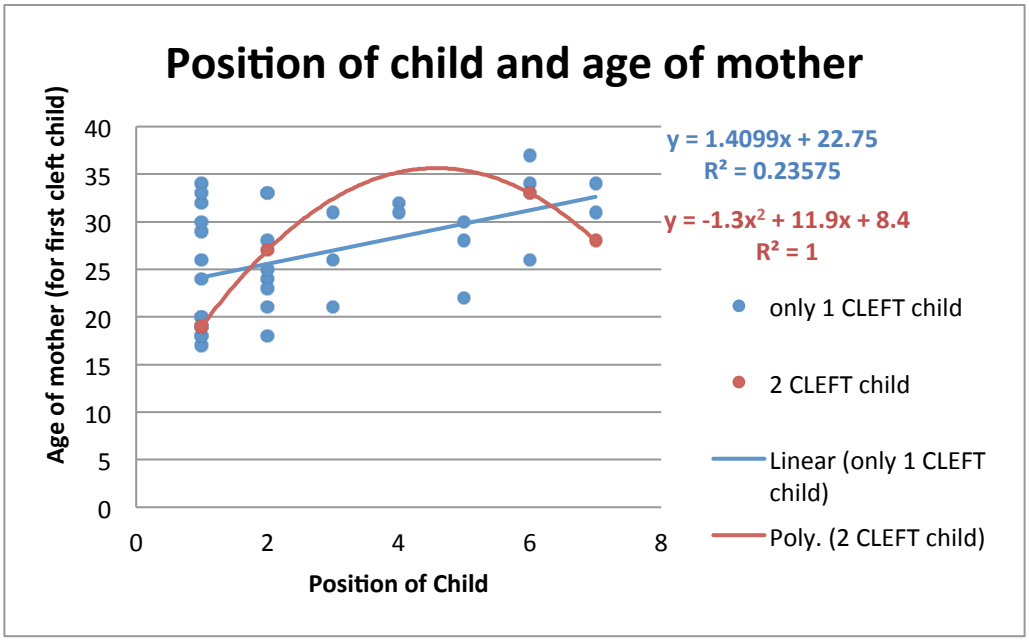


Figure 6.1 Position of cleft child in the family and age of mother

Table 6.2 Family incidence

Position of child in the family born with a cleft[14]											
Born	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		
Case #	**Age of children**									**Current age of mother**	**Age at birth of CLP child**
1	10	9	8	7	6	5	3			34	31
2	15	13	12	10	8	6	3			37	34
3	9	Died 1 mo.	18 mo.							28	26
4	1									25	24
5	15	12	10	6						38	32
6	8	6	4							30	24
7	13	10	6	3	8 mo					34	31
8	7	5	2							33	28
9	4	1 mo								33	29
10	3	1								19	18
11	3	2								23	21
12	1									18	17
13	2	1								22	20
14	4									22	18
15	2									34	32
16	23	20	9	7	5	2				39	37
17	10	5	3							34	31
18	5	2	1							27	25
19	1									27	26
20	1									20	19
21	9	5								39	30
22	20	17	15	12	10	7				38	28
23	21	19	16	14	7	5				39	34
24	26	25	24	22	20	15	14			50	28/36
25	17	15	13	11	7					37	30
26	5									23	18
27	14	12	9	6	1	6 mo				33	32/33
28	4	2								26	22
29	19	18	16	14	6	3				39	33
30	18	16	15	14	8	7	3	2	9 mo	37	21
31	12	8	5							36	20
32	4									28	23
33	8	7								26	19
34	11	10	8	6						45	34
35	4									30	26
36	23	21	19	17	14	12	9	5		45	33
37	1	2 mo								34	33
38	4	2								31	27/29
39	19	16	14	12	9	2				44	42
40	8	5								27	22
41	15	14	11	10	6					34	19/28

[14] A yellow box in this table indicates that the child had a cleft and the orange box that the mother attempted to abort as she did not want a baby.

6.9.2 Government Departments

6.9.2.1 Health departments

6.9.2.1.1 National Health Department, Newborn Screening Act Division

Staff at the Department of Health's new section covering the Newborn Screening Act 2004 RA 9288 were questioned regarding what was involved in this new act. They indicated that it was specifically aimed at testing for a glucose-6-phosphate dehydrogenase deficiency, in relation to the pentose phosphate pathway, co-enzyme nicotiniamide adenine dinucleotide phosphate (NADPH) and cellular glutathione levels. This according to the Health Department could predict future metabolic disorders, such as congenital hypothyroidism, congenital adrenal hyperplasia, galactosaemia and phenylketonuria, however other observations have been made in *G6PD*-deficient mice (Nicol, Zielenski et al. 2000). It was not suggested that the test had any bearing on clefting. It is unclear, apart from cost, why other testing was not considered, given that a blood sample was drawn.

On close questioning it was apparent that this testing was only done in hospitals and that at this stage parents would be asked to pay, as funding had not yet been made available. This was later confirmed by the head of the Quezon City Department of Health. The staff at the Department of Health agreed that it was highly unlikely that every child could be screened and even more unlikely that other tests would be added to this initial one. The researcher concluded that this policy would be almost impossible to implement due to insufficient funding, and the fact that it would only be available to those in hospital who really believed that they needed screening. With almost 70% of children being born outside hospital in the Philippines, screening is unlikely to be far-reaching.

6.9.2.1.2 Quezon City Health Department

The head of the Quezon City Department of Health (and later, the Department of Agriculture and several staff within these departments) believed that the occurrence of CLP was more prevalent amongst the poor, but could not give an opinion regarding the cause. The Department of Health did not collect data on clefting, and it was their opinion that hospitals were the only sources of these data. No effort had been made to co-ordinate any data collected even within the Quezon City area.

During the discussions, however, the question of pollution was raised and all agreed that this was a problem, with many rivers now heavily contaminated, some from mining

effluent, while others were contaminated with human waste, garbage, and effluent from factories. Vehicle emissions were also cited as a major pollution problem. Given that the Department of Health declared that waste appeared to be an issue within the areas where the poor lived, it is quite possible that toxins may spread into water supplies by a number of means and thereby into the food chain, and other pollutants may follow similar paths.

6.9.3 Department of Agriculture

The question was raised with the Department of Agriculture about how the community dealt with the mosquitoes, which, apart from being carriers of dengue fever and malaria, could also carry and transmit some of the water-borne toxins. Little information was gained as it appeared that this was dealt with at a local level and there was little or no national control. It was stated that especially among the poorer areas the communities merely relied on mosquito netting around their houses at best, and at worst just around their sleeping quarters. Apparently some local councils did spray to control the mosquitoes (type not specified), but generally only when an outbreak of dengue fever was imminent (it was unclear as to how this was determined), and even then there appeared no universal method employed.

The purpose of this line of questioning was to determine if chemicals were used which might in some way be connected with known birth defects in general or CLP in particular. The people with whom this was discussed could not name any of the chemicals used. Pingali, Marquez et al. (1994) in a Filipino medical and economic study of insecticide and pesticide use and their effect on rice farmers list many of the substances used. They also found that these impacted negatively on the farmer's health and farm productivity.

6.9.3.1 Airborne pollutants

The city areas of Manila and Quezon City were daily clouded in pollution (personal visual observation). This came predominantly from cars, trucks and motorbikes. Due to the age of many of these vehicles and the apparent lack of maintenance the emission levels seemed extremely high based on the visible emissions coming from the exhaust of the vehicles. For this reason it was considered that lead from fuel emissions may be a pollutant.

6.9.3.2 Lead/mercury

Of the elemental pollutants which could be implicated, lead and mercury were quoted by the Department of Health to be of concern, as mercury in particular had been detected in rice crops in previous years and lead in the least polluted area of Manila – Makati City (Sharma and Reutergardh 1999). This study showed that while high levels of lead were found in playgrounds in Makati City, the highest intake was from the daily consumption of food, presumably from being grown in contaminated soils.

In questioning where the lead could come from, overwhelmingly the answer was from leaded fuel. However, the Philippine government legislated to replace leaded fuel by the year 2000. Therefore the lead is either residual or if it really does come from fuel there are still supplies available. The percentage penetration of unleaded fuel into the market has not been established. Lead and mercury levels near the former Clark airbase were compared to those in other parts of the Philippines (Riederer, Shine et al. 2005), however, results of the study found no difference between areas. The researchers found that the analysis of house dust appeared to be the best media to determine possible exposure levels. In the Naboc River area, Mindanao, researchers (Appleton, Weeks et al. 2006) found high levels of mercury in the rice grown in the area, and fish caught in the river, resulting in 38% of the local inhabitants being classified as mercury intoxicated.

6.9.3.3 Manganese

Considering the level of airborne pollution due to vehicle exhaust, the question was raised whether new pollutants were emitted now that lead had been theoretically replaced. No studies have been undertaken by the Philippine authorities, but it is known that manganese has replaced the lead in unleaded fuels. This is in the form of an organic complex methylcyclopentadienyl manganese tricarbonyl (MMT) (Abbott 1987; Frumkin and Solomon 1997; Pellizzari, Clayton et al. 2001; Zayed 2001) which has been studied for toxicity by a number of researchers. Obviously the level of airborne manganese if it does come just from fuel will be proportional to the volume of traffic in any particular area. Proximity of dwellings to highways would suggest that there would be an unequal distribution within the population but it would perhaps affect both rich and poor and therefore is unlikely to be a direct cause of CLP.

6.9.3.4 Well water testing

The question whether the wells used as a water supply were checked by authorities could not be answered by either the Departments of Health or Agriculture staff. There seemed to be little evidence of any real control. The answer invariably was that they should be analysed each month but no one could confirm that this was done and could not supply data relating to a typical analysis. They did indicate that on occasions some wells were closed for periods of time following a test that highlighted a degree of contamination.

6.9.4 Fertilizer and Pesticide Authority

The Fertilizer and Pesticide Authority (FPA) is a division of the Department of Agriculture and was visited following a comment by one of the staff at the hospital that the division had found DDT in breast milk. This was confirmed by the head of the division, although a copy of the report was not available. It was confirmed that both DDT and dieldrin were no longer allowed to be used but it was stated that both were still very much available on the 'black market'. It was explained by the head of the division that the FPA only had 174 inspectors for the whole of the Philippines. It was therefore impossible to prevent smuggling and illegal distribution occurring across some 700 islands.

Of particular interest was the fact that one of the approved products contains high levels of manganese[15] (Mancozeb, the data sheet located in appendix 6) that is used in both banana and mango plantations. Banana was said to be the fruit of choice for the poor.

In questioning how people were trained to use these products it was indicated that they relied on the suppliers, distributors, and local farmers to train their staff in the appropriate use and handling of such chemicals. No guarantee could be given that this was done.

Evidence of organo-chlorides from farming run-off into the sea has been found in Manila bay in the Philippines (Carvalho, Cattini et al. 2009). High concentrations of these chemicals were found in sediments and in the soft flesh of oysters recovered from the bay. The researchers, however, concluded that the levels detected were unlikely to generate impairment of the marine biota.

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[15] Manganese poisoning produces symptoms similar to Parkinson's disease. None of these symptoms have been seen in women whose child was born with a cleft.

6.9.5 The University of the Philippines

Professors at the University of the Philippines, Diliman Campus, Home Economics Department, specialising in nutrition, were unsure of the cause of clefting and could not give a definitive answer whether nutrition was implicated. One professor, however, considered that malnutrition, combined with living conditions and/or toxins, could be implicated.

A book called *Nutrition in the Philippines* (Florencio 2004) was given to the researcher by a student of nutrition. Professor Florencio, (the author) who was later interviewed, stated that malnutrition was endemic in the Philippines but was unsure how this could be related to clefting. The university had not undertaken any work in this area. The professor also pointed out that the only statistics on the levels of clefting within the community could be via the hospitals and agreed that these figures would not be representative of the community as a whole as most births did not occur in hospitals.

The Newborn Screening Act of 2004 RA 9288 introduced by the Philippine national government was discussed at length. As the professor was in contact with the head of the section administering this, she agreed to discuss the possibility of recording child deformities in their records as part of the screening. She stated that it was unlikely that every newborn child could in fact be screened for possible metabolic disorders, particularly genetic issues, as the test was quite specific and could only be done if the child was in a hospital or close to a pathology laboratory. Likewise, the government appeared not to have properly funded the program.

While Professor Florencio applauded research into clefting it was her belief that the starting point must be either through a hospital where quantitative testing was possible, or in a small isolated community, where perhaps the incidence may be higher. She was unable to point to any such community and was unaware whether the incidence was greater in one particular area or another.

6.9.6 The Philippine Administration

From discussions with all the parties mentioned above and with students at the University of the Philippines, it was possible to obtain some understanding of the administrative structures within the Philippines. These are such that if the government was able to coordinate its activities, it could deliver its policies down to a very small

group of people, as the communities are tiered down from the Republic to small villages, with apparent responsible leaders at all levels. The various levels are:

- Republic or national government
- Regions
- Provinces
- Cities
- Municipalities
- Barangays (similar to a ward within an Australian local government area)
- Barrios (village or small community)
- Puroks (small village or zone)

The problem relating to the provision of appropriate antenatal and general health care appears to be twofold. Firstly, laws to improve health care appear to be made without the funding available for full implementation. This appears to apply more widely than just to health care but seems to be the case in all departments. Given that this is a developing country that has scarce resources and with extremely low levels of income this is not surprising. Secondly, each level of government seems to operate in isolation to maintain its own segment of 'power'. The various health departments appear separate and discreet at all levels of government. At the local level (barangays), a health centre is typically staffed by one to two midwives, one to two nurses, and a group of health volunteers whose main training has been their own experience and what they pick up from the trained staff. A doctor will visit the health centre on a reasonably regular basis to deal with the more serious problems. The health centres report back to the health department in their city. To even consider implementing a national program, and for it to be effective, there would need to be a massive training exercise undertaken.

For government bodies it is easy to mandate that health care should be improved but it is quite another thing in a country that struggles with increasing population growth and high unemployment to fund such a program, and then to ensure that this is followed through. Such is the case with the provision of nutritional supplementation. Of further concern is that supplements seem to be handed out without any investigation in relation to the particular nutritional needs of the person. The basis on which the government has made the assessment of these nutritional deficiencies also appears unclear.

The question of whether improved nutrition education could solve any of the issues in the Philippines is problematic in that the poor, which are the majority of the population,

have limited choices. Rohner, Woodruff et al. (2013) found that stunting and anaemia in children were both associated with low economic status of the parents and their inability to access nutrient-rich food sources.

6.9.7 Living Conditions

The following comments are recorded as personal observations of the researcher.

Within a few hundred metres of the hospital, where part of the study was undertaken, it was possible to view the living conditions of some of the poor in Quezon City. The homes are shanties, or shacks, built from scrap timber and metal, and sometimes plastic sheeting is also incorporated. A house is no more than a few metres square and neighbours are cramped up against each other. In many cases cooking is done on an open fire. Water and sanitation facilities did not appear to exist and the local well or communal tap provides drinking water. One family was seen washing clothes and bathing under a down-pipe from a building after a sudden downpour. Another family was seen doing their washing in the gutter at the same time. Public toilet facilities do not seem to be available except in the larger shopping centres and formal restaurants, both being well away from this area, and clearly not readily within the reach of the poor.

Garbage disposal appears to be a major problem as the local authorities do not seem to have the ability to deal with this. Consequently garbage builds up around homes and in the streets. The frequency of collection appears uncertain.

Air pollution is a problem, with many vehicles being old and in poor condition. Apart from this the roads are continually jammed with cars, small buses called ‘jeepneys’, and a haze of pollution hovers above the streets.

In Quezon City the head of the Department of Health said during an interview that the department preferred to spray the larvae of the mosquitoes where they found stagnant water to prevent them from breeding. They could not say what was used. This is in itself a further problem, as water pools seemed to be used for washing clothes as well as for bathing by the poor. Therefore, any chemical introduced into the water may be inadvertently transmitted to a person in contact with this water.

6.10 Discussion

From the various interviews a number of themes evolved.

- The problem of clefting appears greatest among the poor. Should it be a problem with the rich, it is hidden because they can afford the limited surgery that is available.
- The diets of the poor who attended the hospital with their children were much worse than even the depleted diet of the average Filipino, with not only a reduced protein intake, but very low levels of a number of key nutrients. For example, thiamine and riboflavin (see Table 6.3 below) are well below recommended daily intakes.
- Pollution is accepted as a major problem, both air and water borne, while the inadequate removal of garbage presents further health hazards.
- Clefting appears on the surface to be a random event but most observers point to it being a problem of the poor.
- There appears no significant relationship between the recurrence of a cleft and the position of the child in the family.
- There appears no significant difference between the occurrence/recurrence of a cleft and the age of the mother when the first child was born.

It is difficult to define 'poor' in the Philippines, purely because the vast majority of the population appears to be far poorer than can be imagined from living in OECD countries such as Australia. The discussions tended to suggest that the poor were those people living in 'shanty' type dwellings, where there was limited or no reticulated water supply and/or sanitation (the majority of children presenting for surgery came from living conditions such as these). Clark et al. (2002) carrying out an investigation in Scotland found an association between socioeconomic status and clefting although the difference in the Philippines is that there is a lack of food for the poor, rather than it being just a matter of choice which may be the case in developed countries such as Scotland. They did qualify the findings by indicating that tobacco smoking amongst the lower socioeconomic group may have been higher potentially influencing the results. Mosey et al. (2011) cite several researchers who have tried to confirm the issue of a socioeconomic relationship, but suggest that all of the research is plagued by difficulties in determining socioeconomic status of the participants studied.

As this was a preliminary investigation many of the ideas and most of the issues raised had not previously been considered by the researcher. In this sense they formed the

basis for going back to the literature in certain areas to see whether or not the concepts that developed during this study had merit.

6.10.1 Nutrition

6.10.1.1 Diet analysis

The typical diet of the poor provided by the head nurse was analysed using the Foodworks and Nutricheck analytical computer programs mentioned above.

A summary of the daily nutritional intake of the average population in the Philippines compared to that indicated to be typical of the nutritional intake of the poor is shown in Table 6.3. The data confirmed that the women presenting with their children were more than likely to have a nutritional intake well below the national average which in itself was deficient in many nutrients. While it is impossible from the data collected to predict which nutritional elements may be directly connected with clefting it does suggest that the lack of adequate nutrition may either be directly or indirectly a causal factor.

Vitamin A is a regulator of embryonic development and an excess has been associated with congenital malformations such as NTDs and CP (Ackermans, Zhou et al. 2011). A deficiency in folic acid has been directly associated with NTDs and has been suggested to be associated with clefting as has been discussed earlier in this document (Mastroiacovo and Leoncini 2011). Zeisel (2011) suggests that women eating low-choline diets have an increased risk of having an infant with a NTD or a facial birth anomaly. While cause and effect has been clearly determined between folic acid deficiency and NTDs the same is not as clear for CLP and is therefore yet to be determined.

Table 6.3 Dietary intake of the Filipino poor compared to the national average and recommended daily intake (RDI) USA

Item	Measure	RDI	Philippine national average	% RDI	Diet of the poor	% RDI
Energy	kJ	8905	4970	56	4696	53
Protein	g	45	92	204	40	88
Total fat	g		37		6	
Saturated fat	g		6		1	
Poly-unsaturated fat	g		12		1.6	
Mono-unsaturated fat	g		16		1.5	
Cholesterol	mg		195		49	
Carbohydrate	g		121		224	
Sugars	g		38		11	
Starch	g		84		213	
Water	g		450		1219	
Alcohol	g		0		0	
Dietary fibre	g		7		7	
Thiamine	mg	0.89	0.73	82	0.42	47
Riboflavin	mg	1.34	0.68	51	0.29	22
Niacin	mg		13		8	
Niacin Equivalents	mg	14	31	216	17	120
Vitamin C	mg	3	41	136	21	71
Total folate	µg	200	116	58	89	44
Total vitamin A equivalents	µg	750	256	34	148	20
Retinol	µg		42		10	
Beta carotene Equivalents	µg		1285		823	
Potassium	mg	1950	2313	119	911	47
Magnesium	mg	270	241	89	185	68
Calcium	mg	800	281	35	171	21
Phosphorus	mg	1000	1326	133	568	57
Iron	mg	12	6	49	4	36
Zinc	mg	12	6	51	6	51
kJ from protein	%		31		14	
kJ from fat	%		27		5	
kJ from carbohydrate	%		41		81	
Fat as mono	%		46		35	
Fat as poly	%		36		37	
Fat as saturated	%		19		28	

Source: Foodworks and Nutricheck analysis of Philippine Department of Health statistics and estimates of diet of the poor.

The analysis shows a state of protein and energy deficiency amongst the poor, but more importantly, much greater deficiencies in other nutrients. Further, the average diet indicated that the energy level was 53% of recommended daily intake (RDI), with the protein level being at 88%, the bulk of the latter coming from fish and rice. (One standard cup of white rice contains approximately 4 g of protein and brown rice approximately 5 g). It should be noted that rice is an incomplete protein (incomplete in that it is deficient in some of the essential amino acids) when compared with a similar weight of other proteins, for example eggs and beef (see Tables 6.4 and 6.5).

Table 6.4 Amino acid content in g of 100 g of rice compared to 100 g of egg

Amino Acid	Egg – Whole	Rice – White	Rice as % of Egg
Tryptophan	0.211	0.082	38.86%
Threonine	0.637	0.298	46.78%
Iso-Leucine	0.850	0.356	41.88%
Leucine	1.126	0.655	58.17%
Lysine	0.819	0.300	36.63%
Methionine	0.401	0.137	34.16%
Cystine	0.299	0.103	34.45%
Phenylalanine	0.739	0.382	51.69%
Tyrosine	0.551	0.347	62.98%
Valine	0.950	0.531	55.89%
Arginine	0.840	0.438	52.14%
Histidine	0.307	0.128	41.69%

Source: (Rodwell Williams 1999). It should be noted that this source only provided data on this particular group of amino acids for the egg, rice and beef.

Table 6.5 Amino acid content in g of 100 g of rice compared to 100 g of beef flank

Amino Acid	Beef Flank	Rice – White	Rice as % of Beef flank
Tryptophan	0.232	0.082	35.34%
Threonine	0.879	0.298	33.90%
Iso-Leucine	1.041	0.356	34.20%
Leucine	1.630	0.655	40.18%
Lysine	1.738	0.300	17.26%
Methionine	0.494	0.137	27.73%
Cystine	0.252	0.103	40.87%
Phenylalanine	0.818	0.382	46.70%
Tyrosine	0.675	0.347	51.41%
Valine	1.105	0.531	48.05%
Arginine	1.283	0.438	34.14%
Histidine	0.691	0.128	18.52%

Source: (Rodwell Williams 1999)

The average Philippine diet, which has been published by the Philippine Department of Health, was also analysed in a similar manner. These analyses show that the community as a whole is specifically deficient in iron, zinc, iodine, vitamin A, riboflavin, folate, and thiamine (Florencio 2004).

Certainly deficiencies in these nutrients can lead to a variety of health concerns, however, at this stage there is no evidence to specifically link these to clefting, and, given that poor nutrition is widespread, it is unlikely that lack of any one of the above nutrients is the predominant cause of clefting. None of the mothers who formed part of the study showed any obvious signs of connective tissue damage. A search of the EBSCO databases available through Southern Cross University (New South Wales) indicated that in the date range 1968-2014 there were 125 research papers linking CLP with folate, 12 with iron, 19 with zinc, 6 with iodine, 47 with vitamin A, and 7 with thiamine. The search was based on the nutrient being associated with either cleft lip, cleft palate or a combination of both. In all cases the researchers indicated that the nutrient may be involved in either a biochemical pathway, a genetic interaction, or combined with other (deficient) nutrients in some form which resulted in a CLP. No direct relationship was claimed in any of the papers considered, with the general consensus being that CLP was multifactorial. Obviously had other databases been included the number of papers in each category would have been larger however this was done to illustrate the fact that many researchers have considered nutrition or lack of it as a potential causal factor of CLP.

While the Foodworks and Nutricheck computer programs may indicate adequate levels of protein (88% of RDI) greater consideration must be given to the quality of the protein. In this case, rice has been considered within the program as perhaps a reasonable source of protein. As shown in Tables 6.4 and 6.5, its amino acid content is inadequate when compared to other sources such as egg and beef. When rice is compared with egg the limiting amino acid is methionine (Gropper, Smith et al. 2005), and when compared with beef, it is lysine, although methionine and histidine are also low. While grains can be complemented by legumes to provide more amino acid balance in the diet these are not readily available to the poor, and certainly not in the quantities that would be required. Yoghurt is beneficial where the diet is deficient in methionine and choline, however in countries such as the Philippines this is neither readily available to, or can be afforded, by the poor. Nardone, Compare et al. (2010)

concluded that supplementation which restored gut microbiota by supplementing with lactobacillus paracasei in rats attenuated liver injury in the absence of steatosis.

The rice which was the largest part of the diet was white rice, and while this is more expensive than brown rice the poor would not eat the latter because they believed that it reinforced the impression that they were indeed poor. Brown rice, however, if eaten, would in fact provide better nutritional support, particularly given the higher levels of nutrients as shown in Table 6.6 below.

Table 6.6 Concentration of several elements in rice

Element	Element: mg/kg Fresh Weight of Rice					
	Brown Rice			White Rice		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Copper	2.20	2.00	2.80	1.50	0.77	2.50
Iron	10.00	7.40	18.00	2.50	1.00	5.70
Manganese	42.00	22.00	52.00	10.00	9.00	12.00
Zinc	20.00	13.00	23.00	17.00	15.00	18.00
Selenium	0.20	<0.1	0.40	0.10	<0.1	0.20
Rubidium	6.00	0.64	12.00	3.60	1.20	5.50
Cadmium	0.021	<0.001	0.035	0.020	0.003	0.037
Lead	0.005	0.003	0.007	0.003	<0.002	0.010

Source: "Elements in rice on the Swedish market: Part 1" (Jorhem, Astrand et al. 2008) and "Elements in rice on the Swedish market: Part 2" (Jorhem, Astrand et al. 2008)

Other researchers (Ramirez-Velez, Romero et al. 2011) investigating the role micronutrients play during pregnancy have designed a trial to supplement women with zinc (30 mg), selenium (70 µg), vitamin A (400 µg), alphatocopherol (30 mg), vitamin C (200 mg) and niacin (100 mg) and it is interesting to see that the nutrients proposed for the trial follow closely with those highlighted here as also being important.

With the exception of niacin all of the major nutrients in the Filipino diet of the poor were below the RDI. It should be noted that while the level of protein in this average diet may seem acceptable, it is an average diet based purely on advice given by the nurse and students who were recording the dietary details over a one week period and there is no way of verifying the data. Likewise in this study there were no controls for comparison. All the people this nurse deals with, who have children born with CLP, are from poor families and so the actual dietary intake may be different than that described.

Certainly the average diet, ascribed to the poor by the nurse, is below that of the national average for the Filipino population.

6.10.1.2 Amino acid deficiency and liver detoxification

In relation to the diet of the poor, it appears that protein sources may be limited to that contained in rice and small amounts of dried fish, with some food products high in protein appearing beyond their reach financially. Given this factor, it is possible that amino acid deficiencies could be present which may impact on liver detoxification pathways, reducing the ability of the individual to excrete toxins (Gropper, Smith et al. 2005).

Amino acids are an important supply component to the liver as it is here that a variety of proteins, both structural and plasma-borne, are synthesised. The liver is also where detoxification takes place. Therefore attention needs to be focused in this area to determine what happens to a person who has a limited source of amino acids and is then exposed to toxins of various types. The toxins may be one of two major classes – 'elemental or mineral' or 'organic'. The elemental or mineral toxins, such as the heavy metals, will generally be deposited in the tissue of various organs, e.g. the brain or heart, while the organic toxins will be deposited in fat. Examples of organic toxins are the halogenated hydrocarbons such as DDT.

The liver has evolved very efficient processes for eliminating and neutralising toxins via phase I and phase II pathways (Pizzorno and Murray 2000). When the level of toxins increases, typically as a result of nutritional deficiencies or high level exposure to toxins, the metabolic processes within the body can be severely disrupted. In order to eliminate toxins the metabolic pathways in the liver must be functioning properly. The liver filters the blood, transporting the toxins into bile which is then combined with fibre in the intestine ready for excretion (Gropper, Smith et al. 2005).

Phase I uses a family of enzymes, referred to as cytochrome P450, to convert toxins into products that can be further metabolised in phase II. However, the intermediates thus formed are generally more toxic than the precursors. It is imperative that the phase II metabolic pathways function correctly. If, however, there are excessive amounts of toxins present, the cytochrome P450 enzyme system can be overwhelmed, causing a breakdown in processing via this pathway, resulting in high levels of damaging free radicals (Gropper, Smith et al. 2005). Glutathione, a tri-peptide consisting of glutamate, glycine and cysteine, is an important nutrient required for normal conjugation in this

detoxification phase. Glutathione acts as an anti-oxidant and it is itself oxidized to glutathione disulphide in the process (Cavas and Tarhan 2003). Glutathinone peroxidase is the enzyme involved. It is a seleno-protein therefore dependent on selenium in the diet. Selenium is deficient in some soils and the deficiency which may be transferred via food sources may lead to problems for women during pregnancy (Lantin, Quijano et al. 1990; Tinggi 2003; Vanderlelie, Venardos et al. 2004; Perkins 2006; Perkins 2011; Mistry, Broughton-Pipkin et al. 2012).

If the phase I process is not balanced by the phase II detoxification process (Pizzorno and Murray 2000) this allows the highly toxic intermediates from phase I to build up. In such cases the person may become far more sensitive to environmental toxins, which can then result in severe reactions.

Phase II is principally involved in conjugation (Pizzorno and Murray 2000) which requires protein and involves a number of enzymes which need to be supported by several nutrients, such as glutathione, glycine, S-adenosyl-methionine, cysteine, methionine, taurine, selenium and molybdenum, to name just some. For example a deficiency of glycine through a low protein diet would lead to impaired phase II glycine conjugation (Pizzorno and Murray 2000), resulting in increased susceptibility to toxins. Tables 6.4 and 6.5 show that in the case of methionine, rice only contains 34.6% as much as the equivalent in egg and 27.7% compared to beef. Cysteine, glycine, and glutamine are not present at all. This adds weight to the possibility that in these particular Filipino women who presented with a cleft child, their phase II detoxification process may be impaired due to their reliance on rice as the staple food, which is low in methionine.

Since there is evidence of protein deficiency and exposure to pollutants in those people designated in the Philippines as being most at risk, it is possible that toxins could build up in individual organs or be dispersed throughout the body. These could then be transferred to the embryo across the placenta. These toxins, like procarbazine which was used to create clefts in an animal trial (Bienengraber, Malek et al. 2001), may then cause the clefting. Protein deficiency in itself could be the issue in not delivering sufficient amino acids for protein synthesis and cell replication. Obviously a significant testing regime would need to be put in place to confirm such a hypothesis. This may be an important topic for future research.

6.10.2 Pollution

As has been described above there are many pollutants in the Philippine environment many of which may be the result from early misuse of chemicals such as DDT and dieldrin while others relate to waste disposal and vehicle emissions. The researcher pursued the possibility that manganese which has replaced lead in fuel may be involved but on further investigation found that if manganese poisoning was involved the symptoms in the mothers would have been similar to Parkinson's disease; such was not the case (Boyes 2010). The researcher did not see any evidence of these symptoms in any of the mothers who presented although they may have been too young at this stage for that to have developed. Lead was mentioned as being detected in the playgrounds of Makati City (Sharma and Reutergardh 1999) and had been detected in the food chain. Lead as a toxin has been directly related to neurological disorders such as autism in children (Lech 2001; Lech 2002; Lakshmi Priya and Geetha 2011). Colborn vom Saal et al. (1993) first determined that toxic elements that were present in the mother while not affecting her could pass through the placenta and impact on the developing fetus. Crinnon found (2009) that as the lead concentration increases there is a corresponding decrease in mental function of the child when born.

In Basra, Iraq in the period 1994-5 the incidence of birth defects was 1.37 per 1,000 but by 2003 the level recorded in the same hospital was 2.3 per 1,000 births with congenital heart defects being the most common, followed by NTD and CLP (Al-Sabbak, Sadik Ali et al. 2012). The researchers took samples of hair and toenail from both parents and children. Where possible they also analysed deciduous teeth from the children. Lead was found to be five times higher in children with birth defects and mercury six times higher than controls. The parents of children with birth defects also had higher levels of uranium in their hair sample than the parents of children with no anomalies. The researchers also suggested that emotional stress experienced by the mothers during this period may have also had an impact on the birth outcome. None of the women who gave birth to the affected children took any supplements prior to or during the pregnancy. Water contamination may also have had some impact on the outcomes. A study of the population diets in several Asian countries including the Philippines (Iyengar, Kawamura et al. 2004) failed to show high levels of uranium in the foods consumed. Particularly in the Philippines the study showed that the intake of calcium was low, being probably associated with the low intake of milk and other dairy

products. This was also thought to account for the low levels of caesium and iodine in the diet.

6.10.3 Is Clefting a Random Event or a Family Affair?

It was indeed fortunate to be able to gather the limited statistics available during the interviews being conducted by the students. Their objectives were of a much broader nature and more related to living conditions and food supply. The hospital, however, also recorded the statistics of all parents during their pre-operative interviews when they also gained permission for the team to undertake the operations.

The data in Table 6.2 show an apparent random pattern and after statistical analysis no significant relationships were found within the data. A mother may have had a child with a cleft, and then one without a cleft, or she may have had multiple children, then one with a cleft, and then the next without a cleft. The cause of the clefting may not be due to a specific nutritional deficiency, particular toxin, or a particular genetic deletion or variation, as within this study a large number of variables have been considered. Obviously there could be genetic/environmental or genetic/nutritional issues involved which have not been identified in this study. Where the child was the last of a large number of children in the family it could be questioned whether the mother was physically stressed at that stage in her life, however, she was still able to produce a child. Determining the mother's physical and emotional state at the time of conception was beyond the scope of this study.

6.11 Epigenetics

This study suggests that nutrition or the lack of it and the environment may have an influence on clefting. This then perhaps introduces the concept that clefting may be an 'epi- genetic' event. The term 'epi' means 'above, on, over, nearby, upon; outer; besides, in addition to; among; attached to; or toward,' (Goldberg, Allis et al. 2007) and hence implies that something has occurred that changes the way the gene can act which in the case of a facial anomaly may be a transcription factor. If it could be shown that this was merely due to a nutritional deficiency the term used would more likely be 'nutri-genomic'.

Epigenetics appears to be more clearly defined as the collective heritable changes in phenotype due to processes that arise independent of primary DNA sequence. In other

words gene expression can be changed which is not directly due to alterations in the DNA sequence. Recent research indicates that such genetic changes can be transferred across generations (Chong and Whitelaw 2004; Lui, Li et al. 2008). Arita and Costa (2011) identified a number of metals that have induced epigenetic effects including nickel, arsenic, chromium, cadmium, and cobalt as well as methyl mercury, selenium particulate matter, tobacco smoke, benzene, and polycyclic hydrocarbons. These products are said to have been involved in epigenetic events leading to cancer, pulmonary disease, diabetes, and neurological disorders. Each element or product acts in a slightly different way; for example, in the case where humans were exposed to toxic levels of arsenic in Bangladesh, genomic methylation of blood DNA was positively associated with plasma folate levels (Pilsner, Liu et al. 2007). The researchers in this case indicated that contrary to their prior hypothesis, arsenic exposure was positively associated with genomic PBL DNA methylation in a dose-dependent manner.

It is not merely toxins or toxic-like elements that are involved in epigenetics. Both nutritional deficiency and nutritional excess can instigate an epigenetic event (Lima, Pinto et al. 2011). These researchers showed a link between maternal protein restriction, epigenetic alterations, and metabolic effects in offspring. The mechanism suggested is that protein restriction during pregnancy induces hypomethylation of the peroxisomal proliferator-activated receptor α (PPARA) and glucocorticoid receptor (GR) promoters, and increases expression of GR in the liver. The result is an increased expression of transcription factors. It appears that the mechanisms by which epigenetics impacts on birth outcomes is still far from completely understood but what appears quite clear is that nutrients, non-nutrients (toxins), and other environmental factors can dramatically change the way in which genes are expressed.

6.12 Weighing the Evidence

While this study is based on a limited number of participants the birth pattern in families was random suggesting that the cleft may be the result of a more temporary altered genetic (epigenetic/nutri-genomic) expression which may not in the above cases have become a family trait.

6.13 Conclusions

While this survey had a very limited number of participants, albeit from very diverse areas of interest, some conclusions could be drawn which can now form the basis of further research.

- A general theme has been established suggesting that clefting is an affliction of the poor, however, this could not be quantified and requires larger studies to be carried out.
- Protein deficiency may be an issue because the poor cannot afford protein-rich foods. Indeed, it would appear that in many cases very little food is available to the poor. Rice and small amounts of fish were the basic protein sources. A more detailed study in relation to nutritional differences among the poor needs to be undertaken before a protein deficiency can be linked to CLP.
- While this study did not have evidence indicating that a change from eating white rice to brown rice may improve the health outcome of the population in general and therefore reduce birth anomalies, the promotion of this should be recommended to the Philippine government.
- The general population in the Philippines is undernourished and the poor appear to be living merely on subsistence rations of food.
- Environmental pollutants may combine with a nutritional deficiency to be a trigger which affects the replication and fusion of cells of the lip and/or palate during the early weeks of pregnancy. In the Philippines this may be a combination of garbage around the dwellings, poor quality water supply, air pollution, and chemicals used near the home or sprayed by farmers or the local councils together with poor nutrition. Polluted ground water may be an issue with tailings from mining seeping into rivers, paddy fields and the water table. A much larger and more comprehensive study is required to determine any direct links.
- The use of manganese as an additive in fuel and in fungicides may increase exposure to this element. A specific study would need to be undertaken to determine whether this element is affecting the health of the population.

6.14 Strengths and Limitations

This study by its very nature was an exploratory study designed to give direction for future enquiries. The strength was the fact that the information came from a wide range of sources: those who had a child with a cleft, those who were involved in facial reconstruction, local health-workers, academics, and public servants. The major limitation was that those interviewed merely gave their opinions relating to the cause of clefting as there was little evidence that any had studied the issue in any great depth.

6.15 Recommendations for Future Research

Further similar studies in the Philippines of a much more quantitative nature are required to address the hypotheses generated by this study. Where possible, sampling of blood, urine and hair of parents and the cleft children as well as matched controls would be desirable. In combination with such a study an analysis of the participant's tongue and fingernails may also provide evidence of other nutritional deficiencies.

6.16 Summary

This study has raised more questions than it has answered largely due to the limited number of participants available to be interviewed during the ten days of the ORAL mission.

In the Philippines, because 90% of the population are malnourished (Florencio 2004), the level of pollution is high, and the fact that families do not move far from where they are born, it was not really possible to link a possible causal factor with CLP. It seems that a similar, perhaps more comprehensive, study should be carried out in Australia where pollution levels are comparatively low, food sources clean and adequate, and the population is spread over a wide area, which would give a good geographical spread to any research program.

Chapter 7 Possible Causal Factors of Clefting in Australia

7.1 Research Question

Is it possible that nutrition and environmental toxins are causal factors for clefting in Australian children, as has been hypothesised for the Philippine population?

7.2 Background

The Philippine study indicated the possibility that non-syndromic clefting may be the result of an epigenetic/nutrigenomic event. It was evident that a number of nutrient deficiencies could be involved and perhaps environmental toxins, however, it seemed clear that clefting appeared to be a random event. Pollution, especially water or airborne, may also be present where there is a lack of enforcement of environmental law, and thus be an influence. It seemed logical that any future research should be undertaken in an environment where differentiations could be made or at least where the two possible causal factors (nutrient deficiencies or toxins) could be isolated from each other. The researcher, in taking this approach, is not overlooking the possibility that there may well be genetic predispositions which could be involved in these dietary or environmental situations but is merely focusing on aspects which are within the scope of this particular investigation.

7.3 Introduction

This study was undertaken to investigate a number of health criteria that could have affected a woman planning pregnancy. Families who had a child two years or younger with a cleft were invited to participate in the study. Variables recorded were initially restricted to mother's age, family history of clefting, smoking, alcohol intake, use of supplements, mother's weight and body mass index (BMI). At a committee meeting of the CleftPals organisation the researcher listened to mothers relating their experiences and realised that in some cases the woman had experienced elevated stress at or around conception. This led to the inclusion of questions relating to the mother's coping skills and whether or not a stressful event had occurred in her life at that time.

7.4 Literature Review

A comprehensive literature review was not undertaken as this study was based primarily on the findings of the previous chapter (and the earlier Chapters 1 and 2), where the literature had been searched in an attempt to support the findings in that particular study. However, given that stress had not been considered previously a specific literature search was undertaken to determine whether stress has been considered in relation to clefting.

Researchers have investigated the role of stress using both animal experiments (Fraser and Fainstat 1951), and human stress outcomes (Carmichael and Shaw 2000) (Table 7.1). The stress investigated has been both physical and emotional. Many of the associations with stress appear to be concerned with major life events, for example, an increase in the incidence of CLP in children whose mothers had experienced an earthquake in California (Glynn, Wadhwa et al. 2001). The death of a woman's older child prior to her pregnancy was studied in Denmark (Hansen, Lou et al. 2000) where it was found to have a direct impact on an adverse pregnancy outcome. Hibino, Takaki et al (2009) investigated the effects of an earthquake in Japan in relation to CLP and found an association while terrorism was associated with CLP by Benitzhak and Verny (2004). On the other hand, no clear association exists between maternal and fetal adrenocorticotrophic hormone (ACTH) concentration, and thus another hormone may be implicated (Kosinska-Kaczvnska, Bartkowiak et al. 2012).

Table 7.1 Summary of papers relating to stress and CLP

Country	Event	Reference
USA 9/11	Terrorism	(Benitzhak and Verny 2004)
USA	Earthquake, California	(Glynn, Wadhwa et al. 2001)
USA	Hurricane, Katrina, New Orleans	(Goenjian, Chiu et al. 2011)
Chile	Earthquake	(Montenegro, Palomino et al. 1995)
Czechoslovakia	Russian occupation	(Poradowska and Jaworska 1963)
Iraq	War	(Fathallah 2007) (Al-Sabbak, Sadik Ali et al. 2012)[16]
Japan	Earthquake	(Hibino, Takaki et al. 2009)
Denmark	Death of older child	(Hansen, Lou et al. 2000) (Ingstrup, Liang et al. 2013)[17]
USA	Stressful life events	(Carmichael, Shaw et al. 2007)

[16] Caution should be applied with both of these studies as the issue appears to be a complex mix of toxic pollution combined with reduced availability of food and the stress of being in a war zone.

[17] This latter paper was published after the completion of this study and cites the paper written by the researcher, which was published in the journal *Women and Birth*.

The question that must be decided is whether or not psychosocial stress can be related to oxidative stress and whether the latter can impact on an adverse pregnancy outcome. McEwen (1998) indicates that two factors largely determine individual responses to potentially stressful situations: the way a person perceives a situation, and a person's general state of physical health, which is determined not only by genetic factors but also by behavioural and lifestyle choices. He further points out that one's physical condition has obvious implications for one's ability to mount an appropriate physiologic response to stressful stimuli. Lower total antioxidant status has been cited as a bio-marker in CLP children revealing a higher level of oxidative stress (Aizenbud, Peri-Front et al. 2008).

McEwen (1998) states that if the allostatic load is such that the cortisol secretion does not increase in response to stress, secretion of inflammatory cytokines increase resulting in the negative consequences of an enhanced inflammatory response (oxidative stress). Espel, Blackburn et al. (2004) studied 58 healthy pre-menopausal mothers, 19 of whom had a healthy child (controls) and 39 who had a chronically ill child, who were perceived to have on average a greater environmental exposure to stress. The findings showed that both perceived stress and chronicity of stress is significantly associated with oxidative stress.

Oxidative stress is linked to physical factors such as toxins, nutritional deficiencies and genetic variations but may also be linked to emotional stress (McGinnis 2007). Irie et al. (2002) determined that oxidized nucleic acids in blood correlated with the tension-anxiety scores measured in workers, while other researchers found that participants who practised meditation had much lower blood lipid peroxides than controls (Schneider, Nidich et al. 1998).

Oxidative stress in relation to adverse pregnancy outcomes, has been investigated (Sandman, Wadhwa et al. 1997; Toescu, Nuttall et al. 2002; Moretti, Phillips et al. 2004), with the majority arriving at the conclusion that stress minimisation should be a priority when considering pregnancy. Krabbendam et al. (2005), however, concluded that in a well educated population there was no relationship between stress and adverse pregnancy outcomes. While Krabbendam et al. believed that stress was not a factor there appeared to be sufficient other evidence to consider investigating the association of stress with CLP.

7.5 Ethics Approval

This study was approved by the Southern Cross University Human Research Ethics Committee (HREC # ECN-06-155).

7.6 Research Design

As for the Philippine study reported in the previous chapter this study was a simple qualitative descriptive mixed method research plan involving phenomenology and ethnology. Inclusion and exclusion criteria were as follows:

7.6.1 Inclusions

All families involved had to fulfil the following requirements:

- have a child with a non-syndromic cleft two years or younger
- be domiciled in the eastern states of Australia (this limit was set to minimise the researcher's travel costs)
- be recommended to the program via CleftPals

7.6.2 Exclusions

The following families were excluded:

- families whose children had syndromic clefts
- families who did not have a child with a cleft

7.6.3 Research Method

A questionnaire containing both quantitative and qualitative questions, the latter being the mother's assessment of her coping skills, was developed. The information sheet, consent form, and questionnaire are included as an appendix (see Appendices 8-10).

The quantitative questions were straight-forward but the question relating to the coping skills was the mother's assessment and could not be verified. The part of the questionnaire relating to mental/emotional stress that the mother experienced at or around the time of conception contained the following questions:

- How would you describe yourself?
    - Easy going
    - Worries sometime
    - Worries often
    - Continually worries about the future

- In the period one month prior to conception to two months after conception, was there any event that occurred in your life or your family that caused you anxiety or stress above the normal stresses of life? If yes please describe:

Each questionnaire received was followed up by communication with the mother either face-to-face or by phone to clarify the data submitted.

CleftPals promoted this study on their website and also at their meetings in order to encourage participants to volunteer to be included in this research. All children were born between 2004 and 2006.

7.7 Setting

To overcome the possibility that the incidence of CLP could be related to a specific district or area the study was carried out over a wide area, this being the eastern states of Australia. This also ensured that the families lived in different environmental conditions. Considering that environmental toxins may be involved in clefting, as some researchers have suggested (Shaw, Nelson et al. 2003; Brent 2004; Mekdeci and Schettler 2006), it was essential to ensure that families who were in the study came from different geographic locations so that any localised toxic exposure could not skew the results.

7.8 Data Analysis

All data were entered into an Excel spread sheet and analysed. Where quantitative data were available these were further analysed with the assistance of an independent statistician using the Fisher test to determine the significance of the variable ($p<0.05$).

7.9 Results

Forty-seven families participated, representing 48 children. Two children were included from one family: the first being born with a unilateral cleft lip (U/CL) and the second with a U/CLP. One family had dizygotic twins, one being born with a U/CL and the other with no anomaly.

It was evident that four of the children had syndromic clefts and so those families were excluded. This left 43 families and 44 children in the study.

7.9.1 Mother's Age at Conception of the Child

The mean age of the mothers at conception giving birth to a child with CLP was 32.8 (SD = 5.72) years. The ages ranged from 21 to 44 years.

7.9.2 Position of Child in Family

In the families, 50% of the CLP children were the second born and 50% were the first born child. None of the mothers had more than two children. This may have been due to the fact that they were now concentrating on the medical requirements for the cleft child as none of the mothers had more children after the cleft child.

7.9.3 Sex of Child with Cleft

Of the cleft children 84% were male and 16% female.

7.9.4 Types of Clefts

Eighteen per cent of children had a unilateral cleft lip only and 18% had a cleft palate as the only anomaly. Seven per cent had a bilateral cleft lip and palate and 57% had a unilateral cleft lip and a cleft palate.

7.9.5 Ultrasound Testing

Ultrasound testing during pregnancy did not detect any of the single cleft palate anomalies, and it did not detect five of the cleft lip and palate anomalies, two of these being bilateral cleft lip and palates. However, it did detect all other anomalies no later than 20 weeks into the pregnancy.

7.9.6 Body Mass Index and Weight of Mother at Conception

The average body mass index (BMI) of the mother ranged from 18.4 to 34.3, with a mean of 24.4 (SD = 3.35). Normal BMI is between 18.5 and 24.9, overweight is 25-29.9 and obese is 30 and above, while people with a BMI less than 18.5 are considered to be underweight (Gropper, Smith et al. 2005). The mother's weight at conception ranged from 47.0 kg to 104.0 kg with a mean of 67.88 kg (SD = 11.71).

7.9.7 Length of Pregnancy

Sixty five per cent of mothers carried the child to full term and 4.2% beyond full term by a period of between ten days to three weeks. The mothers who did not carry to full term gave birth between 34 and 37 weeks. The World Health Organization indicates that term is >37 weeks (WHO 2006).

7.9.8 Birth Weight

The mean birth weight of the children was 3.45 kg (SD = 0.63) with a range of 1.2 kg to 4.94 kg.

7.9.9 Smoking

None of the mothers smoked cigarettes containing tobacco or other substances during the pregnancy and only two indicated smoking prior to the pregnancy.

7.9.10 Clefting of the Parents

Three mothers also had clefts and only one of the fathers. In the latter case the mother in that family did not have a cleft.

7.9.11 Clefting in Other Family Members

Apart from the cases mentioned immediately above there were no close relatives with clefts. Examples of other family clefts were first cousin, father's uncle and child's grandfather's cousin on the father's side. In all there were only 14 families who had been able to trace a relative who had previously had a cleft. A genetic predisposition may exist within these families, which perhaps could interact with environmental factors to result in a cleft.

7.9.12 Mental Health/Emotional Stress

In 16 of the 44[18] families (36%) the mothers responded to the questionnaire that at or around the time of conception their lives were highly stressed. In a further 14 cases (31.9%) mothers indicated stress or anxiety but of a milder nature. In all cases where stress was indicated this was discussed at an interview with the participant in order to properly classify the nature of the condition. There was no possible way of quantifying the level of stress as experience of stress is very subjective. However, some level of stress or anxiety was evident at or around the conception period in 68% of cases (n = 30).

7.9.12.1 Grouped stress results

To further analyse whether an association between the occurrence of CLP and one or more of the above factors was present, the cases were divided into three groups. The decision regarding where to group each mother was based on the mother's definition of the level of stress that occurred.

- Group 1 included cases where the mother indicated that there was no particular highly stressful incident and that she coped well with day-to-day stresses.

[18] As two cleft children were born at different times in one of the families they have been included twice.

- Group 2 included cases where elevated stress was noted and again this was confirmed with the mother by the researcher.
- Group 3 included cases where a specific traumatic event, as determined by the mother, had occurred and was verified by the researcher at a subsequent interview.

The results of this regrouping are set out in Table 7.2 below.

Table 7.2 Summary of results

Demographics		**Group 1**	**Group 2**	**Group 3**
Mother's age at birth (years)		30.4 ± 6.1	32.2 ± 5.70	34.3 ± 5.2
Mother's weight at conception (kg)		67.9 ± 11.2	64.9 ± 8.80	70.3 ± 13.9
BMI		24.5 ± 3.3	23.1 ± 3.10	25.4 ± 3.36
Child's birth weight (kg)		3.44 ± 0.81	3.25 ± 0.69	3.62 ± 0.38
Sex of Child	Male	13	11	14
	Female	1	3	2
Position of child in family	First child	7	8	7
	Second child	7	6	9
IVF conceptions		0	3	1
Cleft type	U/CL	3	2	3
	CP	2	1	5
	U/CLP	8	11	6
	B/CLP	1	0	2
Total clefts		14	14	16

Table 7.3 Stress level and cleft type

	Cleft type			
Stress	**B/CLP**	**CP**	**U/CL**	**U/CLP**
No stress	33%	25%	38%	32%
Elevated stress	0%	13%	25%	44%
Traumatic stress	67%	63%	38%	24%

There was no significant relationship between stress level and cleft type ($p = 0.3692$).

7.9.13 Individual Responses

The following responses to open-ended questions are representative of the issues that were highlighted by respondents:

7.9.13.1 Group 3– Cases where the mother considered the stress as traumatic.

7.9.13.1.1 Respondent 1

I went through a very difficult time in my job. I went through a redundancy and then my husband lost his job. I found out I was pregnant the day I left my employment.

7.9.13.1.2 Respondent 2

Undergoing cycles of IVF procedures – injections and medications – anxiety and stress of failure. Confirmation of pregnancy at six weeks – anxiety of discovering twins! Stress involved in planning and welcoming two babies (first-time parents).

7.9.13.1.3 Respondent 3

Extremely stressful job involving bullying, finally leaving the job. Also at the time tension and arguing with the family. I removed myself from the family and now do not phone or visit.

7.9.13.1.4 Respondent 4

Had difficulty getting pregnant and this kept me in a stressed state. My partner then lost his job.

7.9.13.1.5 Respondent 5

I suffer from depression/anxiety and obsessive compulsive disorder (OCD). This was not medicated prior to or during pregnancy. Grandfather died one month prior to conception and moved house almost on the day of conception.

7.9.13.1.6 Respondent 6

Stressful job – worked on community relief for victims of cyclone Larry for 15 months until one month before baby was born. Also got married during this time. Baby diagnosed as a boy, which I did not want.

7.9.13.1.7 Respondent 7

Prior to conception I had a relationship of seven years break down. This was due to the father of my two-year-old daughter and was an ongoing problem for me. Two months after conception I decided that I didn't want to be with the father of my baby and we split up. These two things caused me a lot of stress.

7.9.13.1.8 Respondent 8

Major family disagreement followed by relocation to a new town.

7.9.13.1.9 Respondent 9

Left husband and returned to Australia.

7.9.13.1.10 Respondent 10

Father did not want to have the child and did not accept the pregnancy.

7.9.13.1.11 Respondent 11

Husband left when I was six weeks pregnant. I had worried about his continual drinking.

7.9.13.2 Group 2 – Cases where the mother believed she suffered abnormal stress

7.9.13.2.1 Respondent 1

I got stressed a lot about little things, and when I was getting stressed or angry I got a churning feeling in my stomach.

7.9.13.2.2 Respondent 2

Completely fatigued with second child. Prone to worry and get stressed. Very tired and exhausted.

7.9.13.2.3 Respondent 3

Moved house, built again, took twelve months to conceive. Had morning, noon and night sickness

7.9.13.2.4 Respondent 4

When I was five weeks pregnant began to spot blood and by six weeks had increased to same as a light period. At seven weeks taken to hospital with a major haemorrhage and kept until it subsided. I then bled continuously until thirteen weeks. I also had bronchitis during this time.

7.9.13.2.5 Respondent 5

I worry constantly about the future and consider myself a ‘worry wart’. I had a cleft and was also worried my child would also be born with this anomaly.

7.9.13.2.6 Respondent 6

I had trouble with my boss who has limited experience, and much of the work was left to me which I found difficult and frustrating.

7.9.13.2.7 Respondent 7

Our cat was our child, and it died around the time of my conception. I cried for at least two days.

7.9.13.2.8 Respondent 8

Four to six weeks after conception work was busier than usual and I had more responsibility than usual in this period.

In addition to the specific comments of the group 2 mothers, more than one mother indicated that the child was conceived using IVF (in vitro fertilization)[19] and indicated elevated stress associated with this process.

7.10 Discussion

In hindsight a design flaw of the study was to restrict it to women whose child was under two years of age as it was thought that mothers would best remember the details of their pregnancy if it had occurred in the recent past. This was a male concept, and after many discussions the researcher (a male) came to understand that women remember many details of their pregnancies. The researcher accepts that relying on data recall is not generally considered ideal for data collection but perhaps a longer period after conception may have been useful.

The average birth age for Australian mothers was 27.3 years in 1985 (ABS 2005). In 2009, 294,540 women gave birth to 299,220 babies in Australia,2,295 more births (0.8%) than that reported in 2008. The average age of women who gave birth in Australia has increased gradually in recent years, from 29.0 years in 2000 to 30.0 years in 2009 (ABS 2011). The average birth age of the study group was 31.7 years being slightly higher than the mean age group of mothers giving birth in 2009.

Considering that half the children were the second child to be born into the family it suggests that clefting may be random or that an environmental factor may interact with a genetic predisposition. However, what could have changed for the mothers between pregnancy 1and 2 are factors related to their lifestyle, nutritional status and/or stress levels. Further in a prospective study, the intake of pharmaceutical drugs during pregnancy was associated with CLP births in 28% of the cases (Soltani, Nasab et al. 2014). Pharmaceutical drugs were not a factor in this study.

[19] Infants conceived using IVF have slightly higher risk of birth defects than those conceived normally, Olson, C. K,. K. M. Keppler-Noreuil, et al. (2005).

The results show that the majority of the children born with a cleft are male. Statistics published for Victoria support this (Riley and Halliday 2004; Vallino-Napoli, Riley et al. 2004). No reason to date has been established to understand this phenomenon. It does suggest, however, that one or more of the genetic variant(s) that predispose to CLP may be associated with the X chromosome. As a male has only one X chromosome, any X-linked gene variant may be more damaging to a male fetus than to a female where two X chromosomes are present. Research (Braybrook, Doudney et al. 2001; Andreou, Pauws et al. 2007) has linked the X chromosome gene *TBX22* with isolated CP. It is possible that if *TBX2*2 is not solely responsible for clefting, one closely related to it could be involved in these anomalies (Naiche, Harrelson et al. 2005).

Marcano et al. (2013) discuss the X-linked inheritance of CP and while they acknowledge that there is some evidence to suggest that inheritance exists there are also many cases where this does not appear to be the case. They therefore concluded that due to the variable expression of X-linked inheritance CP is likely to be influenced by modifiers or environmental factors. This suggests that if there is a genetic trait, an external factor has been involved at some time, perhaps oxidative DNA damage (Fenech 2005).

A normal range of BMI for women is considered to be between 18.5 and 24.9 (Gropper, Smith et al. 2005). The women in this study tended to be at the top end of this range, with the variation in weight in this study being quite wide. While obesity has been considered to be a risk factor for CLP and other birth defects by other researchers (Moore, Singer et al. 2000; Cedergren and Kallen 2005; Stott-Miller, Heike et al. 2011; Correa and Marcinkevage 2012; Parker, Werler et al. 2012; Block, Watkins et al. 2013) this study was too small to add weight to that hypothesis. Certainly within the sample group some women were overweight at conception but this cannot be taken as a generalisation. The average BMI of the sample group also suggests that several women may have been overweight at conception. Other factors may have been implicated, although to be certain that insulin resistance is not involved serum fasting insulin analyses would be required. Insulin resistance is the forerunner to type 2 diabetes which in turn is associated with obesity and is associated with an inflammatory state of the body (Pladevall, Singal et al. 2006). Inflammation causes oxidative stress and has the potential to cause DNA damage (Fenech 2002) and thus influences adipose tissue deposition and obesity as inflammation is the hallmark of obesity. Omega 3 fatty acids

are anti-inflammatory dietary sources present in flaxseed, linseed, and fish oils, and thus may be lacking in the diet of these CLP mothers during pregnancy (Casas-Agustench, Lopez-Uriarte et al. 2011).

The individual responses clearly show that the mothers in group 3 experienced quite traumatic events around conception or in the first few weeks of the pregnancy. It is difficult to quantify the level of the stress as each individual copes in different ways. Indeed, even some of the events experienced by women in group 2 could be traumatic in the eyes of the woman involved and yet viewed by others be considered as normal events in everyday life. We can only say that the women themselves considered these as stressful events.

7.10.1 Stress and Pregnancy

There is no doubt that all people from time to time in their lives suffer stressful events and indeed many women who experience a normal pregnancy may also experience stress during that period. In this study a large number of women experienced traumatic stress and some less stressful events. Earlier in this study psychosocial stress was suggested to be a precursor for oxidative stress (McGinnis 2007).

Selye (1956) was credited with being the first to define biological stress, however, it is clear from his book that stress was a common term in use prior to his definition. It perhaps did not have the connotation that it now does, but he points out that stress was the term used to cover all aspects of mental tension. Mason (1975) suggests that stress is a physiological response within the organism elicited by evocative agents, and that stress itself must not be confused with the specific agents which have been titled stressors.

Oxidative damage can result from nutritional deficiencies and/or the presence of environmental toxins (Fenech 2002; Fenech 2005). As comprehensive dietary studies did not form part of this particular study this aspect could not be considered. However, physical and emotional stress, both of which result in altered cortisol levels, could also be involved (McGinnis 2007). Colaiana et al. (2013) induced psychosocial stress in rats by isolating them from their social group and found that oxidative stress was implicated in the stress response. Inoue et al. (2009) used urinary concentrations of 8-hydroxy d-oxy guanisine (8-OHdG) to determine the level of oxidative DNA damage amongst workers in a Japanese factory who believed that they were working under excessive

stress. The researchers found a direct correlation between the female workers' levels of stress and elevated 8-OHdOG. In a further study (Matsushita, Sugunuma et al. 2010) researchers used reactive oxygen metabolites (Diacron ROMs test – Diacron International, Grosseto, Italy) to determine the level of oxidative DNA damage in non-smoking college students in Japan. Their findings suggest that neurotic and anxious female students had high levels of oxidative stress.

Stressors can lead to oxidative damage at the cellular level via hypothalamus – pituitary – adrenal (HPA) axis dysregulation, and high cortisol and cytokine production (Gruner 2006). High cortisol has been linked to abdominal adiposity, insulin resistance and metabolic syndrome (Tsigos and Chrousos 2002), all of which may be involved in oxidative damage. In a study involving diabetic rats the teratological effect of diabetes and increased reactive oxygen species (ROS) have been shown to influence the increased incidence of cleft lip (Eidesio, Wentzel et al. 2012).

Stress levels affect the environment in which the cells reproduce and develop. If stress levels are low or of short duration, physiology and cell development and replication can return to normal, while continuously high stress levels lead to possible abnormal development or cell necrosis (Lipton 2005).

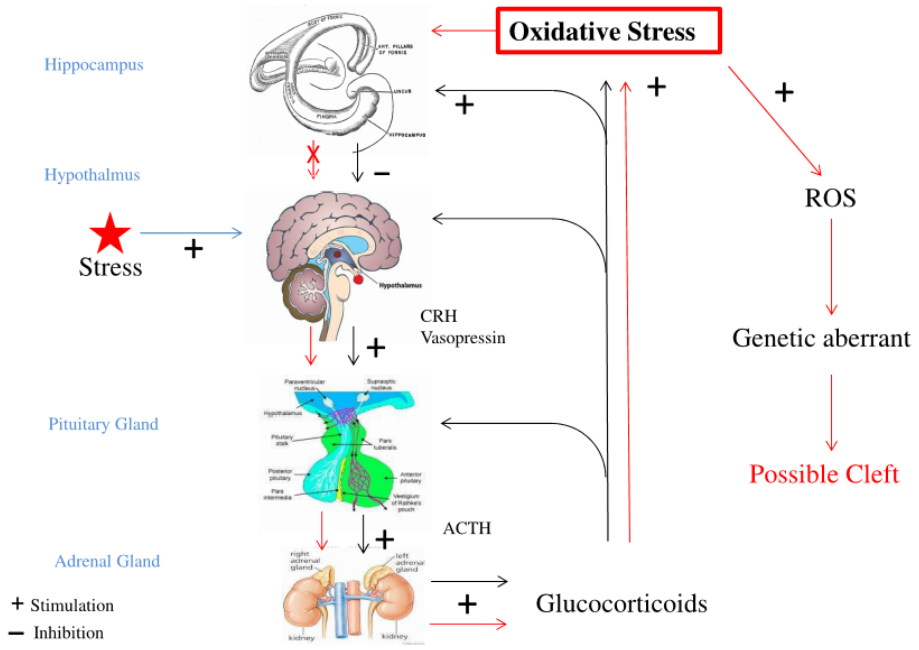

Figure 7.1 HPA axis

This figure was based on one published by Vitale, Salvioli et al. (2013) but modified and added to by this researcherutru. Under normal conditions (the black lines) the

presence of a stressor stimulates the paraventricular nucleus of the hypothalamus which induces the release of ACTH from the anterior pituitary gland. ACTH stimulates the synthesis and release of glucocorticoids from the adrenal cortex. Where the stress is extreme, as in the cases set out in this study, a traumatic stress increases the production of the glucocorticoids increasing the production of ROS leading to a genetic aberration resulting in a possible cleft. Vitale, Salvioli et al. (2013) showed that in ageing situations (shown by the red lines leading to the hippocampus) there is probably also an increase in hippocampal oxidative stress.

The development of fetal tissue and organs is directly proportional to the amount of blood they receive and hence their function. Stress will profoundly alter the distribution of blood flow to the placenta and may change the character of a developing child's physiology (Sandman, Wadhwa et al. 1994; Lesage, Del-Favero et al. 2004). A study conducted in Beijing, China, showed that environmental air pollution elicits increased pro-inflammatory ROS formation when in-vitro epithelial cells were exposed to pollution particles (Liu, Baumgartner et al. 2014). A similar situation may also exist in the Philippines where the poor live close to or near increased concentrations of car, truck and factory exhausts.

There is no doubt that there are some women who experience stress but go through a normal pregnancy and produce a perfectly healthy baby. Indeed, there are many people who work better when stressed and enjoy the adrenalin rush that this provides. An Indian study (Sharma 2011) suggests that the ability to cope is directly related to the personality of the person. It is mentioned here merely to show that the researcher recognises the possibility of a variance in the conclusions.

7.11 Conclusion

Of all of the criteria assessed, mental/emotional stress, as determined by the mother and confirmed by the researcher through a follow-up interview, appears to be a likely risk factor in the occurrence of CLP. To date this has only been considered by a very small number of other researchers.

7.12 Strengths and Limitations

The major strength of the study was the fact that the research was carried out over an exceedingly wide geographical area. All the women involved in this study not only willingly gave time to complete the questionnaire but agreed to more than one interview

in many cases. This added rigour to the study as the researcher was able to revisit particular areas to confirm the data.

As stress is experienced subjectively and reactions to the same stressor are dependent on the coping skills and resources of the individual, there was no possible way in this study to quantify the degree of stress other than qualitatively. Physical stress, on the other hand, coupled with increased ROS formation, reduced GSH, increased ACTH and cortisol levels, may be the physiological stress influence in CLP development. Certainly in any future studies attempts must be made to determine the level of stress quantitatively and have a control group who did not have a CLP baby. It is also necessary to determine how stress affects the biochemistry of the pregnant woman, and the effect it may have on the developing fetus. This could be assessed via stress questionnaires,[20] and from both blood and urine analysis during the pregnancy. Similarly oxidative stress can be measured quantitatively using samples of either blood or urine.

7.13 Recommendations for Future Research

Stress can be mental/emotional, nutritional, and/or physical, and each may have an impact on a developing fetus. Further targeted research of a more quantitative nature is required to determine which contribute to clefting.

7.14 Summary

This particular study began as an investigation into a wide ranging set of parameters only to arrive at a suggestion that stress, whether physical, psychosocial, or mediated by any other form may be involved in clefting. Research since this work was completed tends to support this (Ingstrup, Liang et al. 2013). There is however evidence that an epigenetic response may be involved and that this may be X-linked and heritable.

Australia is the logical place to continue this work as there is a good and relatively pure source of food, a relatively high disposable income across the community when compared to other countries in close proximity, and highly trained laboratory technicians operating in a number of pathology laboratories.

[20] At the commencement of this study stress was not considered. It was not until after some early meetings with CleftPals that stress appeared to be involved and by then it was too late to put in place a sophisticated stress analysis.

Chapter 8 Fetal Clefts and Mother's Nutritional Status

8.1 Research Question

Is there a difference in the nutritional status of women carrying a fetus with a cleft to those whose fetus is clear of all anomalies?

8.2 Introduction

This study is predicated on the hypothesis that poor nutrition, or the limited ability to absorb specific elements/nutrients, could be a deciding factor in the birth outcome. In a recent opinion Glenville (2006, p. 642) states: "We are living in a society that is over-fed and undernourished, with deficiencies apparent from a so-called well-balanced diet."

Individuals who do not understand what a well-balanced diet is may assume that if they consume three meals a day they are eating well. The rise in obesity around the world tends to negate this concept. In a recently published review (Hozyasz 2010, p. 610) comments on the relationship between nutritional intake and other lifestyle factors: "Since the mother is the environment of the developing embryo, interactions between genetic and lifestyle factors are assumed to be involved in abnormal palatogenesis."

The author also states on the same page that:

> In the human genome only a difference of about 1.6% between modern humans and the most developed primates has been found. [Primates however vary significantly in the non-gene coding which is involved in controlling the expression of genes.] In contrast, human dietary habits have markedly evolved since the origin of the species about 2-7 million years ago, especially during the last century. The per capita consumption of refined sugar has increased from 0.5 kg/year in 1850 to about 50 kg/year in the recent decade (Hozyasz 2010, p. 610).

We have no documented evidence that the incidence of clefting is greater now than in 1850, and indeed none going back two million years. The fact that diets have changed so dramatically in recent times may suggest that many of today's health issues could be related to these dietary changes. Indeed some health issues such as diabetes have been shown to be directly related to dietary changes (Mokdad, Bowman et al. 2001) with

obesity being a possible causative factor (Stott-Miller, Heike et al. 2011) albeit a weak one suggesting that it may not be a primary cause.

Studies have been undertaken to identify CLP in non-human species (Goldschmidt, Lopes et al. 2010) with some indicating that clefting occurs more often with other abnormalities. As the objective of this thesis was specifically targeted at human CLP no attempt has been made to understand birth anomalies in other species.

8.3 Background

Previous work undertaken in this thesis and by other researchers (Prescott and Malcolm 2002; Cobourne 2004; Gajdos, Bahuau et al. 2004; Jugessur and Murray 2005) suggests that there are a number of risk factors in relation to a child being born with a CLP.

Among these are the following:

- Physical stress, which may include becoming pregnant too soon after a miscarriage or depleted physical ability due to malnutrition
- Emotional stress caused by trauma experienced at, or near conception
- Poor nutrition and/or the inability to absorb specific nutrients required during replication of the facial structure of the developing fetus
- Environmental toxin exposure

It is hypothesised that all of these factors may lead to oxidative stress resulting in an epigenetic effect, thus potentially resulting in the child being born with a cleft. Pregnancy itself is a state in which oxidative stress is up-regulated with transcription changes occurring (Jiang, Bar et al. 2012).

8.4 Research History

No work appears to have been done to study the health status of the mother at the point the fetus is diagnosed as having a cleft in isolated CLP cases. As clefts are complete at twelve weeks' gestation it seems extremely important to study what has occurred up to this time, rather than to try and make deductions well after the event has occurred. Block, Watkins et al. (2013) considered pre-pregnancy BMI and concluded that there was an increasing risk of birth defect affected pregnancies with increasing pre-pregnancy obesity.

Studies should be undertaken as close as possible to the time that an anomaly occurs otherwise the initiators of CLP may be blurred by other factors during the remainder of

gestation. While no literature could be found where previous work had been undertaken, this study endeavoured to help to fill that knowledge gap in a new and novel way.

An initial study carried out by the researcher in the Philippines provided much of the background information on which the current hypothesis is based (Chapter 6). The incidence of CLP in the Philippines is anecdotally higher than in many other countries, which was the reason for that study. The interviews with government officials and medical personnel revealed the possibility of a nutritional and environmental impact on the developing fetus. Nutritional analysis of the typical Filipino diet showed low protein, low vitamin and mineral levels, largely due to the reliance on rice as the staple food. It was difficult to differentiate between the various likely contributing factors because of the conditions in which the parents of the CLP children lived. Confounding factors included the toxic environment from overcrowding, vehicle exhausts, indiscriminate use of chemicals, and mining residues. In the study reported in Chapter 7, stress was highlighted as a possible causal factor, particularly oxidative stress. To date there appear to have been no studies carried out on the health status of women in early pregnancy in relation to oxidative stress and its predisposing factors.

8.5 Ethics Approval

The study was approved by the Southern Cross Ethics Committee (ECN-12-077).

8.6 Research Design

The research plan considered a combination of factors affecting the health of the mother at or near the time of the diagnosis of the cleft in the fetus. The aim was to recruit pregnant women as soon as they had been diagnosed as carrying a fetus with a cleft (participants). The subjects were matched with women (controls) who were also at a similar stage in their pregnancy but whose fetus had no diagnosed abnormality. Because the diagnosis was based on an ultrasound screening no exclusions could be made on the basis of any further anomalies unless they had been clearly identified. In fact no others were identified therefore all women who volunteered were included.

This study combined both quantitative and qualitative methods therefore making it a mixed method study. The quantitative part involved the sampling of hair, blood, and urine from the participants and having these analysed by commercial laboratories. The qualitative part was in the form of a questionnaire completed by the parents at their leisure. To add rigour to the study all women were interviewed after the questionnaire

had been completed to ensure that they understood the questions and had provided as much information as possible concerning their pregnancy. The qualitative method by its very nature was a phenomenological study in that all women were pregnant. Following the birth of the child the mothers were again contacted to determine the outcome of the pregnancy.

Women who were diagnosed with a fetus having a cleft, or who had delivered a child with a cleft, are referred by the major hospitals to CleftPals the volunteer organisation, referred to earlier. CleftPals had previously provided support to the researcher in the Australian study reported in Chapter 7. That study was purely qualitative, using only a questionnaire. CleftPals agreed to a continued involvement with this project by agreeing to promote the need for participants. Controls were sourced from associates of the cleft women, responses to radio presentations, and speaking engagements at organisations such as Rotary. The information sheet provided to potential participants, consent form, and questionnaire are attached in Appendices 11-12.

8.6.1 Sample Size and Testing

As some of the testing required was quite invasive, the expectation of obtaining a large sample of participants was questionable. Cleft research by others where similar testing was undertaken was considered to determine an appropriate sample size.

Work published (Hozyasz, Chelchowska et al. 2004) using 8-hydroxy-2-deoxyguanosine (8-OHdG) as a biomarker for oxidative stress in a CLP study is one of two studies used to support the size of the sample required. That study used 27 CLP mothers with a mean time post-partum of 6.5 months. Fourteen controls were used in the trial. The CLP cases had significantly higher levels of 8-OHdG compared to the controls. The median was 4.7 ng/ml; range 0.5-8.6 ng/ml compared to the median for controls of 1.9 ng/ml; range 0.1-3.2 ng/ml; $p<0.001$. The mean +/-SD for the subject group was 4.9 +/-2.3 ng/ml, while for the controls the mean was 1.7 +/-1.0 ng/ml. Running a power calculation[21] on the subject group with a power set at 80%, and a significance level of 0.05, a sample size of 4 would be required whereas the same calculation on the control group would indicate that the sample size should be 10. As samples were taken post-partum it is envisaged that oxidative stress levels in the CLP cases could have been higher due to the added stress of dealing with the trauma of

[21] The power calculations were carried out with the assistance of a senior researcher from the Monash Medical Research Centre in Melbourne, Victoria.

seeing the child, the associated problems relating to feeding, and inherent surgical procedures for the child.

In a second study (Hozyasz, Ruszczynska et al. 2005) the researchers involved 35 women whose child had a CLP, 30 with a CP and 31 controls. This study compared the serum zinc and copper levels post-partum between the participants and the controls. The mothers with cleft children had lower serum zinc levels ($p=0.012$) and higher copper levels ($p=0.02$) than the controls. The difference in mean copper and zinc levels in CLP mothers was 61 mcg/L with a SD of 76 (Hozyasz, Ruszczynska et al. 2005). Based on the results, the study could have been conducted with a sample size of 26. The study did not consider excretion levels via urine or measure zinc in the hair tissue. Based on the above studies a sample size of 26 was considered to be the maximum required for this study, with the participant number being ideally between four and twenty-six.

Approximately 300,000 children are born each year in Australia. If one in every 700 has a cleft (AIHW 2004), there are approximately 285 cases of children born annually with CLP. Eighty per cent of these, approximately 230, will be non-syndromic. Given that the distribution follows the normal Australian demographics almost 60% of these are in families who reside on the east coast of Australia, approximately 135. Allowing for the fact that many of these live outside the capital cities where there is no support group operating, it is a reasonable assumption that about 50% will be in the major capital cities – approximately 70 cases.

The rigour planned for this study was considered greater than in both of those above (Hozyasz, Chelchowska et al. 2004; Hozyasz, Ruszczynska et al. 2005) due to the fact that sampling occurred during gestation, and not post-partum, when diet and lifestyle may have changed. The nutritional status of the trial participants was assessed using a blood, hair and urine sample of each. These samples were analysed for biomarkers covering inflammation, toxicity, blood sugar dysregulation, and stress. The tests were designed to highlight possible initiators of oxidative stress which may have led to altered gene expression in the fetus (Jiang, Bar et al. 2012).

The tests were complemented by a questionnaire eliciting information on the setting in which the pregnancy occurred. A copy of this questionnaire is attached (See Appendix 12).

The blood and urine analyses were carried out independently by Healthscope (Gribbles Pathology Laboratory), a commercial pathology laboratory. The blood and urine

samples were taken from participating women at a Healthscope collection centre near to their place of residence.

Of all the tests the most controversial was the hair analysis as this is not used in mainstream clinical practice. Samples were provided by the mother following the instructions provided. The analyses were carried out by Trace Elements Inc. in the USA via Interclinical Laboratories in Sydney, Australia.

Due to the fact that hair tissue mineral analysis (HTMA) is not a mainstream procedure a short introduction follows.

8.6.2 The Science – Hair Tissue Mineral Analysis (HTMA)

Minerals and vitamins can be both synergistic and antagonistic to each other and between each other (Watts 2003), and hence the relative levels of each in the system are important. It is known, for example, that for vitamin B6 to be adequately absorbed there must be sufficient zinc available, and that high levels of iron will suppress manganese absorption (Watts 2003). The question in determining these levels continues to be, how can this be done in a way that is non-invasive? Taking a hair sample was seen as a non-invasive method of sampling to obtain a broad-spectrum analysis of minerals. The blood and urine analysis was limited to relatively few elements firstly due to the limitations placed on the researcher by the Gribbles Laboratory and secondly due to the cost. Further, hair analysis provides levels of elements in a tissue over time while a blood test indicates circulating levels and urine excretory levels so all three provide different data. If it could be shown that hair analysis can provide relevant information on the relative nutritional status of an individual then it becomes a very important research tool, and this alone would be a positive outcome for this study.

Each hair on our body grows out of a tiny pocket in the skin called a follicle. A strand of hair has three layers: cuticle, cortex and medulla. The cuticle is the outer covering. It consists of tough overlapping scales that point toward the tip end. The cortex contains pigment granules. These give hair its colour.

Hairs are keratinous filaments that develop from matrix cells of hair follicles. The follicle is embedded in connective tissue of the dermis and extends through the epidermis. Each hair follicle is a miniature organ with smooth muscle, apocrine sweat glands and sebaceous glands, nerves, and a rich plexus of blood vessels (Leeson, Leeson et al. 1985). The hair shaft is also continuously exposed to trace elements

through contact with secretions from sebaceous and sweat glands (Hinners, Tyrell et al. 1974). In nutritional medicine many practitioners rely on HTMA to determine potential mineral deficiencies and/or the presence of heavy metals (Watts 2003).

Researchers (Leung, Huang et al. 1999) measured the concentration levels of hair elements of calcium (Ca), iron (Fe) and zinc (Zn) in pregnant women from Tianjin China, who had been suffering from deficiencies in these elements as judged by blood tests in mid second trimester or early in the third trimester. Of these cases, 82 patients also had their hair mineral levels measured when the blood tests were conducted. They were then supplied with mineral element nutrients of gluconic acidic zinc (noted as Zn-nutrient), gluconic acidic calcium (Ca-nutrient), and/or ferrous sulphate (Fe-nutrient), which corresponded to the deficient element(s), for more than two months. Eighty-four patients returned to hospital for further diagnoses and had their hair element levels measured for the second time. Finally, in the third trimester or near labour, 13 subjects had their hair element levels measured again. Except for the deficiencies of Ca, Fe and/or Zn, these subjects were all healthy without symptoms of any diseases. The concentrations of hair Ca, Fe and Zn were measured by X-ray fluorescence spectrometry (XRF). The concentrations of these three hair elements measured at three different times were statistically analysed. The hair concentrations of Ca, Fe and Zn reflected the effects of supplementation. The Ca levels increased by 20-40%, Fe 12-30% and instead of the Zn level falling by up to 30% in the third trimester it only fell 10%.

This research indicates that a mineral deficiency can be detected using hair analysis and to determine the effect of having subsequently supplemented the patient with the deficient nutrient. Hair analysis has been used extensively in the study of autism. Autism, like clefting, has always been considered a multifactorial disorder. Researchers (Lakshmi Priya and Geetha 2011) studied 45 children with varying levels of autism and considered the role of copper, zinc, magnesium, selenium, lead and cadmium. There was a direct correlation between the level of copper in the hair of children with autism, together with decreased levels of magnesium and selenium. This work has been supported by other researchers into spectrum disorders, also using HTMA (Marlowe, Cossairt et al. 1984; Shearer, Larson et al. 2005; Adams, Holloway et al. 2006). Researchers investigating other human disorders such as neurological diseases and disorders of the musculo-skeletal system have also used hair analysis (Lech 2001; Lech

2002). David, Holloway et al. (2014) used HTMA in a study to relate peri-conceptual drug use with fetal malformations and while the study showed a high level of drug use during pregnancy they could not link gastroschisis to drug use which was a primary objective.

While there is acceptance of HTMA as a tool to determine nutritional status there are those who take an alternate view (Rodrigues, Batista et al. 2008). In this study the researchers attempted to correlate the HTMA results to whole blood and plasma results. They particularly targeted manganese, copper, lead and strontium levels. Their results showed no correlation for copper, manganese and strontium and a weak correlation for lead. They (Rodrigues, Batista et al. 2008) did maintain that a HTMA analysis presents an attractive alternate biomarker if its accuracy can be established.

Wolowiec (2013), in a meta-analysis covering 66 studies into the use of HTMA, concluded that this might be a useful tool for the early diagnoses of many diseases. Namkoong (2013) investigated the reliability of both intra-laboratory and inter-laboratory HTMA results by comparing these with serum mineral analyses. The researchers confirmed that intra-laboratory results were relatively coherent but suggested caution when comparing between laboratories. They questioned the reliability when compared to blood analysis but tended to overlook the fact that they were comparing a tissue analysis with one taken at a single point in time on a circulatory substance. They recommended continuous refinement for determining reference levels.

HTMA is an accepted analytical tool in nutritional medicine and when the analysis is carried out by experienced laboratories it is reproducible (Pizzorna and Murray 2000).

8.6.3 Recruitment

CleftPals advised mothers carrying a child with a CLP of the research and encouraged them to participate. Cleft Pals had previously demonstrated its ability to recruit volunteers with more than 20 women coming forward over a one month period in the previous study. The control group was recruited from mothers diagnosed as carrying a fetus without any anomalies. These were sourced independently from prenatal groups at various locations that made it known that control volunteers were required. While it was attempted to match the control mothers by number of weeks' gestation this did not

prove to be as easy as originally thought even though a larger pool existed from which to make a selection.

For cleft parents this is a traumatic time and some were too upset or busy preparing for the child such that they withdrew after indicating an interest to be involved. This resulted in a much lower number of CLP mother participants while the number of controls was achieved. It was also acknowledged that some mothers had difficulty accessing a collection centre for the blood and urine samples. In actual fact for every four CLP women who initially volunteered, only one continued to completion. Three women advised that their pregnancy had terminated. In the limited time span of this PhD project it was impossible to recruit the desired number of CLP participants, however, this study made a start in an area where until now research has not been attempted.

8.6.3.1 Inclusions

Only women between the ages of 18 and 45 and living in the eastern states of Australia were included in the study. Only the eastern states of Australia were included as a source of volunteers to minimise costs.

8.6.3.2 Exclusions

Women under the age of 18 were excluded as parental consent would have been required and women over 45 were excluded on the basis that complications are more likely to arise during their pregnancy. As the study was directed at non-syndromic clefting only, those mothers whose children were diagnosed post-partum with syndromic clefts were excluded.

8.6.4 Tests and Procedures

Blood sampling was undertaken at the collection centres of Gribbles Pathology (Healthscope), which are located in every major city in Australia. The samples were forwarded to their laboratory and analysed using their normal methods for each test. The tests planned to be undertaken are listed in Table 8.1 below. For financial and other reasons some tests could not be undertaken, an example being the 8-hydroxy d-oxy guanosine, which Gribbles had taken out of its portfolio of tests and other laboratories were too expensive. The blood samples for this study were in addition to, and separate from, any that the mother's practitioner may have ordered.

A sample of hair was provided by the mother herself, using the instructions provided where the researcher or an assistant could not be present for logistical reasons. In such cases she was provided with a prepaid envelope to return the sample to the researcher. The weight of hair required was 0.3 g. This amount was measured on a simple device included with the instructions. The researcher then sent the sample to Interclinical Laboratories in Sydney who are agents for Trace Elements Inc. in the USA who analysed the samples using an ICP-Mass Spectrometer (Sciex Elan 6100 and 9000 models). The complete details of the method used are attached (see Appendix 17).

The bottles for the collection of urine were given to the mothers by the collection centre at the time the blood samples were collected. They then returned them after the 24 hour sample was taken. Healthscope Limited, the parent company of Gribbles Pathology, provided the laboratory analyses free of charge for this work.

Gribbles were not prepared to include the following tests:

- **C-reactive protein (CRP),** a test that is a marker of inflammation.
- **Adrenocorticotrophic Hormone (ACTH)** acts on the adrenal gland to stimulate cortisol synthesis. This test is more difficult to do than measuring the cortisol level and more expensive and so it was agreed to limit the test to just cortisol.
- **Corticotrophin releasing hormone (CRH).** This test was not undertaken as it requires insulin to be injected into the patient and then samples taken. This was logistically not practical.
- **Total antioxidant.** This would have been desirable however it was not a standard test method, and as we were measuring selenium and manganese in blood, urine, and hair it was agreed not to proceed with this.
- **Beta endorphin.** This is a peptide found in the hypothalamus and pituitary. It would have been useful in completing the network of tests associated with stress but it was not a normal test undertaken and was not measured.

Table 8.1 Tests planned to be undertaken

Test	Screening for	Problems if	Information to be gleaned	Sample tested
Full blood count	GH		Health screening (anaemia, immune function)	Whole blood
Multiple biochemical analysis,	GH		Health screening (electrolytes, kidney, and liver function)	Whole blood
Iron studies including ferritin	OS	↑	Excess free iron interferes with absorption of other minerals (esp. manganese) and causes free radical formation. Low ferritin is an indicator for anaemia. High ferritin can be an indicator of inflammation or iron overload as in hemochromatosis.	Serum/ Plasma
CRP, ESR	OS	↑	Inflammatory markers (if high, oxidative stress)	Whole blood
Fasting glucose, insulin	IR	↑	Pre-diabetic or diabetic state	Serum
Lipid studies	IR	↑	TG and LDL are markers of IR	Serum
Homocysteine	ND, OS	↑	Vitamin B12, folate, methylation	Serum
Thyroid function	ND, IR	↑ (TSH) ↓ (T_3, T_4)	Possible deficiencies of iodine, tyrosine, selenium	Serum, urine
Amino acid profile	ND, IR	↓	Insufficient precursors for hormones, neurotransmitters and immunoglobulins	Whole blood
ACTH Cortisol	PES	↑ or ↓ ↑ or ↓	Physiological stress response High cortisol is linked to IR, inflammation and oxidative stress.	Plasma
CRH	PES	↓	Controls level of corticotrophin in pregnancy and may impact on fetal development.	Whole blood
Total antioxidants	OS	↓	Reduced ability to cope with oxidative stress	Whole blood
Vitamin A and E	OS	↓	Reduced ability to cope with oxidative stress	Serum
Vitamin D	ND	↓	Reduced bone development	Serum
8-OHd-oxy guanisine	OS	↓	Specific biomarker for oxidative stress	Urine. Whole blood
Beta endorphin	OS	↓	An element of the stress array tests	Whole blood
Plasma Mn	OS	↓	Deficiency reduces MnSOD with resultant probable cellular damage	Plasma
Urine mineral status	ND	↑ or ↓	Na, K, Ca, Mg, Mn, Cr, Zn, Se, I_2	24 hr urine
Hair mineral analysis	T	↑	Heavy (toxic) metals	Hair

Key: GH, general health; ND, nutritional deficiencies; IR, insulin resistance; PES, physical/emotional stress; OS, oxidative stress; T, toxins

Apart from the tests mentioned above all of the others were completed. The full blood count and biochemical analyses were performed as part of a normal pathology regime. They were included here to provide insight into the total health of the mother, including the state of her liver and kidneys. Both liver and kidneys are important in screening and preparing toxins for excretion, and should they be impaired a toxic build up could damage the developing fetus (Pizzorno and Murray 2000; Mekdeci and Schettler 2006).

Iron is an essential element for many proteins in the body but its most important role is in the haemoglobin that transports oxygen from the lungs to the tissues. Insufficient iron can lead to depleted haemoglobin and smaller red blood cells (Leung, Huang et al. 1999; Seshadri 2001; Haas, Beard et al. 2005; Angeles-Agdeppa, Capanzana et al. 2008). The complete suite of iron tests was undertaken. Vitamins A and E are important antioxidants and should they be deficient oxidative stress could result (Roob, Khoschsorur et al. 2000; Clagett-Dame and Knutson 2011). Vitamin D was measured as this is important in bone development but also needs calcium and boron present to effectively carry out its role (Mahon, Harvey et al. 2010). Vitamin D is also an important anti-inflammatory agent (Abreu, Kantorovich et al. 2004). While this may or may not be important for the soft tissue differentiation it was thought that it may have an impact on palate development if deficient (Mahon, Harvey et al. 2010). Manganese was measured in the hair, blood and urine. Manganese is important in protecting the mitochondria of the cells from oxidation via manganese superoxide dismutase which is a powerful antioxidant (Hwang, Baek et al. 2003). A manganese deficiency may lead to oxidative stress within cells (Fujimura, Morita-Fujimura et al. 1999). As well as measuring the circulating molecules in blood it was important to see what was being excreted, and hence a suite of mineral analyses was established to trace this via the urine. The HTMA which analysed 37 elements was undertaken for much the same reason.

The questionnaire and interview were used to provide information on participant lifestyle factors. These covered family history, diet, and past medical conditions. It also included other maternal health factors such as height, weight, and medications[22] being taken prior to and during pregnancy (see Appendix 12).

[22] Very few of the participants were taking pharmaceutical medications and many respondents classified folate as a medication. Asthma medications were taken by a few respondents. All cases where a drug was mentioned were followed through with the respondent to determine the dose level and when taken. The

8.6.5 Outcome Measures

A key outcome measure from this research was to quantitatively and qualitatively determine whether or not there were significant pre-existing circumstances relating to the pregnancy or whether a differential health status differed between the cases and controls.

This research endeavoured to determine whether a difference in a single factor or a group of factors relating to oxidative stress and/or a specific nutritional element could be established between the cases and controls. If modifiable risk factors were determined, this would provide preliminary data for a larger study which could provide evidence for changes in advice given to women planning pregnancy on modifiable risk factors to reduce the incidence of CLP (as is the case with folate and neural tube defects).

8.6.6 Statistical Analysis

A controlled study was proposed. Holford et al. (1978) present a logistic model to investigate the effect of several variables simultaneously in the analysis while allowing for the matched design. Brookmeyer et al. (1986) propose a modification of this model when several of the pairs have the same matching criteria in which the pairs are combined into strata and conditional maximum likelihood or Mantel-Haenszel estimates with confidence intervals obtained.

Both designs were reviewed in the context of finalising the approach for this project. However, because the sample size was far smaller than desired the non-parametric Mann-Whitney test was used by an independent statistician. If the p value was <0.05 the two groups were deemed to be significantly different. Once a variable was found to be significant an odds ratio was calculated for that specific analyte.

Side effects, if any, were reviewed with the respondent and these and any drug interactions checked against a data sheet for the specific drug.

8.7 Results

The results obtained from this study have been tabulated below:

Table 8.2 Descriptive statistics – Part 1

Item		Control group (n= 27)	Mother carrying a fetus with a cleft (n=6)
Mother's residential location	Victoria	14	1
	New South Wales	6	2
	Queensland	7	3
		Mean ± SD	Mean ± SD
Mother's age at delivery (years)		34.2 ± 5.0	31.2 ± 4.9
Mother's height (cm)		163.9 ± 6.4	161.3 ± 7.5
Mother's weight at conception (kg)		66.4 ± 13.4	57.3 ± 8.8
BMI		24.9 ± 5.7	22.3 ± 1.0
Weeks' gestation at sampling		20.0 ± 5.2	23.8 ± 2.4
Child's birth weight in g		3482 ± 541	3223 ± 536
Sex of child	Male	13	4
	Female	14[23]	2
Position of child in family	First child	9	5
	Second child	13	1
	Third child	5	0
IVF conceptions * (considered to be stressful)		3	1
Traumatic stress at or around conception		1	4
Elevated stress at or around conception		10[24]	1
Mother's personality	Easy going	6	2
	Worries sometimes	17	4
	Worries often	4	0
	Continually worries	0	0
Child's cleft Type	U/CL	0	1
	U/CP	0	0
	U/CLP	0	3
	B/CLP	0	2
Total clefts		0	6
Mothers who had a previous child with a cleft.[25]		8	0

[23] One of the control infants had very serious birth anomalies and died 5 weeks after birth.

[24] One of the control infants had very serious birth anomalies and died 5 weeks after birth.

[25] These women may not be considered 'clean controls'.

* The IVF figures have not been included with the traumatic stress events in the table unless the woman identified it as traumatic.

Table 8.3 Descriptive statistics – Part 2

Item		Control Group (n= 27)	Mother carrying a fetus with a cleft (n=6)
Family socioeconomic status [26]	Poor	1	0
	> Average	8	3
	Comfortable	16	3
	Well off	2	0
Mother smoking	Yes	3	0
	No	24	6
Partner smoking	Yes	4	0
	No	23	6
Mother alcohol during pregnancy	Yes	8	3
	No	19	3
Medical condition	None	20	6
	Mild	7	0
Miscarriage prior to conception	< 3 months	2	1
	3-6 months	1	1
	6-12 months	5	0
	>12 months	3	0
	Never	16	4
Family cleft history	Mother cleft	1	0
	Partner cleft	0	0
	Other family[27]	7	3
Special diet	Vegetarian	3	0
	Low carb.[28]	5	1
	High protein	1	0
	Gluten free	1	0
	Organic	1	0
	None	16	5
Supplements[29] taken	Yes	26	6
	No	1	0
Drugs taken during pregnancy	Yes[30]	6	1
	No	21	5

[26] The assessment of the family's socioeconomic status was merely the parent's perception of their financial position.

[27] In all cases the relationships were remote, e.g. grandfather's second cousin, great grandfather, sister's daughter etc. There were no father-son or mother-daughter relationships.

[28] Low carbohydrate and weight-loss diets were combined as one group.

[29] In some cases the supplements were not commenced until after conception.

[30] Where IVF cases were involved the drugs taken prior to conception have been included here.

Table 8.4 Data summary 3

Sample	Analyte	Control group (n= 27)	Mother carrying a fetus with a cleft (n=6)
Plasma	Cortisol random nmol/L	563.9 ± 120.5[31]	742.6 ± 123.1
Serum	Zinc µmol/L	10.8 ± 1.3	11.9 ± 2.5
Urine	Zinc µmol/L	4.0 ± 3.0	5.0 ± 2.0
Hair	Zinc (HTMA)[32] mg %	16.4 ± 3.1	22.3 ± 7.8

Table 8.5 Data summary 4 – Mann-Whitney mean rank test; independent statistical analysis[33]

Source	Analyte	Control (mean)	Cleft (mean)	p-value
Serum	Bilirubin (µmol/L)	8.7 ± 4.2	5.4 ± 1.5	0.039
Plasma	Cortisol random (nmol/L)	563.9 ± 120.5	742.6 ± 123.1	0.007
Serum	Vitamin E. Total tocopherol (µmol/L)	35.9 ± 7.3	46.0 ±10.9	0.030
Amino Acid Profile	Asparagine (µmol/L)	73.9 ± 39.3	101.0 ± 23.6	0.038
	Citrulline (µmol/L)	15.8 ± 4.9	18.3 ± 4.6	0.365
	Methionine (µmol/L)	17.6 ± 4.4	21.5 ± 3.3	0.043
Hair	Zinc (mg %)[34]	16.4 ± 3.1	22.3 ± 7.8	0.015
	Nickel (mg %)	0.03 ± 0.03	0.06 ± 0.04	0.051

8.8 Discussion

8.8.1 Residential Location

While neither women in the control, nor cleft groups, were spread directly in proportion to the population of each state there is no cluster of samples, and hence there is unlikely to be bias based on residential location. The distance between the most northerly woman and the most southerly one was in the order of 2,000 km. Such a distance reduces the possibility of any common environmental toxin being involved, especially in the sense that the women were from both semirural and city areas.

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[31] The data are represented by the mean ± SD

[32] HTMA – Hair tissue mineral analysis

[33] The statistical analysis was carried out in conjunction with the independent statistician, Shalem Lee, of Adelaide University. The statistics for all other tests where there was no significance are located in Appendix 19.

[34] Mg % is the term for the mg of the analyte per 100 grams of hair.

8.8.2 Maternal Age

The control mothers were on average three years older than the cleft mothers when they gave birth to their last child. This was as expected as only 33% of the control mothers were having their first child while it was 83% for the cleft mothers.

The birth age of both groups of mothers is not inconsistent with that of the general population which was presented in the earlier Australian study. Maternal age, sex ratio of the fetus, and congenital anomaly associations have been studied by other researchers (Csermely, Urban et al. 2014), however, while maternal age may have impacted on some anomalies, the sex ratio of children born to these mothers did not differ greatly from younger mothers.

8.8.3 BMI

As with the maternal age if obesity was an issue the tendency would have been for it to affect the control group rather than the cleft mothers. It must be said, however, that the BMI of both groups was higher than was expected. The range accepted as being the norm for a female is between 18.5 and 24.9 (Gropper, Smith et al. 2005). In this study the average is nearly 25 for the controls and 22 for the cleft mothers. Cameron et al. (2003) show that the prevalence of obesity in the year 2000 was 2.5 times greater than in 1980. The Australian government Health Department (2013) indicates that in 2005 52% of females aged 25 years and over were either overweight or obese with the prevalence of them being obese being 30.2% of the females surveyed in this age group. Parker, Werler et al. (2012) found that obesity coupled with high dietary glycaemic index appears to more likely increase the risk for some birth defects particularly encephalocele, diaphragmatic hernia, small intestinal atresia/stenosis including duodenal atresia/stenosis, and atrial septal defect.

8.8.4 Weeks Gestation at Sampling

The original objective was to have both the control and cleft mothers' samples taken between 12 and 26 weeks into their pregnancy. This was achieved but it did also mean that some who were above 26 weeks were excluded which in hindsight may have been an error of judgement on our part. The aim however was to sample as close as possible to the 12 week point when the lip and palate formation is complete. Our objective was limited by the fact that for many women the first ultrasound scan was at 20 weeks and so both the control and cleft group were sampled at around that time. The range within

the control group is wider which may also be a factor as they represent a larger sample size. A Pearson test undertaken on the data showed no significant relationship between cortisol levels and gestation days (p=0.822).

8.8.5 Child's Birth Weight

Research suggests that where the mother has a high cortisol level during pregnancy this may result in the child being born with a low birth weight (Phillips, Barker et al. 1997; Bolten, Wurmser et al. 2011). The cleft children were on average 200 g lighter than the controls with the standard deviation in both groups being almost equal. It was expected to see a greater variation in birth weight due to the much higher plasma cortisol levels in the cleft mothers, however, this was not the case in this study. A Pearson test was carried out on the data and there was no significant relationship between birth weight and cortisol level (p=0.368). This will be discussed further under cortisol levels. Birth weight has been associated by researchers with the development of disease in adult life, particularly one study known as the Barker Hypothesis (De Boo and Harding 2006). De Boo and Harding (2006) indicate that birth weight is a very crude measure of fetal growth and that changes in intrauterine environment may not necessarily result in altered birth weight or indeed long term cardiac disease as proposed by Barker. They contend that the altered risk of adult disease may more likely be linked with maternal nutrition status around conception.

8.8.6 Sex of the Child

While this sample was extremely small the higher proportion of male children born with a cleft is consistent with our earlier studies and also with the results of other researchers (Bahado-Singh, Schenone et al. 2011) and many others referred to earlier in this document. By comparison the control group clearly illustrates the difference where the balance between male and female is almost identical.

8.8.7 Position of Child in the Family

Only one of the cleft children was the second child whereas in the control group 33% were the first child.

8.8.8 Stress

Earlier in this thesis stress was discussed in some detail and it was related to clefting. In this study stress also appears to be an important factor in the cleft group but it is not

completely exempt in the control group. Indeed 11% of the control group were IVF conceptions and we have classified those as elevated stress well knowing that to even arrive at the decision to have IVF is a stressful event in the mother's life. In the control group, however, only one mother indicated that at, or near conception, she experienced traumatic stress. In her case the first child suffered from Aicardi Syndrome and the mother had problems dealing with this, and prior to becoming pregnant again, had a nervous breakdown. In the cleft group 66% of the participants experienced traumatic stress. This is also consistent with our previous study. In the first cleft mother's case her grandfather died just prior to her conceiving, and she also moved to a different town. Another mother was under extreme work stress and had to resign her position while at the same time was buying a new house. The third cleft mother was under extreme work stress, was being bullied and finally had to resign from the company where she was employed. The fourth cleft mother bought a new house, moved and started renovating it and found that extremely stressful.

Stress in relation to adverse pregnancy outcomes continues to be investigated by researchers (Carmichael and Shaw 2000; Culhane, Rauh et al. 2001; Toescu, Nuttall et al. 2002; Benitzhak and Verny 2004; Moretti, Phillips et al. 2004; Carmichael, Shaw et al. 2007; Hibino, Takaki et al. 2009; Mostowska, Hozyasz et al. 2011) with the majority arriving at the conclusion that stress minimisation should be a priority when considering pregnancy. Krabbendam et al. (2005), however, concluded that in a well educated population there is no relationship between stress and adverse pregnancy outcomes.

8.8.9 Cleft Type

The type of cleft in this study is generally consistent with past results, with a unilateral cleft lip and palate being dominant. The sample is, however, too small to be sure that such a spread is the norm.

In the control group eight of the mothers had previously had a child with a cleft and can not be considered as clean controls. The reason that we had so many among the controls was that much of our advertising for participants was done through the CleftPals website. Because of this, women who had previously had a child with a cleft, and who were still associated with CleftPals wanted to be involved to further understand why their previous child had been born with a cleft. While it would be assumed that their anxiety levels may be higher in approaching a second pregnancy they all had made lifestyle and nutritional changes prior to conceiving which the researcher believes

overcame to some extent any apprehension they may have had. This is dealt with in much more detail in the following chapter.

8.8.10 Socioeconomic Status of the Family

In our earlier Philippine study the underlying comment from many of the surgeons and bureaucrats interviewed was that clefting was a problem of the poor. In this study we attempted to determine the socioeconomic status of each family.[35] Only one of the families claimed that they were poor, and they were in the control group. All of the cleft group were of average to above average income. Certainly being comfortable does not mean that you always eat the right food or follow a healthy lifestyle, but it does mean that good nutrition can be afforded. It then becomes a matter of choice. Yang, Carmichael et al. (2008) studied the relationship between socioeconomic status (SES) and birth defects in the USA and found that while there were increased risks of birth defects where low SES existed there was no association between individual SES indicators and cleft lip (with or without cleft palate).

8.8.11 Smoking and Alcohol Consumption

None of the cleft mothers admitted to smoking during pregnancy, nor had said that they smoked prior to conception. Some of the control mothers did smoke but even with these women the toxins normally associated with smoking were not prevalent in the hair tissue mineral analyses. This probably confirmed that their smoking habits may have been more social than addictive. Half of the cleft mothers and 30% of the control mothers had some alcohol during their pregnancy. All claimed that their intake was minimal. As guilt may be associated with both smoking and alcohol consumption the fact that some participants may not have disclosed their use of these substances could unknowingly have presented a bias to this result.

8.8.12 Miscarriage Prior to Pregnancy

The World Health Organization (WHO 2006) recommends that there be a rest period of six months after a miscarriage before becoming pregnant again. In this study three women in the control group and two in the cleft group had conceived within the six month period after their miscarriage. Based on this study we cannot therefore claim that a birth anomaly will occur if a woman becomes pregnant quickly after a miscarriage but can continue to suggest restraint to minimise the possibility.

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[35] The socioeconomic status was that perceived by the families and not determined by the researcher.

8.8.13 Family Cleft History

Only one of the mothers had had a cleft as a child and she was in the control group. Several mothers indicated that other family members had had a cleft but in the main these were two generations back or family members quite remote from the mother. One of the cleft mothers had an older sister with Pierre Robin syndrome. None of the fathers had had a cleft as a child. If there was to be a family trait it should have been evident both in the control and cleft group. This was not present.

8.8.14 Special Diets

Apart from the three vegetarians in the control group, all of the other special diets related to weight loss, with mothers either claiming high protein or low carbohydrate. As with most people who go on special diets to lose weight it is not generally something that occurs over a long period, and the term of these diets was not measured. In the case of the vegetarians there may have been more commitment to their regime as often that is more related to a belief rather than personal appearance. Where a vegetarian diet is related to belief rather than science, protein deficiency may result. Losing weight during pregnancy is not recommended. The IOM guidelines (Lu 2013) set out the desirable weight gain during pregnancy for each BMI grouping.

8.8.15 Supplements Taken

Out of all of the participants only one indicated that she had not taken any supplements. This woman was one of the controls and her baby had significant birth anomalies and died five weeks after birth. Her case will be discussed later both in this chapter and the following one. It is not suggested that the lack of supplementation was the cause of this anomaly as the anomalies in this case were extremely complex.

The problem in trying to assess whether the supplements had any benefit, or conversely did not assist the developing fetus, was that the amount, type taken, and when taken, were so poorly described that no reliance could be placed on the data.

Folate was obviously a universal supplement that had been taken except for the one woman who had not taken any supplements, but dosage and timing were difficult to assess. Some did not start taking supplements until after they knew they were pregnant while others had been taking them prior to conception.

8.8.16 Drugs Taken During Gestation

Few women took pharmacological drugs during pregnancy, but those who did took them for minor ailments and none of the drugs were of a type likely to damage the fetus. Other than cigarettes and alcohol no other social drugs were reported as being consumed prior to, or during the pregnancies.

8.8.17 Cortisol

Kalra et al. (2007) used hair cortisol levels to determine stress levels in healthy pregnant women and because a hair sample represents a period of time, rather than a period in time, this may be a better method of analysis for determining whether a person has been stressed. This measurement had not been planned and could not be done for financial reasons.

We are, however, not the first researchers to consider the relationship between pregnancy and cortisol (Phillips, Barker et al. 1997; Kalra, Einarson et al. 2007; Evans, Myers et al. 2008; Giurgescu 2009; Bolten, Wurmser et al. 2011; Entringer, Buss et al. 2011) however, most of the research has related elevated cortisol levels to pregnancy parameters other than CLP. Ingstrup, Liang et al (2013) while not taking cortisol levels in the participants did link CLP with the death of the mother's close relative implying that elevated stress (and hence elevated cortisol) was involved. Evans, Myers et al. (2008) investigated the link between anxiety, depression and cortisol during pregnancy by taking saliva samples at 36 weeks' gestation. Their conclusion was that comorbid anxiety and depression, but not depression or anxiety alone, were associated with elevated cortisol levels. Jung, Ho et al. (2011) conducted a prospective longitudinal study on morning plasma cortisol (total and free), corticosteroid-binding globulin (CBG) and 24-hour urinary free cortisol (UFC) levels in 20 pregnant women during the first, second and third trimesters as well as 2-3 months postpartum. They found a progressive rise in total plasma cortisol, CBG which peaked during the third trimester. The 24-hour UFC and plasma free cortisol levels did not vary greatly from the controls during this period. Sandman, Glynn et al (2006) endeavoured to determine when placental corticotropin releasing hormone (CRH) levels were most responsive to cortisol. They took samples at both 15 and 31 weeks' gestation and found that every unit (µg/dl) change in cortisol at 15 weeks was associated with a 34 unit change in CRH at 31 weeks. This suggests that early detection of stress signals by the placenta which stimulated the subsequent release of CRH increased the risk of preterm delivery.

From the very outset of this study the researcher was surprised to see that the majority of the controls had elevated morning levels of cortisol. The standard reference range accepted by the commercial laboratory used is 120-629 nmol/L (no consideration appears to be given by the laboratory regarding whether the woman is pregnant or not). A simple statistical analysis of the results showed that the controls had a mean plasma cortisol of 563.92 ± 120.49 nmol/L SD, and the cleft mothers a mean of 742.6 ± 123.12 nmol/L SD. The independent statistician provided the same means and a p value of 0.007. Statistically this is extremely significant but what does it really mean? Is stress the sole cause of these elevated values or is there some other mechanism at play?

Cortisol exerts its effects by mainly binding to two types of cytoplasmic receptors namely mineralcorticoid and glucocorticoid (Kalra, Einarson et al. 2007). This study did not consider, nor does the research of others, a proposition that during pregnancy cortisol binding to the receptors, or to plasma proteins such as transcortin, ficolin and hucolin (Sandberg and Slaunwhite 1959; Murray 1967; Werthamer, Samuels et al. 1972; Barton and Passingham 1981; Edgar 1995) may increase, and hence cause an elevation in the results when the woman is tested. Pregnancy alone generates its own stress related response within the body (Allolio, Hoffman et al. 2008). The level of cortisol varies during pregnancy and is dependent on the time of day and number of gestation days (a circadian rhythm). Allolio, Hoffman et al. (2008) studied 10 pregnant women and followed the pattern of their salivary cortisol throughout their pregnancy. They concluded that the elevated salivary cortisol may be explained by glucocorticoid resistance owing to the high anti-glucocorticoid action of high progesterone concentrations.

What can be concluded from the fact that the control women had cortisol levels at the high end of the reference range is that pathology laboratories should perhaps alter the reference range for women who are pregnant if a larger study supports these findings. Likewise medical practitioners should be aware that high cortisol levels in a pregnant woman may not mean that problems are to be encountered. It is perhaps not unexpected that the cleft group had levels well above the reference range. The blood samples were taken soon after these women were made aware of the fact that their child would be born with a cleft and would need almost immediate surgery after birth. It would be an abnormal situation for a woman not to be very concerned and anxious about the child's

future. It is believed that the difference between the control mean and the cleft mean may reflect this level of concern.

All of the above presents a very simple explanation of what may have occurred in both sets of results. Is there perhaps something much more complicated taking place? Cortisol levels have been studied by researchers involved with IVF procedures (Keay, Harlow et al. 2002) as they observed that there was a detectable conversion of cortisol to cortisone following prolonged in-vitro culture of granulosa-lutein cells and that this related strongly to low IVF pregnancy rates (Thomas, Thomas et al. 1998). If pregnancy rates are lowered when cortisol is converted to cortisone, then perhaps inducing a higher cortisol level would indeed aid in the conception process. Nature perhaps ensures that this occurs by ensuring that the woman has differing cortisol levels during her regular cycles.

Cortisol (or hydrocortisone) is the most important human glucocorticoid. It regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions. Synthetic glucocorticoids are available as drugs and these are used either as replacement therapy in glucocorticoid deficiency or to suppress the immune system. Earlier in this thesis (Chapter 1) we referred to these drugs which had been identified as having a relationship to clefting (Kallen 2003). In that situation mothers were being given the glucocorticoids, most probably as anti-inflammatories, especially those who suffered from asthma. The researcher hypothesised above that the increase in cortisol level in the cleft group was due to the increased stress caused by them now knowing that their child would have a cleft, but if the cortisol level had been high at or around conception (not measured in this study) is there a critical point where the cortisol levels increase above a desirable level thus preventing the pregnancy to continue normally? High cortisol levels in mothers have been blamed for other pregnancy issues but we were unable to confirm previous research (Giurgescu 2009; Bolten, Wurmser et al. 2011) as we could not repeat their outcomes.

As mentioned above, research suggested that high cortisol levels during gestation were a predictor of the birth weight of the child. It was proposed that the higher the cortisol level the lower the birth weight (Bolten, Wurmser et al. 2011). Cortisol levels explained 19.8% of the variance in birth weight and 9% in body length. We did not measure the body length and therefore cannot comment on that aspect. In all cases we contacted the mother after birth to get details of the birth including birth weight. Giurgescu (2009)

suggested that a high cortisol level would result in a preterm birth. Our results could not confirm that hypothesis.

The results of this study suggest that caution should be taken in making direct claims based on the level of random cortisol in plasma. The time of sampling during the day is important as is the gestation time. Clearly the pathology laboratories should reassess the reference ranges for pregnant women. Practitioners may consider undertaking hair cortisol levels or more frequent sampling for plasma cortisol if they suspect a woman is seriously distressed. Practitioners should ensure that women of child bearing age be made aware of the dangers of glucocorticoid and corticosteroid drugs if they are considering pregnancy. It is important that practitioners should encourage women to find lifestyle pursuits that are relaxing if they are considering pregnancy within the next twelve month period as well as considering their nutritional status.

8.8.18 Bilirubin

The data indicates that the cleft mothers had lower bilirubin levels than the control group ($p<0.039$). Bilirubin is a naturally occurring antioxidant and as such could have a role in protecting lipids and lipoproteins against oxidation (Stocker, Yamamota et al. 1987; Stringer, Gorog et al. 1989). Bilirubin is also a waste product following red blood cell degradation causing jaundice if not cleared from the blood by the kidneys and if high levels persist such a condition must be treated to prevent brain damage (Pimstone, Engel et al. 1971; Gropper, Smith et al. 2005).

The results suggest that the cleft women may have had less antioxidant capacity than the controls. Most of the papers reviewed regarding bilirubin levels in mothers of new born children, following this finding, related to the development of kernicterus (Harris, Lucey et al. 1958; Cashore, Horwich et al. 1977). Kernicterus is a condition where there are extremely high levels of bilirubin present.

8.8.19 Serum Vitamin E, Tocopherol

The fact that the serum vitamin E level in the cleft group was significantly higher than the controls ($p=0.03$) was surprising. Both groups were within the normal range established by the pathology laboratory but the cleft group was on the upper limit. Vitamin E is an antioxidant and one would expect that this may have benefitted the cleft group. This result appears at odds with the bilirubin levels, discussed above, which

suggests that the cleft group had lower antioxidant levels. There appears to be no simple explanation for this.

Research regarding the role of vitamin E in clefting is extremely limited and there is no conclusive evidence that vitamin E is either protective of, or a promoter of clefting.

Howe et al. (2005) attempted to see if supplementation with vitamin E would inhibit the formation of a cleft in rats which had been administered phenytoin to induce clefts. This experiment used a 'cocktail' of antioxidants including vitamin C, E and co-enzyme Q10 and these were administered both before and during the pregnancy. The experiment failed to indicate a positive effect of the antioxidant in protecting the rats against giving birth to offspring with clefts.

Hozyasz et al. (2006) took post-partum blood samples from women whose child had a cleft (31 mothers whose child had a CL and 29 who had a CLP). There were no control groups. The researchers found that the vitamin E level in the CL group was lower when compared to the CLP mothers. Their conclusion was that the risk factors for each group may be different but that the positive role of vitamin E as protective agent against clefting could not be established.

As with Hozyasz it cannot be concluded from this pilot study that vitamin E played any role positively or adversely in clefting. In a further study (Hozyasz and Chelchowska 2004) the researchers concluded that they believed the ratio of alpha-tocopherol to total cholesterol is more useful as a measure of vitamin status than the alpha-tocopherol level alone.

8.8.20 Amino Acid Profile

Of all the amino acids analysed in this study asparagine, citrulline, and methionine were the ones significantly higher in the cleft group. The asparagine and methionine results for both the control and cleft groups were within the reference range established by the pathology laboratory. The citrulline levels for both groups were below the lower limit.

The citrulline levels reported above are included because Hozyasz (2010) in his review of the potential risk factors in clefting hypothesised that mothers who gave birth to cleft children would have low citrulline levels. This study indicated that all pregnant women tend to have low citrulline levels and that the level is not lower in the cleft group. Citrulline is the product of the oxidation of arginine and is considered a biomarker for intestinal functionality. In this study arginine was also higher in the cleft group but was

not significant when compared with the controls. The arginine levels of both groups were below the lower figure of the laboratory's reference range. No evidence can be found linking either asparagine or methionine to clefting. It is clear that the cleft mothers had higher levels in both cases, however, all mothers were well within the reference range of the pathology laboratory conducting the analyses.

8.8.21 Nickel

Nickel is not known to play any important role in supporting human health. The fact that nickel was borderline significant in the HTMA may be the result of exposure to nickel plated objects and the absorption either orally or via the skin. Nickel is known to cause skin rashes and is often involved in dermatitis (Schnuch, Wolter et al. 2011). Of a more serious nature, nickel has been associated with respiratory cancer (Goodman, Prueitt et al. 2011). The researchers hypothesise that exposure to any nickel-containing substance increases the risk of developing cancer.

There has been no research done linking nickel to clefting but it could be proposed that nickel because of its toxicity may have an impact on the developing fetus.

8.8.22 Zinc

The hair zinc result with p value of 0.015 indicates that the cleft group was significantly different to the control group. A regression analysis found that the risk of cleft for a patient with zinc level of 22.3 (average zinc level in cleft) is 7.9 times higher than a patient with zinc level of 16.35 (average zinc level in controls). Also for every one unit increase in zinc level, it is expected that there will be approximately a 35% increase in the odds of a child having a cleft.

While considering this odds ratio and departing briefly from the subject of zinc to explore the hair analysis of the control whose baby died soon after birth, the hair analysis of that mother showed that many minerals were above the normal range established by the laboratory but specifically the zinc value was 72. This is 3.2 times greater than the cleft average and based on the odds ratio of 1:1.35 there would be an added 66% chance of a cleft or other birth defect. The child had a hole in the heart, only one kidney, and a deletion in chromosome 14. While not being designated as a specific syndrome the combination of anomalies was of a syndromic nature. What the cause was in this case is debateable as the high zinc may have been purely a consequence of the syndrome rather than being causal.

The fact that zinc was so significant in this study was not expected. It was hypothesised that in mothers with CLP children, manganese or selenium, two antioxidants, would be deficient. Such was not the case. We had postulated earlier in this thesis that a zinc deficiency may be involved with clefting and so the high result posed new questions. The early analysis of the statistics showed that the serum zinc in the case mothers was similar to the controls. It was also evident that a significant amount more was being excreted via the urine in the cleft group than in the control. Likewise zinc may have been directed into the hair tissue and perhaps other tissues, not tested, rather than being utilised by the mother and fetus. It is not known whether the zinc was bio-unavailable, suppressed by other mineral antagonism (Watts 2003) or whether the response of the mother's body was such that the zinc was not utilised or that there was insufficient metallothionein to bind it to the liver.

Zinc was first shown to be an essential element for rats and mice in the 1930's, for pigs in 1995 and for humans in 1963 (King 2011). It is a catalyst for more than 50 enzymes and is also involved in maintaining the structure of enzymes such as copper/zinc superoxide dismutase. Zinc is also involved in the regulation of gene expression via a family of metal-binding transcription factors (Cousins 2006). The transcription factors are thought to regulate various genes both positively or negatively depending on the cellular zinc status.

Zinc is a type 2 nutrient which means that it is available for a number of metabolic functions. When an animal or human has a zinc deficiency and the normal balance cannot be restored, additional metabolic adjustments occur which may result in tissue catabolism to restore this balance (King 2011). The researcher indicates that the zinc pool within the body is extremely small and if not replenished clinical symptoms quickly appear and in children can lead to growth retardation.

There have been numerous zinc studies undertaken with animals; one of the earliest (Henkin 1974) reported a direct relationship between elevated plasma cortisol and the urinary loss of copper and zinc in cats and humans. Nockels, DeBonis et al. (1993) demonstrated that when calves were placed under stress there were significant changes in their zinc and copper balances. Researchers at the Linus Pauling Institute at Oregon State University (USA) (Song, Leonard et al. 2009) proposed that low cellular zinc may impair the binding activity of p53, an important zinc containing transcription factor. They also postulated that a reduction in zinc may lower the defence system against

oxidative DNA damage. Their research showed that the expression of OGGI, a DNA repair protein, is dramatically increased by marginal and severe zinc depletion, indicating increased oxidative DNA damage with zinc deficiency.

A study where zinc deficiency was induced in two species of monkeys (Swenerton and Hurley 1980) showed that reproduction was impaired when the zinc deficient diet was fed to the adult monkeys. One monkey aborted on day 48 of the pregnancy. When the fetuses were removed from the monkeys none showed any major congenital abnormalities. Normal reproductive activity returned when the zinc was increased in their diet. By contrast researchers (Apgar and Fitzgerald 1985) studying the effect of low zinc intake in lambs (n=15 plus controls) throughout their pregnancy (Apgar and Fitzgerald 1985) found that in the zinc depleted lambs three aborted, two delivered malformed offspring, one delivered mummified twins on birth, and only three ewes delivered lambs vigorous enough to be put on an artificial feeder but none survived. In an earlier study Apgar (1971) induced a zinc deficiency in rabbits (n=8 plus controls) and of those with a depleted zinc intake only four were able to maintain their pregnancy and those that did had smaller fetuses that did not survive.

The zinc status in humans (a high zinc level in the hair, irrespective of serum levels, can indicate that the zinc is being excreted rather than utilised, which in turn could indicate reduced availability to the developing fetus (Watts 2003)) has also been studied quite vigorously even though it was only first proposed in 1939 (Eggleton 1939) that a zinc deficiency may be involved in human deficiency syndromes.

Bergmann et al. (1980) took samples of hair from women who had given birth to children with spina bifida (n=17 control n=30) and the zinc content was measured. The hair was cut into one centimetre sections so that a plot of the zinc level could be estimated across pregnancy. The results showed that the mothers whose children were born with anomalies consistently had higher hair zinc levels than the controls. The results from that study were graphed and have been included here for two reasons. Firstly, early CLP researchers considered folate as being a likely deficiency candidate in clefting but also suggested that zinc would be important in protecting against NTD. Secondly if zinc is important as described by Bergmann et al. (1980) is it possible that the same two nutrients are required to prevent CLP? Both are definitely required in the one carbon metabolism (folate pathway). Certainly in our study we also found (albeit on a small sample) a similar pattern. In the results graphed below the differential in hair

zinc has increased from10 µg/g to 21µg/g in the first 12 weeks of gestation this being the period during which the clefts are formed.

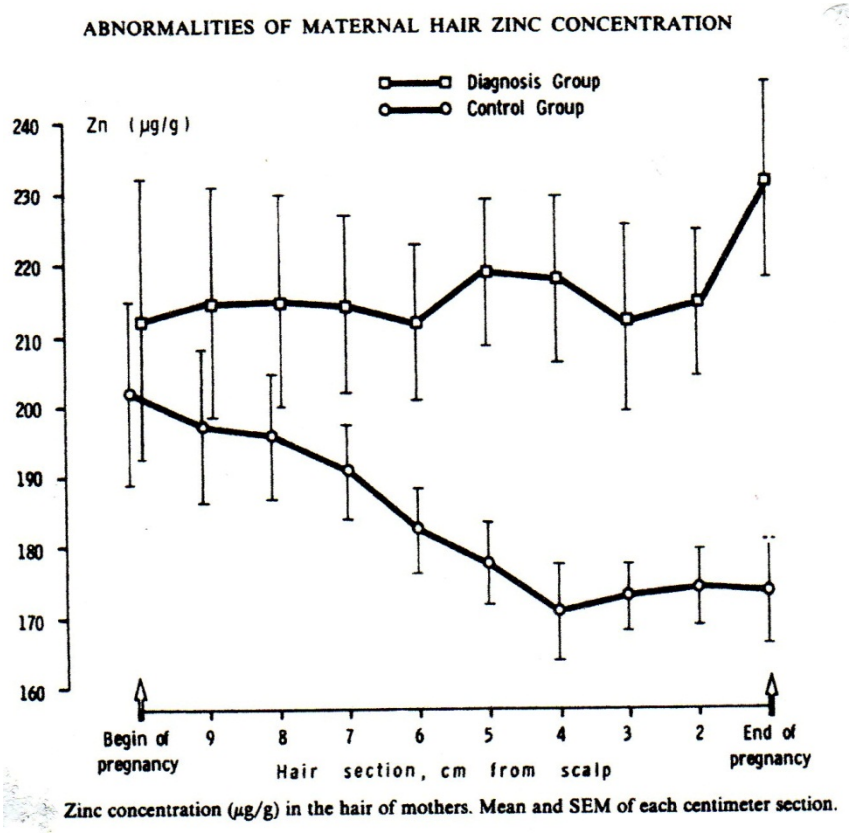


Figure 8.1 Hair analysis of mothers whose child was born with spina bifida compared to mothers whose child had no anomalies.

Source: (Bergmann, Makosch et al. 1980) Permission granted by the publisher to reproduce this figure given that a link to the article is stated - http://ajcn.nutrition.org/content/33/10.toc

In all the studies undertaken we have found that males predominate in clefting. It was proposed earlier in this thesis that it may be that the X chromosome is affected in some way given that males only have one X chromosome and are possibly more vulnerable. Other researchers (Gibson, Smit Vanderkooy et al. 1989) who were investigating zinc deficiency and its relationship to low height percentiles in boys in Southern Ontario selected males for their study because they believed that males were clinically more sensitive to zinc, this perhaps being because of a higher requirement for zinc in males. Favier (1992) showed that zinc was important in reproduction and suggested that zinc deficiency during pregnancy may cause spontaneous abortion, pregnancy-related toxaemia, extended pregnancy or prematurity, malformations, and retarded growth. The researcher also indicated that zinc supplementation had proven beneficial in male sterility and reducing complications during pregnancy. Other researchers (Singh Rathi,

Scrinivas et al. 1999) suggested that a deficiency in zinc had profound effects on rapidly proliferating tissues such as embryo, gonads, skin, and bone marrow. They also indicated that foods that have high fibre and phytate content such as cereals inhibit zinc absorption by the gut. Seshadri (2001) in a review of published research on micronutrient deficiencies in South East Asia concluded that there was evidence to suggest that zinc deficiency is likely to be widespread.

A case controlled study (Krapels, Rooij et al. 2004) investigating the risk factors for CLP considered myo-inositol, glucose and zinc. They found that while glucose was not a risk factor, low levels of red blood cell zinc and myo-inositol were important in the aetiology of CLP. A Filipino study (Tamura, Munger et al. 2005) took plasma samples from 74 mothers with a cleft child and measured the zinc levels compared to 283 controls whose children did not have a cleft. The researchers found that the mothers of the cleft children had lower plasma zinc levels than the controls. While this study supports the findings of our study to some extent, the concern with this research is that the samples were taken up to five years after the child with the cleft was born. Munger et al. (2009), however, in repeating the work that they had carried out in the Philippines in Utah in the USA could not find an association between low plasma zinc levels in women who had given birth to a cleft child. They concluded that poor maternal zinc levels may only become a risk factor when the zinc status is highly compromised.

Uriu-Adams (2010) in their review of zinc and reproduction suggested that women with low zinc levels would have an increased sensitivity to teratogenic insults that could trigger birth defects during pregnancy. From a larger perspective they proposed that women who have poor diets may have elevated risks of pregnancy complications. In their paper they showed the various pathways where a zinc deficiency may have an impact. This has been reproduced below in Figure 8.3 with the permission of the author Dr Carl Keen of the Department of Nutrition, University of California, Davis, California.

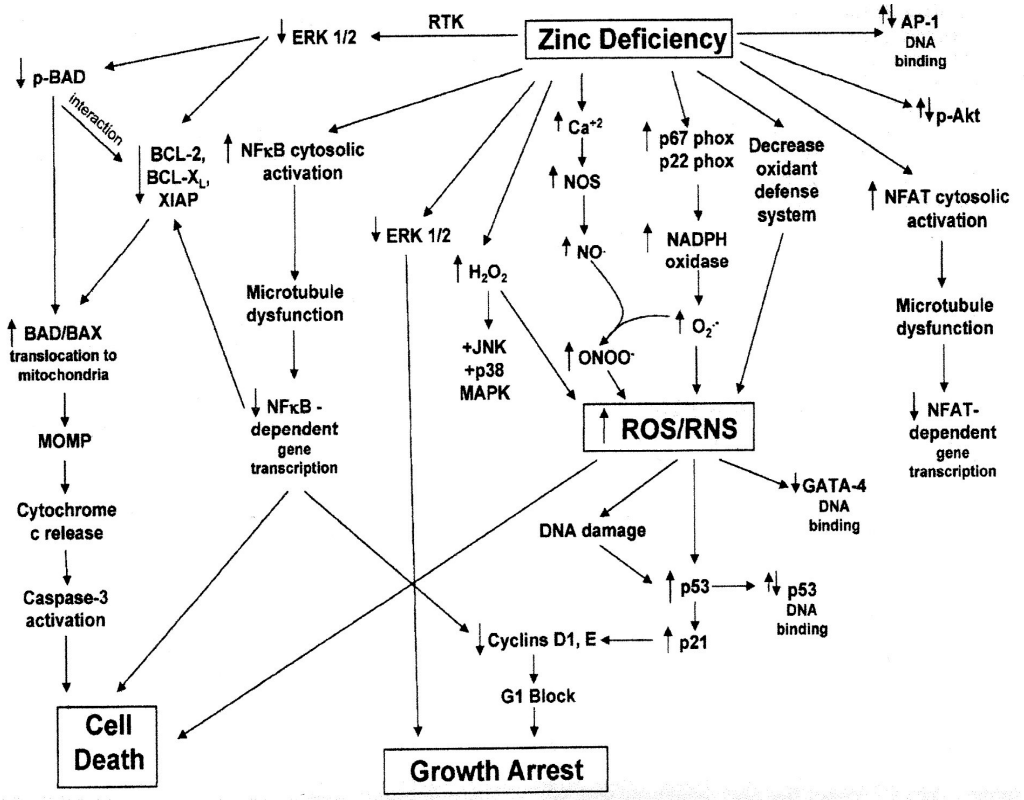


Fig. 2. Conceptal Zn deficiency: multiple signaling pathways are affected that can alter the processes of cell proliferation, cell survival, and cell death. Depending on the cell type, zinc deficiency can increase or decrease some factors, e.g., p-AKT expression and AP-1 DNA binding. The length of time under which cells are exposed to zinc-deficient conditions can also increase or decrease the expression of p53-dependent downstream targets. Note, only select examples of cellular functions reported to be influenced by a deficit of zinc are illustrated. AP-1, activator protein-1; BAD, Bcl-2-associated death promoter protein; BAX, Bcl-2-associated X protein; BCL-2, B-cell lymphoma 2; BCL-X_L, B-cell lymphoma-extra large; Ca^{+2}, calcium; ERK1/2, extracellular signal-regulated kinase 1/2; H_2O_2, hydrogen peroxide; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MOMP, mitochondrial outer membrane permeabilization; NF-κB, nuclear factor-kappa B; NFAT, nuclear factor of activated T cells; NO, nitric oxide; NOS, nitric oxide synthase; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; RNS, reactive nitrogen species; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; p-AKT, phospho-AKT; p-BAD, phosphor-BAD; XIAP, X-linked inhibitor of apoptosis.

Figure 8.2 Zinc deficiency pathways

Source: reproduced with the express permission of Dr Carl Keen, Professor of Nutrition and Internal Medicine, University of California, Davis

Having established that zinc deficiency impacts negatively during pregnancy is there also a link to the high cortisol levels that were also found in women with CLP children? In an animal trial, Watanabe, Tamano et al. (2009) found that the basal levels of serum corticosterone are elevated in zinc deficiency, which may be linked to abnormal neurological behavior. If in fact the two are related this would tend to support information in a very early work (Greene and Kochar 1975) where it was suggested that adrenal glucocorticoids could pass through the placenta (if there is an11-beta hydroxysteroid dehydrogenase (11 beta HSD) deficiency), reach the fetus and affect fetal development at critical times during the pregnancy. The researchers hypothesised that palatal fusion could be impaired. In their review they did not link cortisol levels directly to a zinc deficiency as that was not a part of the review.

Two recent meta-analyses question the effectiveness of zinc supplementation to improve pregnancy outcomes (Chaffee and King 2012; Donangelo and King 2012). Of 20 of the trials considered in one of the two analyses, 15 were where zinc was involved with other micronutrients while five were placebo-controlled trials using zinc alone. The conclusion was there was no evidence that supplemental zinc affected any parameter of fetal growth including risk of low birth weight, length at birth or head circumference at birth. The evidence of preterm birth involving maternal infection was graded low. The researchers in both meta-analyses suggested that the overall public health benefit of zinc supplementation appeared to be limited.

8.9 Conclusion

This study is to our knowledge the only one that has tried to determine nutritional status of the mother while the fetus was still developing. Other researchers have used different time periods for their investigations and therefore are not directly comparable to this study (Leung, Huang et al. 1999).

This study proposes that clefting is related to both a high cortisol level in the mother during pregnancy, and/or a zinc deficiency as defined by elevated levels of zinc in the mother's hair tissue. The subsequent literature review carried out tends to support these findings. While the sample size was small the results were significant, and this demands that further work be done in this area.

It is of interest that pathology laboratories do not have different reference ranges for items such as cortisol when a woman is being tested who is pregnant. Certainly work should be undertaken to ensure that tests undertaken for any person, male or female are appropriate and take into account any conditions that may exist at the time.

This was the first time that CLP research had been undertaken while the woman was still pregnant and it is the most comprehensive, in that blood, hair, and urine samples were taken. The results, however, tend to indicate that if blood and urine testing are used in isolation they should be repeated several times as they only indicate the status at one point in time and therefore a single result cannot be generalised. A much larger prospective study is needed with early gestational sampling of hair, urine and blood before any conclusions can be made. This pilot study can only be used as suggestive of further work to be undertaken.

For nearly 50 years researchers have been using HTMA to study reproduction in animals (Neseni and Koriath 1967; Kuhlman and Rompala 1998). The research leads us to suggest that HTMA is a legitimate tool to be used to determine the nutritional status of a woman who wishes to conceive, particularly if supplementation is required so that it can be provided well prior to conception to ensure a successful pregnancy.

This study also confirms the earlier work carried out (reported in Chapter 7) which suggested that elevated stress at or near conception in the mother is a component in clefting.

CLP is one of the most common congenital malformations, and reducing the incidence of this disorder would be of great benefit to many families across the world.

8.10 Strengths and Limitations

This study presented many challenges, the first being the funding of the laboratory work to be undertaken. After lengthy delays with many companies and government departments being contacted, Healthscope Ltd., a public health care company, agreed to provide blood and urine analysis free of charge. For that we are extremely grateful.

It was impossible, however, to obtain funding for the oxidative stress analysis (8OHdOG). A company that had been providing this test was purchased by Healthscope Ltd., which then discontinued that service. Other companies were contacted, as well as the University of Newcastle, but the costs were prohibitive, and further funding was unavailable. It was therefore impossible to undertake that urine test.

Initially a second hair sample was analysed by Alternate Health Sciences but during the course of the study the company changed the equipment used for the analysis and it was believed that consistency of results could not be guaranteed. This then meant that all the hair samples were analysed by Trace Elements Inc. in the USA, via Interclinical Laboratories in Sydney. These analyses were part funded by Interclinical Laboratories and the researcher.

From the outset the difficulty in recruiting participants was underestimated resulting in a small sample size. There was resistance from women to providing a hair sample, however, this could have been just an easy way out of saying no or that the women would have preferred to give blood and urine rather than hair. The bigger issue was the difficulty of women visiting a collection centre as some lived more than 50 kilometres from the site. Many had extremely bad morning sickness, and as the samples had to be

taken after fasting this proved difficult for them. Finally, some who initially volunteered indicated that the pregnancy had terminated soon after volunteering. While never stated, the fact that the size of the package of information sent to each potential participant was extremely large could also have had a negative impact.

8.11 Recommendations for Future Research

This study was not large enough to provide statistical power and hence larger studies are required. Obviously the problems associated with recruiting participants need to be overcome, this being difficult when the issue contains so many differing emotional responses of the women involved. There are however two courses of action that could be pursued. The first is to attempt in some way to determine if the work done by Bergmann (1980) using HTMA post-partum also applies to clefting and the second to repeat the work contained in this study in an area where CLP is more common, perhaps the Philippines, where the women may be more inclined to volunteer.

8.12 Summary

This work, while only on a small scale, opens the door for other researchers to pursue similar enquiries, and in due time, clefting may be reduced simply by ensuring that a healthy lifestyle and diet prior to pregnancy is followed, or that certain supplements may be recommended to prevent this anomaly, similar to advising the intake of folic acid to minimise the risk for spina bifida. Although this study is too small to make definitive predictions, it is hoped that the results obtained will lead to further enquiry.

Chapter 9 Is Having a Child With a Cleft the End of the Road?

9.1 Research Question

Can a woman who has previously had a child with a CLP confidently proceed with another pregnancy expecting that the child will have no birth anomalies?

9.2 Introduction

The research question discussed in Chapter 8 was: Is there a difference in the nutritional status of women carrying a fetus with a cleft to those whose fetus was clear of all anomalies? Since that study was completed a number of participants who had previously had a child with a cleft delivered a child with no anomalies. This then led the researcher to consider the question of whether a woman can be proactive in any way prior to proceeding to another pregnancy to avoid a second CLP outcome? If so when and how?

In all, eight women who had volunteered for the study discussed in Chapter 8 had previously had a cleft child. All had at some time been associated with CleftPals, and all had been following this research program. While all may not have heard the researcher speak at various functions regarding clefting, all were aware of the issues raised regarding stress, and had been aware of the proposal that nutrition may be a factor. For this study these former control participants (not clean controls as mentioned in the previous chapter) were invited to write their personal stories relating to their pregnancies. All agreed without reservation.

9.3 Background

In the Philippine study discussed in Chapter 6 it was shown that many women who had a child with a cleft went on to have children who had no apparent birth anomalies. At that point it was concluded that clefting is a random event. In Chapter 7 it was suggested that stress may be a causal factor, and therefore if a mother was able to minimise her emotional and physical stressors this may be a preventative strategy. In Chapter 8 we determined that a nutritional deficiency, possibly zinc, may also be a causal factor. We hypothesised in Chapter 8 that stress alters the hormonal milieu in the mother which may impair fetal nutrition. In essence then, there may be several differing

pathways, or causal factors, each potentially limiting micronutrient status of the fetus, which in turn limits the expression of genes involved in lip/palate fusion. The question then is, if a woman is able to address all of these potential initiators or causal factors can she have a child free from anomalies after having already had a cleft child? While it is not possible to prove this from the case history of a small group of mothers their stories my increase the awareness of others to the importance of preconception planning and encourage other researchers to further explore this idea.

9.4 Literature Review

This study was a continuation of the study in the previous chapter, and so a separate literature review was not undertaken.

9.5 Ethics Approval

As this study was merely an additional part of the study previously described in Chapter 8 a further ethics application was not required.

9.6 Research Design

This study like the earlier qualitative studies is a descriptive work combining phenomenology with oral biographies. Each woman experienced the same outcome or phenomenon, and has related their own personal experience in their own words.

Phenomenology has been described earlier in this thesis as a method of qualitative research originally developed in education research (Marton 1994). It is an empirical method to study qualitative phenomena, particularly to investigate the differences between the understandings and perceptions individuals have about the same experience. It utilises a structured interview or questionnaire to acquire perceptions of participants (Marton 1994). The women who had a successful pregnancy, following giving birth to a CLP child, agreed to write down the differences between their first and second, or subsequent pregnancies, in relation to physical, nutritional, and emotional aspects prior to and around conception for each pregnancy. Following the gathering of the women's written accounts semi-structured interviews were composed based on the responses received, and conducted with each woman. This was undertaken to confirm the contents in their narrative. At the completion of each interview, most lasting no more than an hour, although in some cases spread over several days, the interview data for each woman were assembled as a narrative, where necessary, but kept in their own

particular writing style, and re-submitted to each woman for self-audit verification to ensure the rigour of this study.

9.7 Setting

As with all of the other Australian studies the participants were geographically spread over the three eastern states of Australia, namely Queensland, New South Wales and Victoria.

9.8 Results

Narratives from the women follow.

9.8.1 Case Study 1

Mary[36] aged 33 years was born with a cleft lip and palate and has worked as a medical secretary for the past 11 years. Grant, her husband is 38 years old and a management accountant. Their two children are Frank who is three years and Freddie who is six months old. Frank was born with a bilateral cleft lip and palate while Freddie has no birth anomalies.

Mary's first completed pregnancy

> There was a miscarriage three months before Frank was conceived. My emotional state regarding the next baby was that I wanted a baby and the Doctors never said we should wait so we just thought we'd start trying straight away and let fate tell us when, not realising it would take only three months. Of course I was upset but I remember being quite pragmatic about it all.
>
> I took a multivitamin as soon as I fell pregnant with Frank, but while 'trying' I just took a folate supplement (500 µg/day)
>
> At the back of my mind I was concerned that my baby might have a cleft as I had, however I had always eaten healthily: three meals a day, lots of protein, vegies and at least two pieces of fruit a day, no smoking and only social drinking before getting pregnant and then only about 10 glasses of wine the entire pregnancy
>
> Physically, I believed that I was fit – I walked our dog every day and did Pilates before and during the pregnancy until I got too big. I wasn't overweight.
>
> Frank's pregnancy was easy and because I was told there was no cleft lip/palate I would say I didn't stress all that much about the baby/pregnancy. I say "all that

[36] The names of all participants have been changed to protect privacy.

much" because let's not forget I was a first time mum and had no idea what the future held. The future was that he would have an undetected bilateral cleft lip and palate.

Mary's second pregnancy

I found out I was pregnant after over 16 months of trying. I even had a referral for infertility. I cried a lot in the last six months of trying because I wasn't falling pregnant. It turned out that Grant had a problem and it wasn't me. The issue was that Grant's work stress levels were slowing his sperm down. He went on some herbs [these were not identified] to help his sperm activate and I fell pregnant almost instantly.

Two weeks later after finding out I was pregnant Grant found out that moving to Hong Kong was on the cards, and within three or four weeks of this news we had made a trip to Hong Kong to look for houses, meet doctors, visit hospitals, and decide if we would do the move or not. We then began renovating our house to get rent-ready and all that is involved in relocating – a real challenge and very stressful.

To make my stress levels higher I had to have an amniocentesis at 16 weeks, one week before another trip to Hong Kong in January. This test was unusually painful, not at the site of injection, but lower down the stomach. I did worry about the results for the 10 day wait, only to find out it was all good, and simultaneously finding out the lip was fine but the palate was still unknown.

We had one week in an apartment prior to leaving for Hong Kong and I sleep-walked (something I only do when I worry) and fell down about 20 stairs and badly hurt both legs, ribs and my right hand. I had to be taken to Box Hill Emergency for x-rays and fetal heart monitoring. Again all was clear and we flew out for a holiday in Koh Samoi on our way to Hong Kong. By this stage I was five months pregnant.

In relation to the nutrition I still ate healthily [not clarified] although probably not as much fruit as in the earlier pregnancy as I probably gave more of it to Frank. The one difference of course was that in this pregnancy I took a broad spectrum multivitamin and mineral supplement for all of the 18 months since Frank was born but stopped that after six weeks into this pregnancy. I then started taking a specific pregnancy and breast feeding formula.

In Australia I still walked the dog every day the same as in the first pregnancy but had stopped the Pilates. Once I moved to Hong Kong I had to increase the walking as there is no car here, only public transport. I didn't drink as much wine before becoming pregnant and definitely less during the pregnancy.

It really is a mystery to me why Frank had a cleft and Freddie didn't and I still can't help but look at my own medical history given the clefting with me.

9.8.1.1 Discussion

Mary's narrative indicates that the second child, Freddie could be more likely to have had a CLP as both parents were far busier and recognised their abnormal stress levels. Falling down a flight of stairs during the pregnancy further threatens physical and emotional health but would have had no impact on the formation of a cleft due to the gestation time of the accident. Mary did not receive any advice regarding pregnancy spacing which may have also disadvantaged her health for the subsequent pregnancy. Mary's last pregnancy, during a stressful period, was eighteen months after the last pregnancy. The World Health Organisation (WHO 2006) recommends waiting at least two to three years between pregnancies. Mary had, however, been supporting her dietary intake with a broad spectrum supplement containing a range of vitamins and minerals, something she had not done prior to her first pregnancy. Mary may have gained strength and resilience[37] in the period between her pregnancies to then have a successful pregnancy and deal with complex stressful issues.

9.8.2 Case Study 2

Lillian is a 31-year-old administration assistant married to Ken an account manager aged 33. Lillian has three children; a boy Leslie aged four years with no birth anomalies and Paul aged two years born with a unilateral cleft lip and hard palate and a bilateral cleft of the soft palate. Mary's newborn third boy, David, has no anomalies. Neither Lillian nor Ken have a CLP nor does any direct family member.

Lillian narrates the three pregnancy experiences below.

Lillian's first pregnancy

- Emotional state – Great. We had just returned from a four-month holiday in South America.
- Physical state – I would say that I was quite fit and healthy due to our trip.
- Nutritional state – maybe not so good. I didn't take any pre-pregnancy vitamins (in fact, I wasn't taking any vitamin supplements). I probably didn't eat as well

[37] Obviously in all these cases the last child may not have had the genetic variants that may be associated with CLP. We cannot be sure if a genetic variant or nutritional deficiency is the primary cause unless both form part of the same study. Genetic studies were not conducted for this thesis and so no decisive claims can be made regarding causality.

as I should. A big problem I have is eating enough protein every day. Eating a well balanced diet of fruit and vegetables is also not easy in most South American countries.

- Experience during pregnancy – Great. Uncomplicated pregnancy. No stress, worked throughout.

Lillian's second pregnancy

- Emotional state – We had a one-year-old when I fell pregnant and it was unexpected. We were happy but having a one-year-old at the same time was hard work.
- Physical state – I would say no worse nor better. I wasn't doing any exercise though.
- Nutritional state – Again no pre-pregnancy vitamins. I was probably more deprived of nutrients as I was still breastfeeding our first son, so that would have been an added drain on me nutritionally.
- Experience during pregnancy – We were happy to be having another baby and again the pregnancy went well until we found out about the cleft at the 20 week scan. The last 20 weeks were therefore stressful and full of worry.
- The other thing that happened during the first trimester with this pregnancy was we moved into our new house. We painted the house and I do recall cleaning the bathroom with 'Exit Mould' (or something like that) and thinking that I should not have been doing it. I think at the time I was around 6-8 weeks pregnant.

Lillian's third pregnancy

- Emotional state – Great. We got married and this baby was planned.
- Physical state – I had been exercising prior to and during the first trimester. I was probably the healthiest at this time as I had been working with a Naturopath to get my health back on track. This involved eating better, taking vitamin supplements [clarified below] and exercising.
- Nutritional state – Much better than the other two, as mentioned above.
- Experience during pregnancy – Great. Uncomplicated pregnancy. No stress. Worked two days a week.

9.8.2.1 Discussion

Lillian did have a previous miscarriage before she had any children. Neither she nor her husband smoke, and during this last pregnancy she only had a glass of wine socially about once per week. She did not disclose what her previous alcohol consumption had been.

During this latest pregnancy she was prescribed Amoxil and erythromycin due to a bout of pneumonia, conjunctivitis, and a urinary tract infection by her doctor, and had taken these for about 10 days during the first trimester.

Lillian reported that the supplements included a herbal formula to support liver function and to build up her immune system (her words). She also took vitamin C, zinc, docosahexaenoic acid (DHA), a pro-biotic, an antioxidant formula, and a broad spectrum (multivitamin/mineral) prenatal product (identified by brand name).

She seemed determined to have her body in the best physical and emotional condition as possible prior to becoming pregnant. The nutritional supplements that she took, in particular the antioxidant formula, complemented the other products taken.

9.8.3 Case Study 3

Kaitlyn is aged 33 and her partner Andrew is 39. Andrew works as an economist and Kaitlyn worked as a financial researcher for ten years prior to having her first child, a girl aged two and a half years, born with a unilateral cleft lip and palate. After the birth of their first child Kaitlyn did not return to the workforce and has now had another little girl with no birth anomalies.

Kaitlyn's pregnancy experience

> During my first pregnancy I was working long hours in a quite stressful corporate environment and also spending a long time commuting back and forth to the city. I always felt that there was not enough time for everything so I did a lot of rushing around.
>
> I always had to be strict with my diet as I am a Coeliac and so have been on a gluten free diet for many years. My sister had had problems in conceiving and so I was careful to ensure that before the pregnancy I not only took folate, but also a broader spectrum nutritional supplement.
>
> I did have an inkling, that apart from the stress encountered prior to and during my first pregnancy, that something else was not right; however, it was not until six months after

the birth of my child that it was confirmed that I had an underactive thyroid. I did have blood tests, in the years before I became pregnant, checking my thyroid but they always came back negative.

I resigned from my job after I had my first daughter, so during my latest pregnancy I was much more relaxed, less tired and therefore felt overall healthier.

I started taking medication (Oroxine) for an underactive thyroid about six months after my first daughter was born and was under the watch of a specialist
during my latest pregnancy, which again made me feel less tired.

This last pregnancy was far less stressful but I do not feel there was any difference nutritionally between the pregnancies.

My diet was the same for both pregnancies with regard to the amount of meat, fruit and vegetables etc. that I consumed. I also consumed the same amount of vitamins – particularly folic acid, in both pregnancies, taking these for 5-6 months before I conceived.

The major difference between the two pregnancies was that in the second my stress level was low compared to the first, and I was in a stable home environment without the work and travel stresses.

9.8.3.1 Discussion

Kaitlyn's underactive thyroid condition remained undetected until after the first pregnancy. The stress which occurred continually during that first pregnancy may have contributed to the outcome. Kaitlyn made two changes to her life: first reducing her stress levels by leaving the work environment, and also placing herself in the care of a naturopath and a physician, to ensure that she was healthy prior to the second pregnancy.

9.8.4 Case Study 4

Odelyn is 39 years old, and her partner who is 40 years old is a management accountant. She is no longer employed but had worked in the banking industry for six years prior to the birth of their first child two years before this current birth. The first child was conceived by IVF[38] and she left work during that pregnancy.

My first child was conceived via an IVF program. Emotionally I was under a lot of stress, working long hours with little support, in a middle management banking position

[38] Age and IVF placed Odelyn in a higher risk category for birth anomalies.

prior to conception. At 32 weeks I got shingles and suffered cramping at work [this would have had no impact on the clefts given that she was now 32 weeks, but the stress earlier leading up to the shingles may have]. At this point I resigned and have not returned to work. My child was born with a cleft of the soft palate, and some other issues that are similar to Gordon's Syndrome.[39]

It is not my belief that my nutritional status was any different either during, or preceding, either pregnancy. I did take a well-known brand of antenatal supplements prior to, and during, this last pregnancy. My second child, a boy was naturally conceived and has no medical issues.

9.8.4.1 Discussion

The fact that Odelyn also contracted shingles during her first pregnancy perhaps adds substance regarding her claim that she was so stressed that she had to leave work. Shingles often occurs when individuals are stressed (Irwin, Pike et al. 2004; Glaser and Kiecolt-Glaser 2005). The IVF process itself is not without stress and indeed the decision to go through that process must of itself be stressful.

There is also no doubt that some ongoing stress may have existed in the lead up to this second pregnancy, albeit to a lesser extent, as she still had to deal with the medical issues relating to her first son. The supplement regime may have helped but more likely the absence of trying to hold down employment and the home-life may have been the key to success in this latest pregnancy.

9.8.5 Case Study 5

Ann is 27 years old and is an administrator in a doctor's surgery. Her partner who is 28 years old is the general manager of a family owned business. Their first child was born with a unilateral cleft lip and palate. Her second child, a boy, was born with no birth anomalies. Ann relates her story below.

I really cannot see that there were any differences between the two pregnancies.

Both children were naturally conceived. Nutritionally there was really not a lot of difference except that I did take antenatal supplements both before and during this latest pregnancy.

[39] Gordon's Syndrome is rare but is known to be inherited as an autosomal dominant genetic disorder. In this case the issues were only similar to Gordon's.

If anything I was more worried about the second pregnancy because of the first child having a cleft but I am not a 'stressed' person generally. I can say that my emotional state was similar in both pregnancies.

In this last pregnancy I was much more nauseous than in the first and survived on toast and dry crackers plus my supplements from week six or seven through to week 16.

9.8.5.1 Discussion

In this case there seems little reason for the cleft in the first child, and certainly it would appear that on the basis of stress or worry alone it should have been the second child with a cleft. A genetic predisposition may have been involved here as there seems no other logical causal effect. Ann however while being more nauseous with the second child appears to have been rigorous with her supplementation. She could not recall if she was as regular with that prior to the first pregnancy.

9.8.6 Case Study 6

Pauline is 40 years of age and had been working as a marketing manager for eight years before her first son was born. Her husband is a fire safety engineer. Her first child had a unilateral cleft lip and palate and is now four years old. Her second pregnancy was via IVF. She tells her story:

In 2005 I lost weight for my wedding thanks to Lite and Easy and was going to the gym 4-5 times a week. I hadn't eaten meat for years but was taking iron and multivitamin tablets. In 2006 I was losing weight and getting fitter but had a miscarriage so added chicken to my diet.

In 2007 I was in a very stressful job in change management and was the subject of a lot of bullying. I was pregnant at the time and went to VCAT to have an action taken against the company. I won but left that job and continued with contract work until eight months into the pregnancy. At about three months I had a major fight with my mother-in-law and my family, and cut off all relationship with them. CleftPals members were my real family at that time and they cared for me from week 2. I still have close contact with the women that helped me and will continue to do so for many years to come.

In 2010 I decided to become pregnant via IVF and commenced taking multivitamins, folate, herbal remedies and even had a lot of acupuncture. My husband has been my great support through all the issues that I have had to face. This last pregnancy was a breeze in that while some of the emotional issues still live on I am now able to put them

into the context of my life. I have grown up and now know how to deal with them. I now have a wonderful little girl with no birth issues.

9.8.6.1 Discussion

While Pauline outlines the issues that she faced with her work and her family, the text does not do justice to the trauma that she outlined during the interview which it is not intended to detail here. She suffered severe trauma during that first pregnancy and it was obviously only by having such a good husband that she survived emotionally intact. At the first interview which was while she was still pregnant she was calm and composed and spoke openly about her journey. Had the early problems still been an issue these would have been noticed but she was very much in control of herself at this time. One can only speculate that stress played a part in the issues in her first pregnancy. The researcher has met with her on two occasions since her last child was born and she is now certainly in full control of her life.

9.8.7 Case Study 7

Amy is 37 years old and had previously worked as a compliance officer and team leader for an insolvency firm. Her husband aged 35 is an Internet web designer. Amy's first child was born with a unilateral cleft lip. She tells her story below.

> Prior to my first pregnancy I had a miscarriage at six weeks and then became pregnant again 2-3 months later. At the time I was not taking any supplements but after 13 weeks into the pregnancy began taking supplements.
>
> About six months before becoming pregnant I was under a lot of pressure at work and finally resigned from my position. At the same time we had booked a trip to Europe, and were in the process of buying a home. The collective stresses were overwhelming. From conception to just past the six week period I experienced more stress than at any other time in my life. When I was about six weeks into the pregnancy I complained of stomach pains to my Doctor, and not knowing I was pregnant at the time took some prescription medicine for that. I do not recall what they were. I was quite active during this first pregnancy particularly walking and cycling.
>
> My second pregnancy was much easier. I took prenatal and pregnancy supplements prior to becoming pregnant and during the pregnancy. I took time out for relaxation and was not working at all during the pregnancy. I was nowhere near as active as during my first pregnancy mainly driving to appointments rather than walking or cycling. I now have a beautiful little girl so have one of each. Life is good.

9.8.7.1 Discussion

There are many things that we can look at in this case. Amy highlights stress but perhaps the miscarriage before the first pregnancy and the lack of supplementation in the early weeks of the pregnancy may have been involved. A miscarriage brings with it both physical and emotional stress. The most important thing was that she consciously made a decision to change her lifestyle and the end result was positive, perhaps she was just lucky.

9.8.8 Case Study 8

Teena is 32 years old and her husband Mark 33. She no longer works in paid employment but her prior employment was in the area of anti-money laundering compliance. Mark is a senior operations manager. Her second child, a boy was born with a unilateral cleft lip and palate.

She relates her story:

> We have three children, all boys and all three conceptions and pregnancies were quite different, at least in the early stages. With our eldest (now 7) I was 24 and working in a full time, stressful job which at times required me to travel overseas, studying part time and I wasn't doing a lot of activity. We would get home late and eat fast, but home cooked meals. Lunches were usually bought and I have to say, not always healthy. I was about six kilos heavier than I am now. I have polycystic ovaries so when my husband and I decided we were ready to try for a baby, we went to my gynaecologist and started on clomiphene to induce ovulation. We were lucky and fell pregnant the first time I ovulated. I was really sick the first three months with morning sickness and was still working full time and doing late night conference calls. I ate a lot of watermelon and rockmelon, especially in the early days, as it was the only thing that would settle my stomach. I lost three and a half kilos between six and twelve weeks.
>
> With our second son, we were on clomiphene for a year with no success this time so we were referred to a fertility clinic and began IVF. We found out my husband's sperm morphology and motility wasn't great so we had ICSI, injecting the sperm into the egg. To our delight, I fell pregnant straight away. Not long after I fell pregnant, I got a terrible cold accompanied by typically for me, a horrible cough from tracheitis. I advised my GP I was pregnant and was prescribed Pulmicort in an inhaler. I didn't have as much morning sickness so the times I was sick I still relied on melon but was able to eat a little more normally, although, was still fairly nauseous. I also had a fair bit of stress at the time from marriage trouble. Regarding diet, I was cooking all our meals as I

was a stay at home mum but did indulge in maybe a little too much chocolate and sugar. I had another little boy but on this occasion he had a bilateral cleft lip and palate.

Our third conception was completely different. We didn't think I could fall pregnant naturally so were leaving it in fate's hands and I wasn't really thinking about it. I was going to the gym, eating healthy and studying full time whilst also being a mum to my other two children. My husband and I were in a much better place in our marriage and we went on an incredibly relaxing family holiday to Fiji for a couple of weeks. A month or so after we returned, I discovered I was pregnant. Because I didn't know I was pregnant until around eight weeks, I had been doing a lot of things you shouldn't when pregnant. I ate soft eggs, smoked salmon and took cold and flu tablets when I got a couple of terrible colds. I didn't have much morning sickness so was eating fairly normally all the way through (although once I knew I was pregnant I followed the eating guide for pregnancy no- nos. I was studying full time and I fell pregnant right around assignment time and two days before my exams. Of course my law exam was first off the rank. Thankfully, I passed everything. I started pregnancy vitamins from around eight weeks, once I found out I was pregnant.

9.8.8.1 Discussion

In the second pregnancy there are two issues which may link to Teena being in a highly stressed state although she does not imply that, either in her writing or interview. The decision to go through an IVF process[40] is not an easy one to take, and is both physically and emotionally stressful. Further the actual process is rigid and a strict timetable must be maintained. This in itself raises the stress levels in both partners. Teena at the same time was having relationship issues which she calls 'marriage trouble'. Obviously the depth of these has not been disclosed but all relationship issues bring with them heightened stress levels. Stress therefore may be implicated in the second child having a CLP. Certainly her diet appeared to be constant in all three cases, and it is unlikely that any of the pharmaceutical products taken had any impact on the second child.

9.9 Conclusion

While the risk factors in each of the above cases seem to be different, each woman in their latest pregnancy believed that they should reduce their stress levels, and enhance

[40] Olson, Keppler-Noreuil et al. (2005) found that of 1,462 IVF-conceived children 90 had birth anomalies, of which 10 were orofacial, compared to 8422 controls of which 369 had birth anomalies, 43 being orofacial. Their conclusion was that there was a slightly higher risk of birth defects in IVF children but that a larger study was required.

their nutritional state before becoming pregnant again. In each case the woman gave birth to a normal healthy baby. They are in that sense unusual as research shows that there are "low levels of knowledge and behaviour related to preconception care in women of reproductive age, particularly with regards to adherence to nutrition, and lifestyle recommendations for planning a pregnancy" (Mazza and Chapman 2010). The randomness of CLP may be reflected in this study rather than the improved nutritional and emotional state of the women. However, ensuring that a woman is healthy and emotionally stable prior to conception is important in every pregnancy, especially where there has previously been an adverse outcome.

9.9.1 Publication

This chapter and that preceding led the researcher to consider ways in which information regarding stress and nutrition could be easily communicated to women of child bearing age and their partners, and perhaps also to those who may be considering sexual relationships. This was a specific target audience and other health researchers have used the term 'knowledge translation' (Choi 2005; Swinburn, Gill et al. 2005; Armstrong, Waters et al. 2006) to describe ways in which research outcomes can be put into practice in the public arena, and the importance of transferring this knowledge.

In this case an idea was born to see how the effect of nutritional deficiencies, imbalances in nutrients, and the presence of toxins could be made interesting to the general public, using music. The Royal Melbourne Philharmonic Orchestra and choir agreed to be involved in the project, although the researcher was limited to a one off session, this being not more than 20 minutes. From this music session a DVD was produced, *Genes at Work and Play – Making Babies*, a copy being included with this thesis. While this is amateurish in its production, it has been used successfully with parenting groups, Rotary, and other organisational speaking engagements, and has assisted in obtaining state and national radio opportunities to discuss the issues surrounding clefting. The DVD has also been uploaded to YouTube however this communication channel has not proved to be as successful as had been expected, perhaps because parents only search for information after a specific event rather than in anticipation that it might occur.

9.10 Strengths and Limitations

The strength of this study comes from the willingness of the women to provide information of a very private nature, honestly and in some detail. As seen in the letter in

Chapter 5 from a mother who stated that it was difficult to share her thoughts with a complete stranger, this takes a strength of character that many of us do not have. The limitations of the study lie firstly in the small number of participants and the fact that we cannot be sure whether the same birth outcome may have occurred had they taken no preventative action.

9.11 Summary

This study presented a rare opportunity to discuss with women their pregnancy outcomes, particularly where one had been difficult for them to deal with. The work has no real depth and was not intended to be more than a glimpse into their pregnancy histories. It has however shown that where women take extra care in planning a pregnancy there is a possibility that the result will be very positive. Other researchers (Tolarova and Harris 1995; Wallace and Hurwitz 1998; Mossey, Davies et al. 2007) have also suggested that planned pregnancies are associated with a lower risk of orofacial clefts, and hence any promotion increasing preconception planning is likely to have both social and financial benefits for the community.

The researcher, however, was able to use this knowledge to design and produce a DVD which has been subsequently used to encourage other women to consider a number of criteria when approaching a pregnancy. It is hoped that other researchers and health professionals may also consider novel ways to promote this idea in a much more professional way.

Chapter 10 Pregnancy and the Mother's Mineral Status

10.1 The Research Question

Is the nutritional status of a woman who has had a child with a cleft different to that of a woman whose child has no birth anomalies?

10.2 Introduction

An earlier study (Chapter 7) suggested that traumatic stress around conception may be involved in clefting. The results from that study were published in the journal "Woman and Birth" (see Appendix 18). It was proposed that the hormonal change in the woman, who had been exposed to stress, may have caused a change in the way nutrients were delivered to the fetus, possibly leading to the fetus being deprived of essential nutritional elements during this critical time.

The study reported in Chapter 8, while based on small participant numbers, suggests that zinc may be involved. With zinc it would appear that while a serum analysis may show a slight reduction in intake of this nutrient, elevated excretion via the hair and urine, suggest that the woman may not be able to utilise the available zinc. An analysis of the earlier data indicated that a woman with elevated hair zinc is eight times more likely to have a child with a cleft than a woman whose hair zinc level is lower. The reference range for each element has been established by Trace Elements Inc. in the USA, which is the company used by Interclinical Laboratories to undertake the analyses (Watts 2003). Obviously when comparing the test data to control data the reference range is of secondary importance.

The early indication based on the findings in Chapter 8 is that by using the non-invasive testing of hair in this study we may be able to add strength to the previous data.

Subsequently contact was made with various people around the world to see if a study using HTMA could be carried out in different countries to add rigour to the research. Initially researchers in Cambodia and Hong Kong indicated interest in being involved. The researcher visited the contacts in both countries but they subsequently withdrew from the project, mostly due to cost. ORAL has a contact in the Philippines who locates children with clefts and organises them to be assessed for surgery. He is located in Cebu

in the centre of the Philippine archipelago. It was agreed that the researcher could travel with him to Cebu, Mactan, and Bohol islands where parents (generally, just mothers) could be met, and interviewed once their child had been committed for surgery. Travelling with the researcher and the local operator would be a female translator, and it was proposed that all contact would be made with these parents at a local health centre where nurses were available to be party to any contact that was made.

CleftPals agreed to advertise the Australian component of this study through their website and literature so that women in Australia could participate. While it was expected that Australian women may be more reluctant to participate, as was described in Chapter 8, having a wider geographical spread of participants added rigour to the outcome.

10.3 Background

The research outlined in a previous study (Chapter 8) suggested that a zinc deficiency may be involved in clefting. It also suggested that the deficiency could be detected using HTMA. This was first suggested by Bergmann (1980) who took hair samples from women postpartum whose child was born with spina bifida. Bergmann cut the hair into one centimetre sections, analysing each section to provide a picture of the nutritional status throughout the pregnancy in relation to zinc levels. In the previous study the results were taken during the pregnancy and therefore only gave the zinc status at one point in time. To undertake a study along the lines of Bergmann is extremely expensive and beyond the scope of this research project. However taking a hair sample post-partum and analysing it, gives an average zinc level close to the end of pregnancy which can be compared to controls. If a similar result could be obtained from this study to that described by Bergman et al. (1980) it would add weight to the hypothesis that hair tissue mineral analysis may provide a useful biomarker for congenital anomalies with potential to indicate deficiencies.

As there is evidence for the validity of HTMA as a tool for measuring mineral deficiencies in a variety of human health-related conditions (see Chapter 8), and since the study reported in that chapter shows a possible correlation between clefting and mineral imbalances/deficiencies (as determined by HTMA), it is proposed that HTMA may be a useful antenatal screening tool to detect such imbalances. If so, measures can then be put in place before, or early in pregnancy, in order to avert certain health problems, including clefting, in the offspring.

10.4 Literature Review

This study is a follow on study from Chapter 8, and therefore a separate literature review was not undertaken. To the knowledge of the researcher there have been very few previous studies using HTMA to determine a causal relationship between birth anomalies and nutrition (Bergmann, Makosch et al. 1980; Saner, Dagoglu et al. 1985).

10.5 Ethics Approval

The Southern Cross University Human Research Ethics Committee gave approval for this study (ECN-12-077). A letter from the governor of Bohol endorsed the work to be carried out in the Philippines and is attached in Appendix 20.

10.6 Research Design

This study like the former reported in Chapter 8 is a mixed method involving phenomenology and ethnology, and while some of the results are of a qualitative nature the main outcome is quantitative. The mineral status of participants was determined using hair tissue mineral analysis (HTMA) where the analyses were conducted by Interclinical Laboratories in conjunction with Trace Elements Inc. – USA. Approximately 35 nutritional elements as well as non-essential and toxic elements were measured. Hair samples were taken from women who had a child born with a cleft lip and/or palate within the previous 12 months and the results compared to controls whose child had no birth anomalies. The study also surveyed the participants, to seek information about age, number of pregnancies, general lifestyle, diet, and supplement use. The information sheet, consent form and questionnaire are located in appendices 14-15.

The data obtained from the HTMA analyses were statistically analysed using the Pearson bivariate correlational and Mann Whitney U statistical tests.

In both of the countries where this research was carried out the sampling was definitely opportunistic, but this was the only cost-effective way of achieving a reasonable sample size. In Australia the researcher continued working through CleftPals, and in the Philippines worked through surgical missions including ORAL, the health centres, and hospitals that they are associated with.

In consideration of the potential vulnerability of the participants, nurses at the hospital or health centre in the Philippines, rather than the researcher himself, sought consent from the participants, helped the participants complete the questionnaire, and also took

the hair samples. The document showing how to take a hair sample was the basis for teaching the nurses the procedure to follow. (Taking a Hair Sample – see Appendix 30.) The researcher estimated which part of the sample grew within the last period of the woman's pregnancy by trying to relate the child's age with the position where the sample was taken.

In Australia volunteers were recruited through CleftPals as they have been for the other studies. The researcher did not make any direct approaches to women to be involved in any of the studies but merely advertised through the CleftPals newsletters and their website. Women have then contacted the researcher volunteering to be involved. No change was necessary to that procedure for this study.

In the Philippines a very different structure had to be pursued as such an organisation as CleftPals does not exist and so it was decided to recruit participants whose cleft child was presenting for surgery. The HREC suggested that we should approach the potential participants after their child's surgery was completed. This is not practical as the parent is the primary care giver after surgery, and the child is generally discharged the following day in the case of a cleft lip and as soon as practical after a cleft palate surgery. Therefore the mother is focused on and giving her full attention to the child. The reason for this is that the hospitals do not have sufficient beds to cope with the numbers. In the case of the General Malvar Memorial Hospital in Quezon City the recovery ward is a large room with makeshift beds where the parent often sleeps on the floor beside the child after surgery.

The procedure for a mission consists of the following:

1. A preliminary review of the available resources regarding the necessary equipment for the surgery at the Malvar (GMMH) or target hospital is conducted by at least two members of the Australian medical team.
2. The mission is advertised through charity groups such as the Rotary Clubs with a date set for parents to register their child for assessment.
3. The local medical team screen the children to determine their suitability for surgery and, if selected, a blood sample is taken, and the parent signs a consent form. At this time the nurse or doctor outlines to the parent what will happen during surgery and also tells them to bring food and water for themselves for the time that they will be at the hospital. The parent is given a date to be at the hospital with their child.

4. On the day of the surgery the children is checked by the surgeons prior to commencing the operations to ensure that they have not contracted any bronchial infection or have other health issues which may affect the surgery.

Therefore the best time to inform the participants (mothers of cleft children) of the research project and ask for their involvement was after the pre-screening and confirmation that the child will have an operation. The nurses helped the participants to complete the questionnaire and take a hair sample. Because of the limited literacy in the Philippines it was important for the local nurse to explain to the participants what the research was about rather than provide a translation of a written document. Likewise, when the interview/survey was conducted it was far better that the participant be interviewed by someone who speaks the mother's language (even if this was English), rather than just handing out papers which could be misinterpreted or just not understood. Therefore the research was conducted via a nurse and/or translator who were both fluent in English and the language of the local people.

As it was essential to have controls in each country a small monetary contribution was provided in the Philippines so that the nurse could collect data and samples from mothers whose child did not have a cleft in the days after the mission had been completed.

10.7 Funding

Applications for funding were made with some success. Limited funding was provided by Nordic Naturals Inc. (USA), a company producing fish oil supplements. Several meetings were held with potential organisations but only Nordic Naturals Inc. committed to the project.

Interclinical Laboratories continued to provide the hair analyses at a 50% discount and so the funding from Nordic Naturals Inc. covered some of the cost of the hair analysis.

10.8 Setting

The Philippines, the Islands of Cebu, Mactan, and Bohol and the eastern states of Australia.

10.9 Results – The Philippines

10.9.1 The Philippines – A Commentary

Operation Rainbow Australia Limited has a resident volunteer in Cebu (Mr Piet Treitjel) who is in the process of setting up a database of all the children requiring surgery for a CLP. The objective was to accompany him and his translator so that we could interview mothers whose child was less than 12 months old, who had been confirmed via a local health centre that the child was ready for surgery, and to take a hair sample from the mother. All mothers whose child had not yet had one operation were offered a time for their child to be operated on in either October 2012 or May 2013. In all but a few cases the interviews were conducted in the local health centres. When this was not possible we went with a nurse and the translator to the mother's home.

The work was carried out on three islands, Cebu, Mactan, and Bohol. Mactan is joined to Cebu by a bridge and the Mactan area is little different to Cebu. Bohol is between one hour and two hours distance by fast ferry from Cebu depending on which part you go to. On Bohol the research was mainly carried out in the North and Central areas of the island. Bohol is one of the poorest parts of the Philippines.

All of the mothers were poor and lived in conditions that are difficult to describe. One family lived in a house approximately five metres by three metres with the walls made of woven palm leaves, and the roof constructed of sheets of corrugated iron. The floor was compacted earth. To get to this house we had to walk from the road for about one kilometre up a narrow path on the side of quite a steep hill. This family did have some land around them and were growing a type of cucumber. They used the leaves of a tree as another green vegetable. The leaves were rather bitter when eaten raw, but presumably tasted better when cooked. We were later advised by a local doctor that the leaves contained a variety of vitamins, and that he had them encapsulated to give to malnourished patients. The family also had some chickens.

Another family in a similarly remote area had a slightly larger house with concrete walls up to about one metre, and then a similar structure to the former from there up. This house had a concrete floor. In this case the mother was a teacher in the local community but had no qualifications for doing this. A third mother lived in what is referred to as a 'squat' area, which was considered too dangerous for us to enter, and so she came with her child to a shelter near the road. The shelter was made from bamboo.

All of these women were living well below the poverty line. An average wage for a Filipino worker is 300 pesos per day ($AU6.80). If the house is rented, and the ones above were not, the rent would be 2,000 pesos per month. For this amount of rent you get a house similar to that described above where the teacher lived. A kilogram of white rice costs 33 pesos (there are 44 pesos to one Australian dollar). At a health centre in Ubay on the island of Bohol one woman whose husband was the local policeman said that her husband received 170 pesos per month ($AU3.86). This was an unbelievably low income from that source and while not indicated there had to be other income from somewhere. The staff at the health centre could also not believe that this small income was all that the family received. We did not ask questions about the financial status of any of the families, as we believed that it could have been demeaning to the mothers. We knew their status without having to ask. It should be noted that the highest salary for an employee in the health centre was 300 pesos per day so that the staff were in many cases not much better off than the women we interviewed. From these figures it is easy to see that families struggle to survive.

Being in the health centres had both positive and negative impacts on the study. The positive was that there were several health workers in attendance, and they all took an interest in what was going on, were supportive, and wanted to make a contribution. This meant that the women interviewed would have been well looked after should there have been any emotional issues, but none occurred. On the negative side was the fact that the participant women knew everyone at the centre, and likewise the health workers knew the women quite well. When the more personal questions relating to stress issues were being asked we quickly learned that facial expressions were more important than the answers. Many of the women were suffering from stress but obviously did not want to voice it in front of other locals. This meant that stress levels may have been understated. If they did not indicate that stress existed we recorded that as stated.

Most of the stressors were financial in nature but others related to family issues, particularly in relation to the husbands drinking, or physical abuse. Two women indicated that they wanted a divorce but because the Church would not allow this they were locked into the current situation. Physical stress was evident in several who had had in excess of nine children. One woman indicated that she had attempted to abort, using a product that she got from the grocery store, but could not recall what it was.

Several women were overweight but indicated that diabetes had never been diagnosed. While this is not the only indicator for diabetes it was not verified probably because no one at the health centre was capable of doing this, and the nurses were unable to take blood or urine samples. The women may not have had regular access to a medical doctor and certainly could not afford any fees that may be charged for a proper diagnosis. Many of the women ate bread to supplement their diet, which was mainly rice and some vegetables. The bread in the Philippines is cheap and very sweet in taste, and most of the other bread-like items in the local bakery were also extra sweet. The coffee was undrinkable (in the opinion of the researcher) as the standard coffee contained milk, generally powdered or condensed, and it always seemed to have at least three teaspoons of sugar in it.

In spite of all the difficulties that were obvious in the lives of these women, they always smiled, and seemed grateful that someone had taken an interest in them.

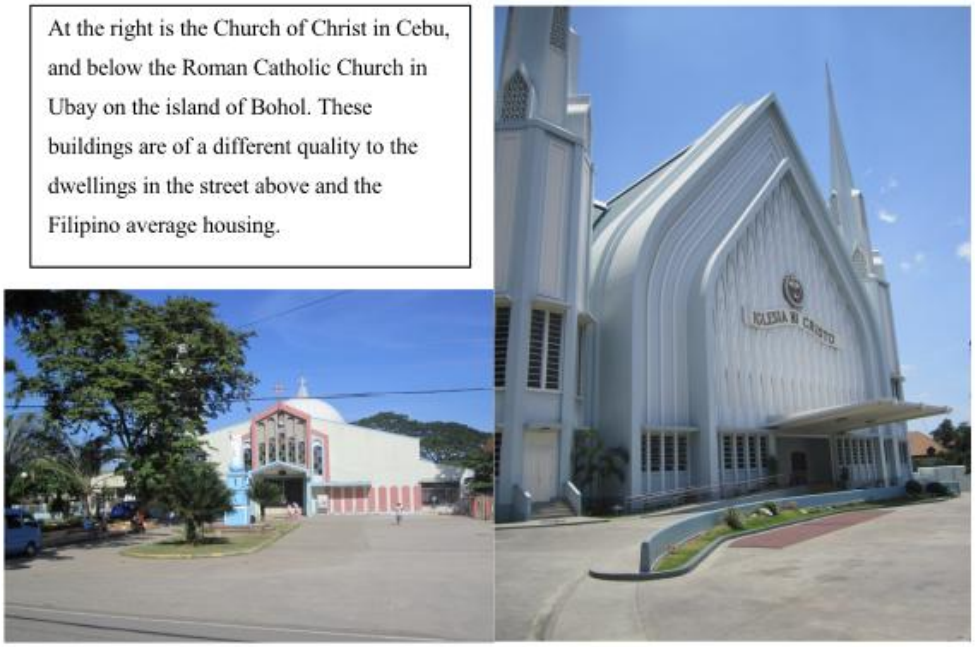
The photographs below illustrate the gap between rich and poor and also indicate that the suburban councils (barangays) have few funds to help the poor, given that their own facilities are substandard. In the civic centre shown, the state of the plumbing in the toilets was appalling not only in relation to installation but maintenance and cleanliness. The health centres offer little privacy for those attending, and while the staff are provided with uniforms this merely covers up the state of the centre as a whole. It should be noted that the sign beside the Civic Centre is promoting the drug naproxen which can cause clefting if taken during pregnancy (this was highlighted in Chapter 1).

NB: All of the photographs taken and listed below in figures 10.1, 10.2 and 10.3 where mothers and/or children have been included were taken with the specific permission of each mother with agreement for them to be included in this thesis. Permission was not sought to photograph the churches, shop fronts or the health centre as they werere considered public buildings.

On the bottom left is the Maguikay Barangay Civic Centre, above is an advertisement for a drug with known clefting side effects adjacent to the Civic Centre (See Chapter 1, Drug use during Pregnancy). The top three pictures were taken in the street of the Civic Centre. The musicians were from a local school who came to the Health Centre to raise funds for their school, probably knowing we were there.

Figure 10.1 The Philippine environment (1)

At the right is the Church of Christ in Cebu, and below the Roman Catholic Church in Ubay on the island of Bohol. These buildings are of a different quality to the dwellings in the street above and the Filipino average housing.



Mothers with their children in the Health Centre in Ubay, on the island of Bohol. Some had been operated on in May, 2012 by ORAL, and were being scheduled for either a further operation, or in some cases, their first. Thirty three children were brought, and eight were under twelve months old. Some of the pictures below were taken in Ubay and other Health Centres. All mothers gave permission to be photographed.

Figure 10.2 The Philippine Environment (2)

Figure 10.3 Women with their children who attended the health centre in Ubay on Bohol

10.9.2 The Philippine Participants' Results

As soon as the HTMA results came back from Interclinical Laboratories two things were clear:

- Most women including the controls had HTMA nutritional patterns quite different from those that are normally encountered in Australia. Obviously this had to do with the women's diets and the fact that food sources are limited, and in general of poor quality. This is not an indictment of their food choices but merely highlights the difficulty they have in surviving. It seemed clear that the mineral balance in their bodies was reflecting their poor nutritional intake and appeared to be less than optimal. This confirms that where samples are being taken in a particular country the controls must come from the same area.
- From the outset it was recognised that it was going to be difficult deciding which section of the hair sample represented that which grew during the last few weeks of the pregnancy. Some criteria had been established for taking the sample but the results show that this was not accurate enough, and hence the results obtained by Bergman (1980) could not be repeated in this instance. It was thought that an average result may be obtained towards the end of the pregnancy but the span of results shows that it was not achievable or that there is significant variability between women and hence a larger number of women must be included in the sample. While this means that the findings of Chapter 8 cannot be confirmed, other information is still of interest and requires further confirmation.

10.9.3 Data Analysis

Over and above the descriptive statistics the data were analysed using the following methods:

- Shapiro-Wilk normality tests
- Mann-Whitney U test of equal proportions
- Logistic regression
- Factor analysis
- Classification analysis
- Location difference

The complete data analysis can be found in Appendix 19

Table 10.1 Philippine descriptive statistics

Item		Control Group (n=26)	Mother with a cleft child (n=25)	P value
Mother's age		27.31 ± 6.97	31.72 ± 5.92	0.022
Mother's height (m)		1.53 ± 0.03	1.52 ± 0.06	NS
Mother's weight prior to pregnancy		48.4 ± 11.6	54.7 ± 15.8	0.040
BMI		20.5 ± 4.8	23.6 ± 6.0	0.003
Number of children in the family		2.54 ± 2.53 Range 1- 13	3.6 ± 2.63 Range 1-11	0.027
Age of last child in weeks		19.3 ± 16.5	35.1 ± 15.9	
Socioeconomic status of parents		Poor	Poor	
Sex of last Child	Male	13	21	
	Female	13	4	
Mother's stress at or around conception		7.7% (2)	40% (10)	0.017
Alcohol consumption during pregnancy		19.0% (5)	12.00% (3)	
Mother smoking during pregnancy		3.9% (1)	20% (5)	
Mother diagnosed medical issue		0%	25.% (4)	
Supplements/medications taken by mother		100% (26)	88.0% (22)	
Family CLP history	Mother	0%	0%	
	Partner	0%	0%	
	Family	0%	60% (15)	
Child's cleft type	U/CL	0	2	
	U/CP	0	0	
	U/CLP	0	23	
	B/CLP	0	0	
Total clefts		0	25	
Mothers who had a previous child with a cleft		0	1	
Mother's personality	Easy going	10	6	
	Worries sometimes	15	14	
	Worries often	1	5	
	Continually worries	0	0	

The data were analysed using the Mann-Whitney U test and test of equal proportions (cleft vs. control). Of the data analysed few showed a significant difference between the controls and the cleft sample. In the two tables shown the items marked NS showed no significant difference between cleft and control. The figure for zinc has been included merely to show

that the difference between the results for the cleft group was not significantly different from the control.

Table 10.2 Mineral status

Variable		Control (mean)	Cleft (mean)	P value
Calcium	(mg per 100 g of hair)	268.08	204.08	NS
Magnesium	(mg per 100 g of hair)	14.47	23.69	0.032
Sulphur	(mg per 100 g of hair)	3939.12	3810.40	0.035
Zinc	(mg per 100 g of hair)	39.31	45.84	NS
Arsenic	(mg per 100 g of hair)	0.01	0.02	0.053
Mercury	(mg per 100 g of hair)	0.07	0.05	0.033

10.10 Discussion

10.10.1 Age

It was not possible to match the ages of the control group to the cleft group because we were not being selective with either group but accepted participants as they came forward. The age difference also relates to the fact that the control group on average had fewer children.

10.10.2 Number of Children

This statistic is misleading as it really does not highlight the range in the family size. Only one mother was over the age of 40 and she was 42. The youngest mother was 18. In the cleft group the family size ranged from one to 11 with a slight bias towards the top end while the control group ranged from one to 13 children with a distinct bias towards the lower end.

10.10.3 BMI

As indicated above, the weight of the mothers was surprisingly high in relation to their height with the cleft group of mothers having a significantly higher BMI than the controls. This is clearly evident in some of the photographs above. Given the fact that these women were all poor this must clearly be due to the available food sources. While it would need verification it is proposed that this is due to the high level of carbohydrate and sugar intake in their diet.

Cedergren and Kallen (2005) in Sweden studied 1686 mothers of cleft children. They divided them into four groups depending on their BMI. They found that mothers whose BMI was above 29 had an overall increased risk of having a child with a cleft (OR 1.3, 95% CI). In the

Filipino mothers who had a cleft child only three had BMI's above 29 (i.e. 12%) and so this alone cannot account for the majority of the clefts in this current study.

10.10.4 Calcium

The role of calcium had not previously been considered in this thesis. From a nutritional point of view it may normally be associated with the development of bone, but it does play a larger role in nearly all processes in the body and should not be discounted in future research. Calcium was lower in the cleft group although not significant. It has been reported that the sequencing surrounding the *JAG2 A657H* mutation site is likely to be a calcium-binding epidermal growth factor domain (EGF) (Vieira, Avila et al. 2005). The EGF receptor induces cell proliferation. Further enquiry is required if low body calcium levels do have an association with this mutational site.

10.10.5 Magnesium

Magnesium is significantly higher in hair from cleft mothers than in the control mothers. Magnesium is the fourth most abundant cation in the body and the second most abundant cation in intracellular fluid. It is a co-factor for about 300 cellular enzymes and has an important role in energy metabolism. Only about 0.3% of the total body magnesium store is located in the serum and hence assessing magnesium status using a blood sample in isolation is problematic (Elin 1967). Only one paper can be found where magnesium has been considered in relation to clefting (Krapels, Van Rooij et al. 2004). This paper did not deal with the maternal load but merely with dietary intake. The researchers proposed that where a woman was deficient in magnesium, supplementation may assist in avoiding having a cleft child. It is therefore difficult without further work to propose what impact this higher HTMA magnesium result had on the pregnancy outcome.

10.10.6 Sulphur

The lower sulphur result in the cleft women will certainly be related to the dietary intake of the individual. Sulphur is normally obtained from proteins such as meat, poultry, eggs, milk and cheese. None of these would rank high in the diet of the Filipino women taking part in this study.

Sulphur is contained in two important amino acids namely methionine and cysteine (Gropper, Smith et al. 2005, p. 177). Cysteine plays an important role in stabilising extra-cellular proteins, however, a much more important role is that it is thought to enhance zinc absorption

(Gropper, Smith et al. 2005, p. 421). In the Philippines zinc is deficient in the soil and inherently low in rice, therefore it is important that whatever zinc is available to the mother is fully absorbed and utilised. Should there also be a cysteine deficiency the zinc absorption could be further retarded. Much more work would be required to prove that this is in fact the case.

Methionine is important in the absorption of chromium. A deficiency of methionine could indirectly be related to the increased BMI of the study population. Chromium complexes with nicotinic acid to form the glucose tolerance factor (GTF). The GTF is thought to initiate the bridging between insulin and the insulin receptor (Gropper, Smith et al. 2005, p. 447). Therefore if chromium absorption is decreased insulin resistance may result leading to type 2 diabetes.

As can be seen sulphur plays an important role as a mediator in two of the areas that may be related to clefting particularly in the Philippines.

10.10.7 Zinc

As has been briefly discussed above it was disappointing that this study could not confirm the earlier findings of Bergmann (1980), despite the fact that his related to spina bifida, but as stated previously there was a deal of guesswork in determining the segment of hair that had grown during the last part of the gestation period. The role of zinc has been adequately explained earlier in this thesis and every effort should be made to have other researchers follow this line of questioning.

10.10.8 Arsenic and Mercury

Arsenic is higher and mercury lower in hair from women with cleft children, both results being significant. Both elements are toxic and will impact somewhere on the body's metabolism, neither having any beneficial effect. The fact that one is higher in one cohort may have a lot to do with the physical location of the individuals within that group and the source of their drinking water. Toxins are widespread throughout the Philippines but there appears to be no uniformity across the landscape. Researchers investigating the presence of mercury and arsenic in Filipino formula milk found that two out of three products tested had levels of these toxins above the level set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee (Cruz, Din et al. 2009). A further study found high levels of both toxins in fish from the Marikina River which flows through the main city of Manila. These researchers found that the levels in fish were the same when sampled from

both upstream and downstream of the city indicating that the pollution was widespread (Lam and Sua Su 2009). In Cebu the researcher spent some time with orthopaedic surgeon, Felix J. Vicuna Jr. who indicated that as well as CLP, he saw a number of clubfoot cases in children, which he believed may be nutritionally related. Researchers in Chile (Boston and Arriaza 2009; Madden and Arriaza 2009) determined that high levels of arsenic did affect fetal growth and that both clubfoot and CLP were possible outcomes. Bangladesh which has a high incidence of both CLP and clubfoot (Bari, Haque et al. 2002; Islam, Siddika et al. 2013) has struggled with arsenic in its water supply since a disastrous campaign in the 1970s to bring clean water to the county backfired horribly. Millions of tube wells were drilled to provide villagers with clean water, but many of them were dug into shallow layers of ground that had naturally occurring arsenic thus contaminating the water. Uddin, Harun-Ar-Rashid et al. (2006) used HTMA to determine the level of arsenic in residents of a community in Bangladesh still relying on groundwater for drinking purposes. They found that the levels were much higher in residents over 12 years old suggesting that this toxin accumulates in the body. They also indicated that arsenic was present in groundwater in several other countries including the Philippines. It would seem from their description that where flooding occurs following a dry period the water leaches the toxins through the subsoil layers into the groundwater, which subsequently flows into the wells. The method of growing rice may exacerbate this effect as the land surface is flooded during the growing season. Leaching of this water through the subsoil could have the same effect as if the ground had been flooded. Monsoon rains occur in the Philippines also, and obviously the Bangladesh problem may be being repeated where wells are being used for drinking water.

Goenjian, Chiu et al. (2011) suggested that there may be a link between heavy metal contamination and clefting. This was based on an increase in the incidence of clefting in New Orleans following the 2005 hurricane Katrina. However, they put more weight on stress being the causal factor and indicated that additional work would be needed by others to connect heavy metals with clefting before such a claim could be confirmed.

10.10.9 Stress

The fact that stress appeared so prominent was a surprise as the Filipino people in general are a happy ‘laid back’ group and seem to accept their lot in life. Obviously they, like everyone else, have family issues and these definitely came to the fore. The stress of not being able to get a divorce was obvious, as was the financial situation of mothers struggling with multiple children. Many had no local breadwinner, many relying on funds flowing in from an overseas

relative. Throughout this thesis stress has been indicated to be a possible causal factor in clefting and the Philippines has merely added to that possibility. In the last paragraph mention was made of the increase in the incidence of clefting in the USA since hurricane Katrina (Goenjian, Chiu et al. 2011). These researchers reported that within the 12 months following the hurricane the incidence had risen by almost 78% but they could not clearly indicate whether this was completely due to stress or an increased level of toxins in the environment due to the hurricane or perhaps some other factor. Other researchers have also confirmed that the incidence of clefting increases after a stressful event (Hansen, Lou et al. 2000; Benitzhak and Verny 2004; Hibino, Takaki et al. 2009; Goenjian, Chiu et al. 2011). The fact that stress has again appeared in this study adds weight to the earlier studies undertaken in this thesis.

10.10.10 Sex of Cleft Children

This data supports other data previously outlined in this thesis and by other researchers previously mentioned (Dixon, Marazita et al. 2011) that clefting affects males more than females. Dixon, Marazita et al. (2011) indicated in their investigation that there is a 2:1 ratio of males to females involving CLP and a 1:2 ratio for isolated cleft palate. This particular study showed a greater percentage of males which may be biased by the small sample or another factor relating to the fact that the geographic spread within the Filipino sample was small.

10.10.11 Alcohol Consumption

While many parents – both the women whose child had a cleft and those whose child did not – admitted to alcohol consumption this was extremely minor in terms of the quantity consumed, mostly because they could not afford it. From the various discussions it seemed that where alcohol was used in a family situation it was most often the male rather than the mother. Typically for the mother it was one or two glasses of beer during the pregnancy however there could have been a wide variation in both cohorts.

10.10.12 Tobacco Smoking

The association between maternal smoking and orofacial clefts has been assessed in many studies (Kallen 1997; Sargent 2005) however these have had a number of limitations including the inability to examine a dose–response effect and an inadequate sample size. In our study we too suffered from these same problems. While the association tended to be more with the cleft mothers, as with alcohol, the consumption was minor and the dose unable to be

measured. Typically the mothers referred to their consumption as half a leaf each week or at a maximum one leaf, as they purchased the dried tobacco leaves and made their own cigarettes. Where a woman smoked a commercially prepared cigarette she referred to how many 'sticks' a week she smoked as they can buy cigarettes singly. Typically the answer was one or two per week. Honein, Rasmussen et al. (2007) concluded that heavy smoking of 25 plus cigarettes per day was modestly associated with CLP. None of the women in our study smoked nor could afford to smoke that number of cigarettes and hence this factor on its own would be unlikely to be associated with their child's cleft.

10.10.13 Diagnosed Medical Condition

Four per cent of the cleft mothers indicated that they had a diagnosed medical condition but none of the controls. In all of the cleft cases the condition was predominately asthma and they intermittently used medications to control this when they could get it. As the timing and naming of what they had taken was not disclosed it is impossible to draw any conclusions from this data.

10.10.14 Medications/Supplements Taken

While 80% of the cleft mothers and 100% of the controls indicated that they took either a multivitamin or folate/iron combination, either before or during pregnancy, a closer investigation clarifies the position. None of the cleft mothers took anything prior to conception and only two of the controls. The vast majority took nothing prior to three months' gestation and some not until six months. Part of the reason for this may have been that they did not go to the health centre until then, but more likely because supplies at the centre were short or they may not have had access to the supplements at that time. During the research period the health centres had little stock of a folate/iron supplement, and much of what they had was out of date. Health centres have very limited funds and they do not have the power to demand from the local mayor or provincial government that they must have antenatal supplements.

10.10.15 Family History

Sixty per cent of the cleft women knew others within their extended family who had had clefts. None of these were direct relations, and if they were they were two generations previously. Most were aunts, or cousins. As mentioned in an earlier chapter on a previous Philippine study it is difficult to claim a direct genetic connection as the families have always been poor, and always lived in polluted surroundings, and so the nutritional supply and

environment may have much more to do with these clefts than a specific gene, or a family trait. Only one cleft mother had had a cleft herself. As none of the studies in this thesis undertook genetic assays it is not possible to indicate whether any of the cleft women had genetic predispositions which may have impacted on their children.

10.10.16 Personality

There were slightly more mothers in the cleft group who indicated that they worried either sometimes or often. In the latter case this included those who had definite family issues. As stated earlier when interviewed all women were happy and relaxed but this may have been more because they knew that their child was to be operated on to correct their cleft.

10.11 Results – Australia

Table 10.3 Australian descriptive statistics

Item		**Control group (n=10)**	**Mother with a cleft child (n=11)**
		Mean ± SD	Mean ± SD
Mother's age		32.9 ± 3.8	32.18 ± 3.8
Mother's height in metres		1.66 ± 0.1	1.64 ± 0.1
Mother's weight prior to pregnancy		68.1 ± 14.0	65.45± 13.1
BMI		24.8 ± 4.7	24.4 ± 4.0
Number of children in the family		2.1 ± 0.6	2.3 ± 1.1
Age of last child in weeks		17.8 ± 18.0	19.3 ± 12.1[41]
Socioeconomic status of parents	Poor	0	1
	Comfortable	6	7
	Above average	4	3
Sex of last Child	Male	7	6
	Female	3	5
Mother's stress at or around conception		30% (3)	64% (7)
Alcohol consumption during pregnancy		20% (2)	36% (4)
Mother smoking during pregnancy		0	9% (1)
Mother diagnosed medical issue		80% (8)	27% (3)
Supplements/medications taken by mother		100% (10)	77% (8)
Family CLP History	Mother	0%	0%
	Partner	0%	0%
	Family	10% (1)	18% (2)
Child's cleft type	U/CL	0	2

[41] The last child had the cleft.

Item		Control group (n=10)	Mother with a cleft child (n=11)
	U/CP	0	2
	U/CLP	0	4
	B/CLP	0	3
Total clefts		0	11
Mothers who had a previous child with a cleft		2	0
Mother's personality	Easy going	4	2
	Worries sometimes	3	3
	Worries often	3	4
	Continually worries	0	2

10.11.1 Data Analysis

Over and above the descriptive statistics the data was analysed using the following methods:

- Shapiro-Wilk normality tests
- Mann-Whitney U test of equal proportions
- Logistic regression
- Factor analysis
- Classification analysis
- Location difference

None of the analyses found any significance between the control and cleft groups. The Mann-Whitney results can be found in Appendix 21.

Factor analysis attempts to represent a set of observed variables in terms of a number of common factors plus a factor which is unique to each variable. It allows the researcher to reduce a large number of variables to a smaller number of broad concepts called factors. In this case as one element could not be clearly defined to be associated with a cleft, could a number of elements collectively explain why a cleft occurred in one group and not in another?

Discriminant analysis was performed on the factor to determine a discriminant function which helps to identify those hair minerals most likely to classify a cleft. This showed that participants who had high hair levels of factor 1 were more likely to be associated with a cleft.

Using a factor analysis, however, two factors explained approximately 99.2% of the variation between cleft and control in the Australian results. The two factors are F1 and F2 in Table 10.4.

Table 10.4 Factor analysis result

F1	F2
Calcium	Sulphur
Magnesium	Cobalt
Strontium	
Iron	
Sodium	
Barium	

Performing the same analysis on the Philippine data gave less convincing results. In that case 11 factors explained approximately 77% of the data variation:

Table 10.5 Philippine factor analysis result

F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Potassium	Thallium	Iron	Magnesium	Cadmium	Nickel	Arsenic	Selenium	Uranium	Bismuth	Beryllium
Sodium	Titanium	Molybdenum	Strontium	Lead	Zinc	Vanadium	Aluminium	Cobalt	Zirconium	
Chromium	Tungsten	Germanium	Manganese	Tin						
Rubidium		Barium								
Lithium										

Further analysis indicated that participants with higher HTMA levels of the following minerals may be prone to have a cleft child. Obviously in both the Australian and Filipino results the data does not show a cause and effect relationship with the analysis undertaken merely suggesting possibilities which in themselves have far too many variables to be of great value.

Table 10.6 Philippine predominant factors

Potassium	Magnesium	Vanadium
Sodium	Strontium	Bismuth
Chromium	Manganese	Zirconium
Rubidium	Cadmium	
Lithium	Lead	
Iron	Tin	
Molybdenum	Nickel	
Germanium	Zinc	
Barium	Arsenic	

10.12 Discussion

The Australian results like those obtained from the Philippine study are disappointing in that very little can be concluded from the data except that a pattern of data similar to what Bergman (1980) found in relation to NTD was not apparent.

However, the descriptive statistics show that the controls were well matched to the cleft mothers in terms of age, number of children in the family, socioeconomic circumstances, sex of last child, and smoking history.

The main differences were:

- The control mothers had significantly more diagnosed medical issues, with the predominance being asthma. Research shows that women with asthma have more preeclampsia, preterm birth and, in the case of a female fetus, decreased growth (Clifton 2005). This is brought about by alterations in glucocorticoid metabolism and downstream changes that occur in the pathways regulated by glucocorticoids.
- Little strength can be applied to supplementation in this study because the large majority in the Philippine group did not commence taking supplements until the pregnancy was confirmed while all the Australian cleft and control groups who indicated taking supplements commenced before trying to become pregnant.
- In relation to the mother's assessment of her personality there was a very slight tendency of the cleft mother to worry more than controls but this was not significant.
- Reported stress events were much higher in the cleft group (64% compared to 30%) however the Mann-Whitney analysis did not find this significant. Despite this the higher number of stress events in the cleft group is consistent with previous work.
- The sex of the cleft children was significantly different to those in previous studies and to literature already stated in earlier chapters in that the split between male and female was almost equal. In past studies males were predominant.

The HTMA results did not provide any insight into the aetiology of clefting. Perhaps, as has been stated in the discussion on the Philippine results above, the researcher was not able to judge the hair section that had grown during the last three months of the pregnancy and had the hair sample been sectioned different results may have been obtained. As explained in earlier chapters the cleft is completed within the first three months of gestation and so a hair sample taken in the last three months of the pregnancy was merely trying to determine whether a similar pattern to Bergman (1980) could be determined (i.e. high hair zinc for the

CLP group). It was impossible to determine a common element between both Philippine and Australian data which could have been implicated in clefting. Certainly in the Philippine study where a larger sample set was collected there were elements that warrant further exploration.

10.13 Conclusion

It was difficult to draw any conclusions from this study apart from the fact that socioeconomic and lifestyle factors may be involved in clefting, but even then, there may well be significant variations within a country as well as between countries. Stress may have played a role in some of the cases, however, larger numbers of women are required to confirm this. It may be that if hair samples were taken closer to the end of the pregnancy or during pregnancy as outlined in Chapter 8 HTMA may be a useful diagnostic tool.

10.14 Strengths and Weaknesses

The strength of this study again was with the participants who voluntarily nominated to be involved. It was satisfying in the Philippines to know that every child would receive an operation free of charge, and even more pleasing to know that this has occurred since the study was completed. One hundred and three operations were carried out by ORAL in May 2013 with several of the children whose parents were participants receiving their second and final procedure.

The weaknesses are all too obvious; small number of participants and the inability to accurately sample the section of hair that grew in the last three months of the pregnancy. Ideally antenatal hair sampling and further sampling during the pregnancy would be more useful.

10.15 Summary

This study aimed to determine whether HTMA studies undertaken by an earlier researcher investigating NTD's may also be relevant to clefting. Clearly this did not occur. The earlier researcher (Bergmann, Makosch et al. 1980) was trying to understand why women who took a folate supplement still had a NTD child. His conclusion that zinc was also involved suggested that optimal one carbon metabolism requires zinc to be present with folate to avoid an adverse outcome. This may also be the case in clefting; zinc plus folate and perhaps other elements, including vitamin B12, and perhaps others need to be available to avoid a child being born with a cleft. Obviously further studies are needed to test this hypothesis and HTMA should further be assessed for its utility as a diagnostic or screening tool.

Chapter 11 Is HTMA a Useful Investigative Tool?

11.1 Introduction

This chapter does not aim to report on a particular study but merely to highlight data obtained during the course of this thesis to pose the idea that HTMA may be a tool that could be more widely used to predict adverse pregnancy outcomes, or better still, as an antenatal screening tool to provide nutritional direction for prospective parents.

The appearance of an individual's hair has long been thought to reflect their diet and nutritional status (O'Connell 2005, p. 175), and with the advent of sophisticated scientific analyses it has been realised that diet and nutritional information can also be gleaned from the chemical and elemental composition of hair. Metals in hair can be considered nutritionally desirable or toxic with the former being primarily derived from diet. While hair is most relevant to metals toxicology as a biological medium or biopsy material for analysis it is also of importance as a bio-monitor (Kales and Christiani 2005, p.125). Hair analysis is most informative when the metal of interest is xenobiotic and the exposure route is ingestion and the hair analysis reflects an internal dose of the metal and not external contamination of the hair (Kales and Christiani 2005, p.125).

The assessment of dietary intake and the assessment of nutritional status are two very different issues, diet being that which is intentionally consumed by an individual including both food and water while nutritional status is the degree to which an individual has a certain level of nutrients for optimal metabolic function. It is possible for individuals to consume widely different diets and yet be equal/similar in their nutritional status. The nutritional status of an individual may be assessed by measurement of the nutritionally necessary elements (O'Connell 2005, p. 175-193) in which hair can play an important role. Hair is an ideal biopsy material since it is available from most individuals and is easy and painless to sample. Its physical and chemical stability means that no special storage between sampling and analysis is required and its external growth in a linear fashion provides a longitudinal measure of body tissue synthesis and therefore potentially of variation in diet or nutrition over a long period of time. It is important to recognise that trace metal concentrations in the body are not solely dependent on the metal concentration in the diet but are dependent on other dietary constituents (Gershoff, McGandy et al. 1977; Yeleswarapu and Rao Nallapu 2012), for

example dietary phytate and fibre levels are known to affect zinc absorption (Reinhold, Faradji et al. 1976). Ascorbic acid increases the intake of inorganic iron but reduces copper absorption and competitive absorption in the gut between iron and manganese and between copper and zinc will affect the body levels of these pairs of elements (O'Connell 2005, p. 175-193). One of the most successful applications of hair analysis in assessing nutritional status has been monitoring Keshan disease, a myocardial disease resulting in heart failure and often death. A large scale study (Yang, Chen et al. 1984; Tan, Zhu et al. 2002) in China determined that residents in an area where Keshan disease was prevalent had low hair selenium levels. Subsequent work demonstrated that while low selenium was not the cause, supplementation with selenium reduced the incidence significantly.

Some hair studies involving zinc status have produced unexpected results. For example the hair of seriously malnourished children in Turkey contained greater amounts of zinc than did a healthy population (Erten, Arcasoy et al. 1978). This suggests that the zinc may not be in an easy to utilise form or that other dietary substances are blocking its utilisation, e.g. phytates (Reinhold, Faradji et al. 1976). The situation may be similar to methylenetetrahydrofolate reductase (MTHFR) variants (mutations) that a percentage of the population carry, which make them more at risk of folate deficiency, needing to have much higher doses of folic acid to maintain proper function of the folate (one carbon metabolic) pathway. Indeed during the course of the studies for this thesis similar unexpected results have been encountered, which suggest that while the evidence can be taken as indicative much larger studies need to be undertaken before association can be established.

During the course of this research project HTMA appears to have provided information that is not readily available from other testing regimes. It is far less invasive than other regimes, and provides information regarding mineral tissue levels that could only otherwise be obtained by a much more intrusive biopsy.

The purpose of this chapter is merely to highlight examples of hair analyses of women, some who were volunteers in one of the studies conducted for this thesis, and one who was not related to the research program to show the birth outcomes related to the HTMA results. Obviously the graphs illustrated below cannot be taken as generalisations as there were variations in each group of participants within the studies as every participant was different to some extent. However, it may be possible in a large study to determine whether there are patterns in HTMA data that predict risk or associate with adverse outcomes.

11.2 Analytical Results

The HTMA analytical method is outlined in Appendix 17 and the individual results reported in each study (chapter) have been grouped and tabulated. Graphs similar to those displayed in Figure 11.1 below are provided by the laboratory for each hair sample submitted for analysis. The purpose of the four figures is to show the data context of the birth outcome of that participant and for other researchers to contemplate the utility of HTMA.

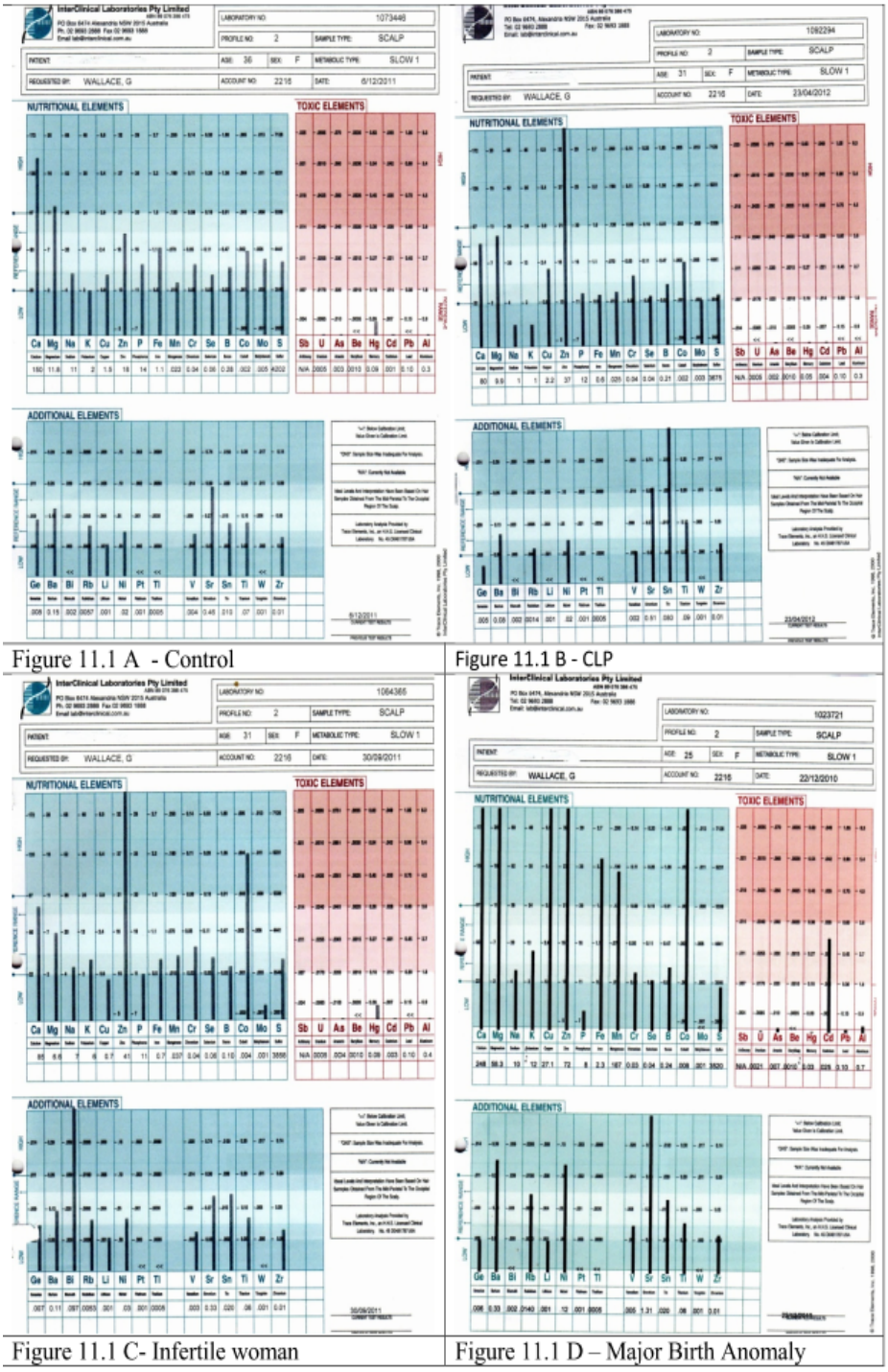


Figure 11.1 HTMA reports

With the exception of the graph for the infertile woman, the graphs shown above have been taken from participants who were involved in the study covered in Chapter 8. Figure 11.1 A shows data from a mother who had taken a broad spectrum nutritional supplement together with a folate supplement for some months prior to conception. She was rigorous in her diet and compliant with her supplementation regime. She subsequently had a child with no birth anomalies.

Figure 11.1 B shows data for a mother who had a child with a CLP. She had taken a broad spectrum nutritional supplement irregularly prior to, and during her pregnancy, but had experienced, in her opinion, extreme stress.

Figure 11.1 C shows data for a mother who had been trying to conceive for four years. She had not taken any supplements when the hair sample was taken. Following this analysis she began taking a broad spectrum nutritional supplement, together with a commercial brand zinc supplement. At the time of writing this document she was pregnant and scans indicated that there are no anomalies in the fetus. (This case is discussed in more detail below.)

Figure 11.1 D shows data for a mother who presented as a control. She indicated that she was a vegetarian but very occasionally ate fish. This woman had not taken any supplementation prior to or during the pregnancy. When the result came back from the HTMA laboratory a copy was immediately sent to her doctor (who may or may not have known how to interpret this data), as it was believed that there may be an issue, as the nutritional balance appeared to be quite abnormal. The doctor was not given advice on this but merely had the results referred to him. The child was born with a hole in the heart, one kidney, a deletion in chromosome 14,[42] and died five weeks after birth. There may have been other contributing factors other than nutritional imbalance, but none was declared to the researcher.

11.3 Case Study

The infertile woman (Sally) asked for guidance in approaching a pregnancy. While this was not planned to be a part of this thesis it did provide the researcher with an opportunity to look more closely at whether an improved diet via supplementation could assist with the pregnancy. The question in the researcher's mind was: Is this a cleft waiting to happen?

At the time of taking the initial hair sample Sally was 31 years old and married. She was of Khmer heritage and her husband was Australian by birth and heritage. She was born after the

[42] Chromosome deletions or part deletions are well known causes of miscarriage and birth defects.

end of the Vietnam war. The family lived in a region of the Mekong Delta which had historically been part of Cambodia but is now a part of Vietnam. All of the people in the village are Khmer and speak Khmer as their primary language. Many of the men in the village worked as interpreters for the Americans during the war but were cast aside by both the Americans and Vietnamese after the war ended. Sally's parents lost their land and lived a subsistence life after the war ended. Her eating and sleeping patterns have until very recently been irregular as they, as a family, used to scavenge for food at night. She never went to school but speaks three languages as well as a little Mandarin. She moved to Phnom Penh in Cambodia for work as there was no work in her local village. This is where she met her husband who was living there at the time. Four years ago they moved to Australia and while the food sources were better she continued to eat a Cambodian style diet of mostly rice and vegetables with a small amount of pork and fish.

When we met it was suggested that we undertake a HTMA to see what her nutrition was like as she desperately wanted a child. It appeared that she was not absorbing and/or utilising the zinc that was being taken in via the food. Her serum zinc level was within the normal range as was her urinary zinc. This appeared to suggest that the zinc was being stored in tissue as indicated by the HTMA.

An initial regimen of supplementation was based on a product from the USA called a Woman's Pure Pack. This product is marketed in a daily sachet of nine capsules covering all aspects of a woman's nutritional needs as set out by the Australian National Health and Medical Research Council in their dietary guidelines (NHMRC 2013) but also included rhodiola rosea and lemon balm titled as a Stress/Calm Blend. The downside of this product, in the researcher's opinion, was that it did not provide sufficient zinc – merely 7.5 mg compared to the Australian National Health and Medical Research Council Guidelines of 11 mg/day (NHMRC 2013). It was suggested that she add a local product to the regimen called Bio Zinc. This product contained 25 mg zinc from 125 mg of zinc chelate as well as 25 mg of magnesium, 2500 IU of vitamin A and 50 mg of vitamin B6.

Within two months of being on this supplementation program she commented that her hair skin and nails had noticeably improved and that she felt stronger. Because she felt that this was all due to the supplementation it ensured that she continued with the program for another four months. While she believed that it may just be the supplements it was noted that she had started to add more Western foods to her diet also. After six months on the program she was still not pregnant and so she elected to proceed with an IVF intervention. At that point the

supplementation protocol was changed by dropping the imported product and adding a locally available product called Conceive Well.[43] In addition to this the Bio Zinc was retained in the program. She continued with this regimen for the next four months.

Sally commenced the IVF program but could only provide five eggs of which only three were viable. Two embryos were transferred and they failed. She did not have a period the next month and found later that she had conceived naturally. Once this was confirmed the regimen was again changed replacing the Conceive Well with a local branded product called Pregnancy and Breast Feeding Gold (sourced from the same company as Conceive Well) but continuing with the Bio Zinc.

During her pregnancy, Sally who is slight and just 1.5 metres in height, increased her weight from 41 kg to 55 kg. There were many scans during the pregnancy and all indicated that it would be a normal birth. Towards the final days of her pregnancy it was clear that the baby was in a breech position and that a caesarean section was required, which is quite a normal approach in this situation. The baby, a little girl, weighed three kg. The doctors believed that the baby did not have enough room to turn, perhaps because of Sally's slight proportions. Mother and baby are both well.

On visiting Sally after the birth she agreed to a further hair sample being taken. This was taken from the scalp and cut into three sections of 2.5 cm to represent the period of the pregnancy. This method is consistent with that used by other researchers (Bergmann, Makosch et al. 1980; David, Holloway et al. 2014). Sample A was closest to the scalp and therefore represented the last three months of the pregnancy while sample C represented a time around or soon after conception. Sample B was obviously mid-pregnancy. The results are set out below in Figure 11.2.

If we first consider the HTMA zinc, the level before supplementation was 410 parts per million (ppm) which increased to 540 ppm. This can be easily understood by the fact that she was ingesting more zinc and obviously at that time may not have been utilising the extra zinc which was also being sent off into tissue. Once she entered the second trimester she was using part of the zinc stores and the HTMA level had started to fall, being 360 ppm. At full term her zinc level was down to 170 ppm. This pattern is consistent with the work of Bergman (1980) which was discussed in Chapter 10.

[43] Conceive Well and Bio Zinc are proprietary names for a major Australian producer and marketer of nutritional supplements and may not be available in other countries.

The final HTMA graph shows how the pregnancy has used up the store of elements that perhaps had been sequestered in tissue. They are all now well balanced. She is continuing with both the Pregnancy Gold and Bio Zinc supplements. One important and difficult item to explain is the appearance of the cadmium and mercury in samples C and B. It is possible that the increased zinc levels have mobilised the toxins allowing them to be excreted via the hair, and possibly, although not quantified, via the urine. Zinc is an antagonist to both mercury and cadmium (Watts 2003) and therefore the high level of zinc in the system could have been responsible for this reduction or conversely zinc which is bound to metallothionein has been replaced by heavy metals (Zhang, Georgiev et al. 2003).

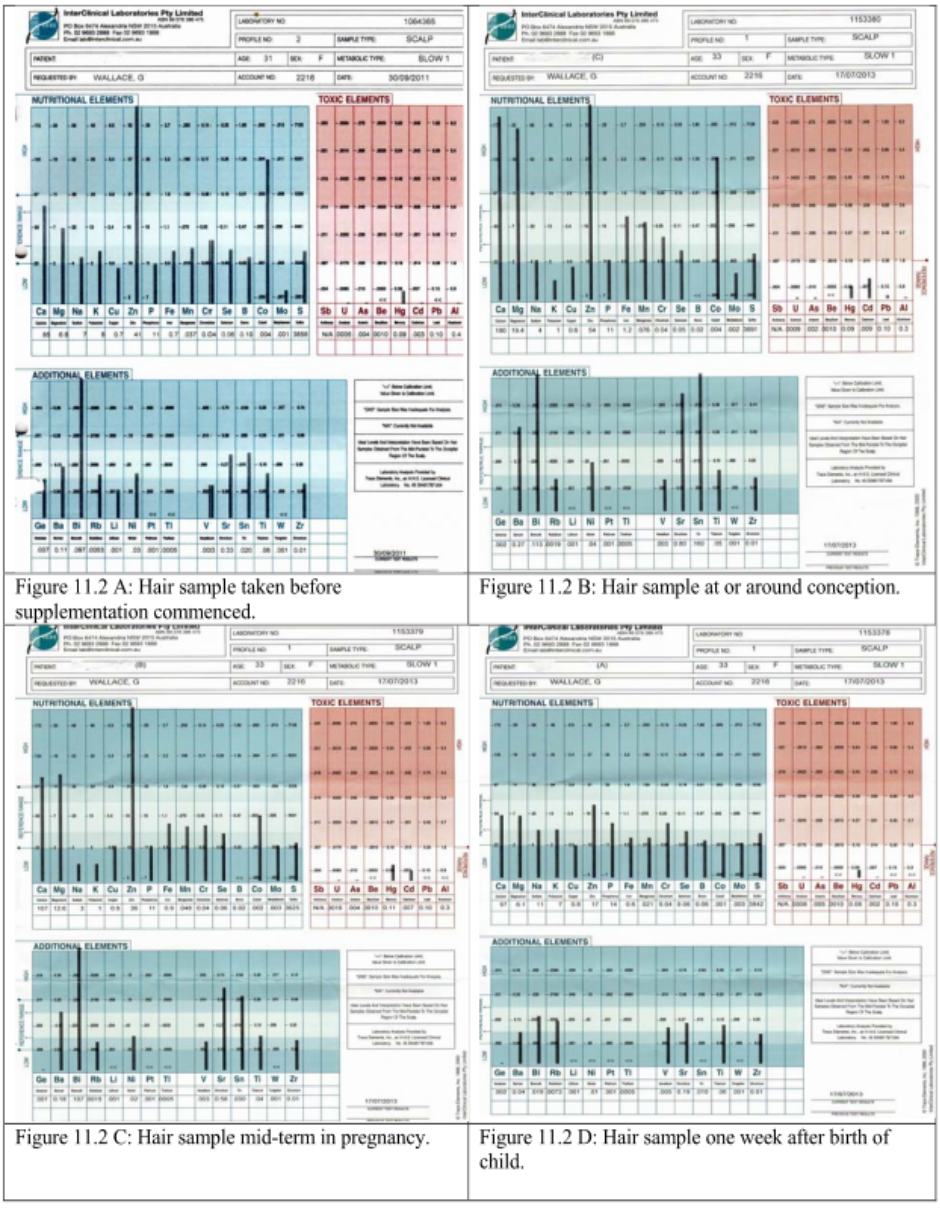


Figure 11.2 A: Hair sample taken before supplementation commenced.

Figure 11.2 B: Hair sample at or around conception.

Figure 11.2 C: Hair sample mid-term in pregnancy.

Figure 11.2 D: Hair sample one week after birth of child.

Figure 11.2 Hair sample from participant having difficulty to conceive

11.4 Conclusion

None of the above can be taken as conclusive evidence for the accuracy of HTMA in predicting adverse pregnancy outcomes as a sample of one can never prove a hypothesis. These samples were merely to illustrate the point that HTMA may be a useful tool to assist the practitioner. However, in situations where a practitioner may consider a woman to be at risk of having an adverse pregnancy outcome, be malnourished, reside in an area which may be toxic, or be having trouble to conceive, such a test may be useful. If such is not the case it may still be useful by providing a guide to any supplementation that a mother may require prior to conception. As mentioned above, it is presented here merely for other researchers to contemplate the use of HTMA.

Chapter 12 Conclusions

12.1 Summing Up

Each study in this thesis has added a colour to our rainbow indicating not only that another aspect of clefting has been investigated but that further knowledge has been gained by the researcher. An analogy may be that the researcher throughout this study was moving from darkness to light. The darkness of ignorance to a better, and yet dim light, of understanding the chemistry of procreation and reproduction, as well as the innermost thoughts of people who had experienced the trauma of an association with cleft lip and palate. However, having arrived at the end of the rainbow where fictionally it is said there is a pot of gold, we find that the rainbow is only the part of the spectrum that we can see and that there is much more to be determined as we enter the ultra-violet segment. Indeed this is true of this thesis as we have not been able to determine a cause and effect but have merely scratched the surface of a difficult question: Why does clefting occur?

The researcher witnessed first-hand the poverty in some countries where clefting is more prominent than in Western societies. Discussions with other researchers were undertaken when the researcher visited Hong Kong, Cambodia, and Poland.

Finally, in coming to an understanding of the possible links to clefting the researcher used music to try to communicate some of his hypotheses to parents who may be considering a pregnancy, and to the public in general. The DVD attached does not aim to portray a detailed analysis of the subject, but as this thesis is embedded in primary health where health promotion is a key ingredient, it is presented to encourage other researchers to also find innovative methods to promote their work and in this case, pregnancy planning. While this DVD is based on ideas developed during this body of work it cannot be considered authoritative due to the small sample sizes involved in each study.

The researcher's knowledge gained from the various studies, which need more extensive investigation, led the researcher to propose a hypothesis for others to contemplate.

12.2 The Hypothesis

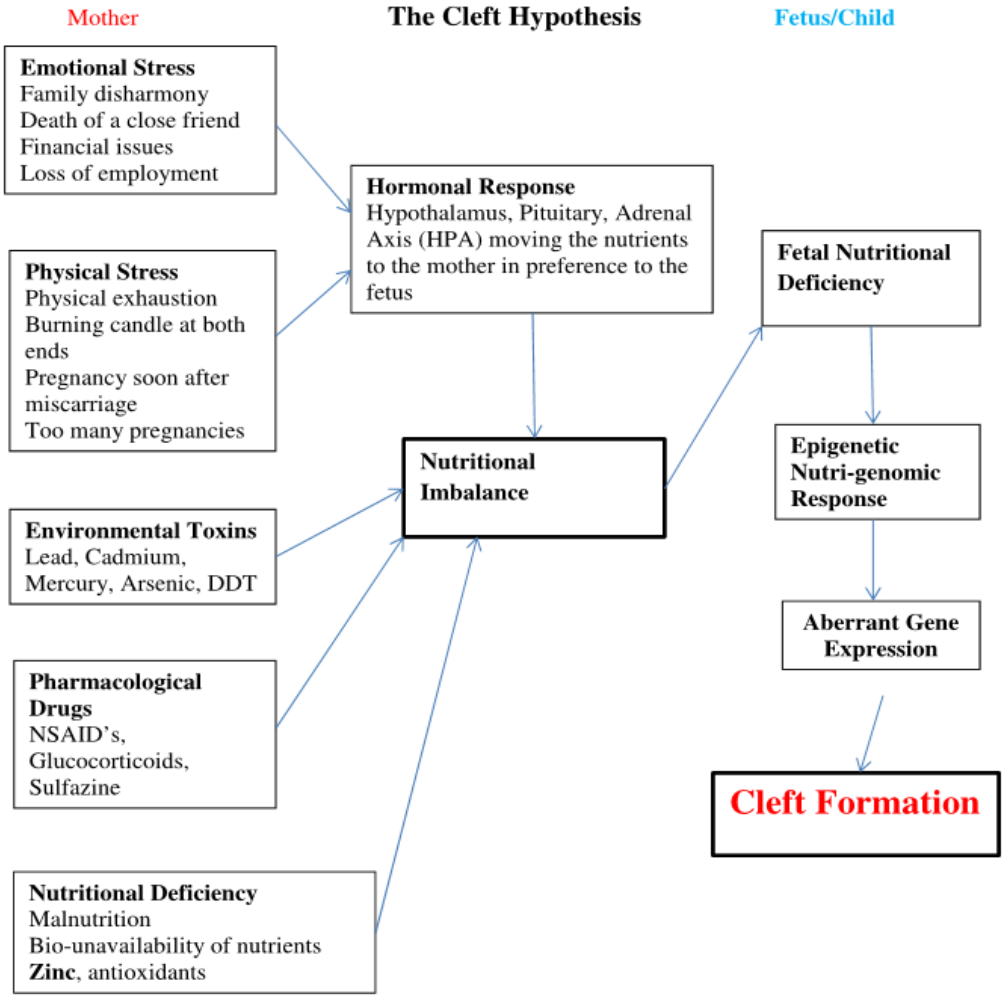


Figure 12.1 The hypothesis

Based on the work done for this thesis the researcher suggests that while there are several possible casual factors their impacts may all follow a similar path as shown above. It would seem that in the case of stress the hormonal response is to provide nutritional support to the mother in preference to the fetus, thus denying the fetus nutrition at a critical time. One possible deficiency may be zinc which is required in a multitude of important metabolic pathways including cell replication. Environmental toxins and pharmacological drugs must by their very nature impact on the nutritional balance, hormone response, cell-signalling molecules, with either inflammation or anti-inflammation in the body. Obviously a nutritional deficiency that is already existent in the mother will have a similar impact. It is hypothesised that if such a deficiency occurs, the genes involved in cell differentiation and replication may not be able to carry out their designated function.

This may be referred to as epigenetics or nutrigenomics. Epigenetics is the study of heritable changes in gene activity that are not caused by changes in the DNA sequence but rather by changes such as DNA methylation, histone methylation and acetylation. These changes in epigenetic state alter gene expression and sometimes cellular phenotype. In a broad sense then, epigenetics is the bridge between genotype and phenotype (Goldberg, Allis et al. 2007). It is stated that cell differentiation may be considered an epigenetic phenomenon largely governed by the 'epigenetic landscape' rather than alterations in genetic inheritance (Goldberg, Allis et al. 2007). In essence then there is something above (epi) the gene that causes it to either switch on or off. Environmental factors such as diet, micronutrient supplementation and exposure to toxins can induce these changes.

Nutrigenomics is the understanding of molecular-level interaction between nutrients and other dietary interactives with the genome. In the past decade, nutrition research has undergone an important shift in focus from epidemiology and physiology to molecular biology and genetics (Muller and Kerston 2003). Muller and Kerston (2003) further state that there has been a growing recognition that micronutrients and macronutrients can be potent dietary signals that influence the metabolic programming of cells, and have an important role in the control of homeostasis. Within this thesis however no claim can be made regarding the absolute nature of how a gene has been impacted upon. It can only be said that the data suggest that a nutritional deficiency or hormonal imbalance may contribute to clefting.

As it appears that clefting affects males more than females, gene variants associated with it could be X-linked and any DNA damage sustained on the X chromosome by adverse environmental factors will therefore preferentially affect males more than females.

Other findings were:

1. The medical fraternity in general is completely unsure of the cause of CLP. Most consider it multi-factorial.
2. Children born with a cleft in Australia tend to cope with life in general with a very positive attitude. Their progress through to adulthood follows a normal path, except for the surgical procedures required.
3. The parents of Australian children born with a cleft experience trauma but this is reduced to some extent by the professionalism of the plastic and reconstructive surgeons and support staff.

4. In the developing countries of Cambodia and the Philippines medical practitioners and health workers believe it is a problem of the poor.
5. Traumatic stress experienced by susceptible individuals at or around conception may be related to birth anomalies and CLP in particular.
6. Physical and emotional stress following a miscarriage and then pursuing pregnancy immediately after may be related to a birth anomaly. In countries where family planning is restricted, having a pregnancy after multiple children (8+) appears related to birth anomalies in following pregnancies. These women may be physically worn out, poverty also being a major factor.
7. Smoking and alcohol consumption during pregnancy while not common in the participants of this study, may cause oxidative stress, and therefore should be avoided prior to and during pregnancy.
8. Zinc appears to be an extremely important element in procreation. In women who bear a child with a cleft this element appears either bio-unavailable or is being excreted, and thus not available during facial development. This takes the form of elevated zinc levels in the hair. Zinc deficiency alone, however, may not be the cause as there may well be a need for both zinc and folate sufficiency to be available to avoid a birth anomaly.
9. Elevated cortisol levels were found in all pregnant women but much more so in women who were carrying a fetus with a diagnosed cleft. This latter fact may be related to the fact that the women knew that their child would be born with a cleft.
10. Other elements which may be of importance are the antioxidants manganese and selenium. Supplements containing these may be useful prior to pregnancy.
11. Hair tissue mineral analysis (HTMA) may be an appropriate method to identify biomarkers of a woman's nutritional status and her ability to produce a normal healthy child.
12. In the interviews with the Australian women who had a cleft child none ever raised the issue of postnatal depression. It is thought that the early support by the medical profession (surgeons and other health workers) together with the support of CleftPals assisted in removing some of the burden these parents may otherwise have experienced.
13. It is difficult to see how the conclusions from this work can be utilised in developing countries where there are limited funds, rapidly expanding populations, high unemployment and poor education. This conclusion has also been supported by others who have undertaken studies in developing countries (McMichael, Waters et al. 2005). Improved health systems in these countries may not be improved by systematic reviews

alone as more direct action from policy makers including social and economic changes will be required if real progress is to be made (Garner, Kale et al. 1998).

14. Food fortification specifically in relation to rice could be worthwhile in the developing Asian countries or in others where rice is a major part of diet.
15. The source of water in developing countries should be continually monitored to ensure that toxins such as arsenic are not present.
16. Agricultural chemicals and their use in all countries must be monitored to ensure that they do not enter the food chain.

12.3 Thoughts at the Macro Level for the Community and Health Professionals

In the past decade we have seen huge changes in the way in which people communicate. The changes have been largely the result of the Internet whereby a large amount of information previously only available to a few is now in the public arena. Web pages exist for many health issues and there is no doubt that these are widely read. However, in the past five years the technology has expanded even further, bringing information onto the mobile phone with the younger age groups communicating via Facebook, Twitter, and other applications. As information on pregnancy must reach those in the younger age groups the challenge is, how can these new communication vehicles be used to promote a responsible approach to conception? In this study the researcher suggested music may be one way of communicating and while this may be appropriate for one group it could not be seen as being the universal solution. Those involved in health promotion must now look at new and novel ways to involve and engage the young in a dialogue on conception and pregnancy.

12.4 Strengths and Weaknesses of the Study

12.4.1 Strengths

Having a chemistry background, and an understanding of nutritional medicine was of benefit. Being able to investigate clefting from a position of little knowledge of clefting and then build on studies incrementally to gather sufficient information on which to base a hypothesis allowed each step to be rigorously pursued.

In the overseas locations female translators and interviewers were made available to assist the researcher. The fact that more than one person was present all the time who spoke the participant's language/dialect when the participant had no English ensured that what was said was a true translation, and therefore the rigour of the interview was not compromised.

12.4.2 Weaknesses

From the outset funding was the main issue, and that led to parts of the proposed research being cut from each of the individual studies undertaken.

The Australian governments irrespective of their political persuasion, had seemingly little interest in this research,[44] two NHMRC applications for funding being rejected. This could be construed as complacency because money has been provided for other health care research, which they obviously deem as sufficient, but the fact is that very little is provided for primary health care and early intervention studies, which perhaps should be their focus. It is acknowledged that many other researchers have the same difficulties in obtaining funds and while it was disappointing not to have support, a substantial part of the planned work was achieved.

An accepted disadvantage was that the researcher was male delving into a very personal issue which affected females more than males. It was accepted from the start that having a cleft child was a traumatic emotional event in any mother's life and an empathetic understanding of this was required to be taken in every conversation with parents. The researcher admits that this was at times very difficult and often confronting. Also, for reasons not fully understood, the women, both cleft mothers and controls, were concerned about providing hair samples, and many who initially volunteered withdrew when they were fully aware of the details. In addition the package of information and the extent of the questionnaire, may have been a factor in women declining to be party to the research, although this was never stated. In the study where blood and urine samples were being taken the distance of the mother from a collection centre also impacted on the participant numbers.

12.5 A Final Statement Regarding Where to Next

12.5.1 Recommendations for Future Research

This body of work points to the concept that clefting in Australia may be reduced by the improved dissemination of preconception information. This may have a beneficial side effect of encouraging people beyond this cohort to improve their health status. Certainly research must be undertaken to determine innovative ways to achieve this. In this body of work music was seen as one way to get a message out to parents who the researcher came in contact with,

[44] Numerous letters were sent to government agencies and politicians with few responses. Politicians who pledged support failed to deliver on any undertaking. Two formal applications were submitted to the National Health and Medical Research Council (NHMRC) for support and both were rejected.

and to obtain leverage into the media to obtain radio and press exposure. A more professional approach is required which involves technology that is being used by young people in the child bearing age group.

In the developing countries, and especially those like the Philippines, research has to be undertaken to see how food fortification and supply can be improved. Perhaps it may be possible to increase zinc levels in rice, or to promote the eating of brown rice instead of the more refined products. Research has already commenced in this area (Johnson, Kyriacou et al. 2011) with the researchers being able to increase both the iron and zinc levels in rice using a single gene strategy. This work is still being pursued at a laboratory level but shows great promise to improve the nutrient intake of people across the world whose primary food source is rice. It may also be necessary to work with local agricultural groups to ensure that the soil where these crops are grown is enhanced with these nutrients so that this new strain of rice can in fact extract the nutrient during its growth period. Work must be undertaken in developing countries to ensure a clean water supply free from toxins such as arsenic.

It is not possible here to define all the areas of research that may flow from the studies covered in this thesis but it is considered that this work has opened up new areas for future work, which hopefully will lead to the reduction in the number of children being born with facial anomalies.

12.6 Known Positive Outcomes from these Studies

The following are some of the known positive outcomes from the present studies:

- The published paper "Cleft Lip and Palate-Could Stress be a Causal Factor?" has been regularly visited online and cited, as recorded by the Research Publication Monitor – Research Gate.
- The DVD *Genes at Work and Play – Making Babies* has been uploaded onto YouTube but is not getting the exposure expected. Obviously someone more experienced in the social media area is required to exploit this.
- Prospective parents who have been following these studies have had children with no birth anomalies.
- Professor Claire Roberts, NHMRC Senior Research Fellow of the Robinson Institute, School of Paediatrics & Reproductive Health, University of Adelaide, South Australia, a supervisor of this work, was influenced by these studies and that of other

researchers to undertake further research into the roles of micronutrients in pregnancy outcomes.

Appendices

Appendix 1: Questionnaire – Community Awareness

Non-Syndromic Cleft Lip and Palate Study Questionnaire

Post Code ……..

What is your age? 20 – 30 ☐

30 – 40 ☐

40 – 50 ☐

50 – 60 ☐

60 + ☐

Sex: Female ☐ Male ☐

Do you have children of your own?

Yes ☐ No ☐

What is your current or previous occupation?

What is your highest education?

1. **Are you familiar with the condition in a child known as a cleft Lip and/or Palate (hare lip)?**

Yes ☐ No ☐ ➔ stop questionnaire here

If Yes, what do you know about this condition?

……

……

2. **What do you believe to be the cause of a CLP?**

……

……

3. Do you know if this problem is prevalent in Australia?

Yes ☐ No ☐ Don't know ☐

4. Do you think this condition can be corrected?

Yes ☐ No ☐ Don't know ☐

If Yes, explain how?

……

……

5. How many operations do you believe each child would need on average to correct:

- A Cleft Lip ……..
- A Cleft Lip and a cleft Palate ……..
- A Cleft Palate ……..

6. Do you know if the Government provides support to families of these children?

Yes ☐ No ☐ Don't know ☐

If Yes, in what form does this take? Please describe.

……

……..

7. Do you know if these children require Speech Therapy?

Yes ☐ No ☐ Don't know ☐

If yes, Should the Government provide financial assistance to the parents?

Yes ☐ No ☐ Don't know ☐

8. Do you know how much the correction of this problem may costs?

(…….) *create your categories*.

9. Should the Government support research into this problem?

Yes ☐ No ☐

Southern Cross University

Participant Information Sheet

My name is Graeme Wallace and I am studying at Southern Cross University (SCU) and completing a PhD qualifier. As part of this study, I am conducting research to try to identify possible causes of cleft lip and palate in children in the Philippines. My Supervisor at SCU is Dr Tini Gruner. She is available at the following contact numbers.

Dr Tini Gruner
Lecturer
School of Natural and Complementary Medicine
Southern Cross University
PO Box 157
Lismore
NSW 2480
Australia
Ph: (02) 6620 3349
Fax: (02) 6620 3307
Mobile: 0416 175 153
e-mail: tini.gruner@scu.edu.au

At this stage we hope that be talking to you and others we may be able to find some common thread which will direct our attention to the cause of the problem.

To date most research has been carried out on surgical procedures and speech therapy. A small amount of work has been done trying to determine if folate deficiency is involved with this problem but it would appear that this has not been the case. Most of this work has been done using animal studies.

From my research it would appear that very few people have actually carried out a field study in an area where anecdotally the incidence of this problem is higher than in other countries.

I would like to conduct an interview with you based around some questions that may be relevant to your knowledge. For your information a copy of these is attached. The interview will take approximately one hour. I would like to conduct this interview at your office so that you will not be inconvenienced.

I must point out that your involvement is on a purely voluntary basis and you are free to withdraw at any time should you not wish to proceed.

Any information provided by you no matter what form this takes would be returned to you if you subsequently felt that you did not want it included as part of my research.

We assure you that your involvement as a participant in this program does not involve any risk to you at all with all information provided being confidential and none will be disclosed to third parties.

If you wish to receive any feedback in relation to the research program and its progress please contact me at the address which is listed below.

The ethical aspects of this study have been approved by the Southern Cross University Human Research Ethics Committee. The Approval Number is ECN-05-163. If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the Committee through the Ethics Complaints Officer, Ms Suze Kelly, (telephone (02) 6626-9139 or fax (02) 6626 9145, email: skelly1@scu.edu.au

Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome.

My contact details are as follows:

Graeme H. Wallace

Unit 1, 13 Elizabeth Street

Doncaster East

Victoria 3109

Australia

Telephone: 61 3 9848 7890 (Office)

61 3 9848 3514 (Private)

Facsimile: 61 3 9848 7839

Email: graemew@bigpond.net.au

While in the Philippines: C/- Sulo Hotel, Quezon City

Appendix 3: Consent Form – Philippine Study

Southern Cross University

Consent Form

Thank you for agreeing to speak to me regarding my research into the cause of Cleft Lip and Palate. This is an important piece of research and given that we do find the reason for this problem, women all around the world will eventually benefit from your input.

It is important for both of us that we agree on the terms of this interview and accordingly ask that you read the following consent statements and if these are acceptable to you sign at the bottom of the form.

Terms of the agreement

I agree to have notes taken by the researcher during the interview and the information provided to be included in any subsequent document that the researcher produces.

I understand that the researcher will provide me with feedback on the research project should I so desire. In such a case I will contact the researcher whose details appear below in writing.

I understand that I can freely withdraw from this research at any time I understand that neither my name nor any identifying information will be disclosed or published, except with my permission.

I understand that the Southern Cross University's Ethics Committee has approved this project.

I am aware that I can contact the researcher at any time after the interview.

If I have any further questions about this study, or have further information to contribute, I am aware that I am free to contact the Research Supervisor whose details follow:

Dr Tini Gruner
Lecturer
School of Natural and Complementary Medicine
Southern Cross University
PO Box 157
Lismore
NSW 2480
Australia
Ph: (02) 6620 3349
Fax: (02) 6620 3307
Mobile: 0416 175 153
e-mail: tini.gruner@scu.edu.au

The ethical aspects of this study have been approved by the Southern Cross University Human Research Ethics Committee (HREC). The Approval Number is ECN-05-163

If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the HREC through the Ethics Complaints Officer, Ms Suze Kelly, (telephone [02] 6626 9139, fax [02] 6626 9145, email: skelly1@scu.edu.au. Any complaint you make will be treated in confidence.

I understand that I will be given a copy of this form to keep.

I have read the information above and agree to participate in this study. I am over the age of 18 years.

Name of Participant: ……...

Signature of Participant: ……

Date: ……

I certify that the terms of the Consent Form have been verbally explained to the participant and that the participant appears to understand the terms prior to signing the form. Proper arrangements have been made for an interpreter where English is not the participant's first language.

Signature of Witness ……....

Date: ……

Researcher

Graeme H. Wallace
Unit 1, 13 Elizabeth Street
Doncaster East
Victoria 3109
Australia

Telephone: 61 3 9848 7890 (Office)
61 3 9848 3514 (Private)
Facsimile: 61 3 9848 7839
Email: graemew@bigpond.net.au

Appendix 4: Questionnaire – Philippine Study

Southern Cross University

Non-Syndromic Cleft Lip and Palate Study Questionnaire

Contact Number …….

Do you believe that there is a high incidence of Cleft Lip and Palate in the Philippine community?

☐ Yes ☐ No

What information do you have which gives you this impression?

……

……

Is there any particular group within the community where this appears more prevalent?

☐ Yes ☐ No

What information do you have which gives you this impression?

……

……

Do you have any idea as to why children are born with facial defects?

☐ Yes ☐ No

If Yes what do you think is the cause?

……

……

If it was suggested that the cause may be one or more of the following:

Genetic influences
Nutritional Status of the parents
Environmental toxins,
given your own particular skill base and experience could you elaborate on why you think any one of these areas might be implicated?

☐ Yes ☐ No

If "Yes" please outline your reasons for believing this to be a factor.

……

……

……

It has been stated by the Philippine Government that many families do not have a clean water supply or any appropriate sanitation. If such is the case how do these families obtain their water and what sanitation facilities would they use?

……

……

Could these factors have any impact on the problem?

☐ Yes ☐ No

If "Yes" why do you think this?

Do you believe that there is a high level of pollution in the Philippines?

☐ Yes ☐ No

What form does this take?

Air ☐

Water ☐

Effluent ☐

Garbage ☐

Other ☐

……

……

Could one or more of these be implicated?

……

……

Additional comments:

……..

Appendix 5: Questionnaire for Non-parent Interviews

Questionnaire for non- parent interviews

Name of participant, place of work, department and title

This was done to determine their position within the establishment in which the interview was conducted and their authority and ability to contribute valid information to the study. This information was not recorded on the Questionnaire but was cross-referenced to a Participant Number (Contact Number) only known to the researcher to ensure confidentiality.

Do you believe that there is a high incidence of Cleft Lip and Palate in the Philippine community and what information do you have which gives you this impression?

Is there any particular group within the community where this appears more prevalent and what information do you have which gives you this impression?

These questions were asked to open up a discussion as to the severity of the incidence and the possibility that it was confined or at least dominant within certain areas or groups of people.

Do you have any idea as to why children are born with facial defects, and if so what do you think is the cause? (This question was followed by the next if people were uncertain or said that they had no idea, just to see whether they saw any logic in progressing down this path).

If it was suggested that the cause may be one or more of the following:

- *genetic influences*
- *nutritional status of the parents*
- *environmental toxins*

Given your own particular skill base and experience, could you elaborate on why you think any one of these may be implicated?

These questions were asked to elicit comments regarding both concept and experience in relation to the person's contact with the problem. This was particularly important in relation to the medical staff.

It has been stated by the Philippine Government that many families do not have a clean water supply nor any appropriate sanitation. If such is the case, how do these families

obtain their water and what sanitation facilities would they use and could these factors have any impact on the problem?

Do you believe that there is a high level of pollution in the Philippines?

What form does this take and could one or more of these pollutants that you have identified be implicated?

Appendix 6: Mancozeb Label and Data Sheet

(Extracts only full data sheet available at:
http://www.syngenta.com/country/au/SiteCollectionDocuments/Labels/INNOVA%20MANCOZEB%20750%20FUNGICIDE%20Label.pdf)

CAUTION

KEEP OUT OF REACH OF CHILDREN

READ SAFETY DIRECTIONS BEFORE OPENING OR USING

innova

Mancozeb 750

FUNGICIDE

ACTIVE CONSTITUENT: 750 g/kg MANCOZEB

GROUP FUNGICIDE

For the control of certain fungal diseases of field crops, fruit, ornamentals, tobacco, turf and vegetables as per the Directions for Use

25 kg NET

Syngenta Crop Protection Pty Limited

Level 1, 2-4 Lyon Park Road, North Ryde NSW 2113

APVMA Approval No: 61356/25/0906

IMa0806

®

®

DIRECTIONS FOR USE

For use in All States where appropriate for crop and/or disease

Restraint: DO NOT incorporate this product with an integrated mite control program

NOT TO BE USED FOR ANY PURPOSE, OR IN ANY MANNER, CONTRARY TO THIS

LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION

WITHHOLDING PERIODS

Harvest

Custard Apples, Mangoes, Pawpaws (Papaya):

DO NOT HARVEST FOR 1 DAY AFTER APPLICATION

PAPAYA LEAVES MUST NOT BE MADE AVAILABLE FOR HUMAN CONSUMPTION

Beans, Carrots, Celery, Cole crops, Cucurbits, Garlic, Green Legumes (green crops), Onions, Peas, Pulse crops (green crops), Tomatoes:

DO NOT HARVEST FOR 7 DAYS AFTER APPLICATION

Almonds, Beetroot, Capsicums, Cotton, Fennel, Lettuce, Passionfruit, Peanuts, Pome fruit, Rhubarb, Silver Beet, Spinach, Stone fruit:

DO NOT HARVEST FOR 14 DAYS AFTER APPLICATION

Green Legumes (grain crops), Pulse crops (grain crops):

DO NOT HARVEST FOR 4 WEEKS AFTER APPLICATION

Grapevines:

DO NOT HARVEST FOR 30 DAYS AFTER APPLICATION

Poppies:

DO NOT HARVEST FOR 7 WEEKS AFTER APPLICATION

Bananas, Citrus, Potatoes, Tobacco:

NOT REQUIRED WHEN USED AS DIRECTED

Grazing

Cotton:

DO NOT ALLOW LIVESTOCK TO GRAZE TREATED COTTON CROP, STUBBLE OR GIN TRASH

Papaya:

DO NOT USE OR SUPPLY TREATED PAPAYA INCLUDING LEAVES FOR STOCK FOOD

Beans, Peas (for fresh consumption):

DO NOT GRAZE OR CUT FOR STOCK FOOD FOR 7 DAYS AFTER APPLICATION

Green Legumes, Peanuts, Pulse crops (grown for dry beans):

DO NOT GRAZE OR CUT FOR STOCK FOOD FOR 14 DAYS AFTER APPLICATION

GENERAL INSTRUCTIONS

INNOVA MANCOZEB 750 is a protectant fungicide for the control of certain fungal diseases in many crops.

For best results, apply as a regular spray program.Thorough coverage of the treated crop is essential.

Mixing

Slowly add the required amount of product to the spray tank as it is being filled or thoroughly premix in a

nurse tank for concentrate or aerial spraying. Add other products after INNOVA MANCOZEB 750 is in suspension.

Continue agitation while spraying to prevent the product settling out and to ensure a uniform spray mixture. Rinse out spray tank, pumps and nozzles at the end of the day.

When preparing spray solutions for use in a hand sprayer, premix as a slurry in a small container, and then add to sprayer containing ⅓ to ½ the desired final water volume.

Compatibility

INNOVA MANCOZEB 750 may be harmful to *Typhlodromus pyri* and its use in orchards where integrated pest control is practiced should be avoided.

This product is compatible with most commonly used insecticides and miticides such as azinphos, carbaryl, dicofol, endosulfan, pirimicarb, propargite, sulphur powder, wetting agent or spreader-sticker.

Also compatible with D-C-Tron.

As formulations of other manufacturers' products are beyond the control of Syngenta and water quality varies with location, all mixtures should be tested prior to mixing commercial quantities.

Surfactants

The addition of a surfactant will improve initial spray deposits, fungicide redistribution and weatherability.

Refer to manufacturers label instructions for further details.

Application

Dilute spraying: Use a sprayer designed to apply high volumes of water up to the point of runoff and matched to the crop being sprayed. Set up and operate the sprayer to achieve even coverage throughout the crop canopy. Apply sufficient water to cover the crop to the point of runoff. Avoid excessive runoff.

The required water volume may be determined by applying different test volumes, using different settings on the sprayer, from industry guidelines or expert advice. Add the amount of product specified in the Directions for Use table for each 100 L water. Spray to the point of runoff. The required dilute spray volume will change and the sprayer set up and operation may also need to be changed, as the crop grows.

Concentrate spraying: Use a sprayer designed and set up for concentrate spraying (that is a sprayer which applies water volumes less than those required to reach the point of runoff) and matched to the crop being sprayed. Set up and operate the sprayer to achieve even coverage throughout the crop canopy using your chosen water volume. Determine an appropriate dilute spray volume (see *Dilute spraying* above) for the crop canopy. This is needed to calculate the concentrate mixing rate. The mixing rate for concentrate spraying can then be calculated in the following way.

Example only:

1. Dilute spray volume as determined above, eg 1500 L/ha

2. Your chosen concentrate spray volume, eg 500 L/ha

3. The concentration factor is 3 x (ie 1500/500 = 3)

4. If the dilute label rate is 10 mL/100 L, then the concentrate rate becomes 3 x 10, ie 30 mL/100 L of concentrate spray.

The chosen spray volume, amount of product/100 L water and the sprayer set up and operation may need to be changed as the crop grows. For further information on concentrate spraying, users are advised to consult relevant industry guidelines, undertake appropriate competency training and always follow Industry Best Practices.

For fruit trees and some row crops apply as a high volume dilute spray.

The spray volume will vary according to the type of crop to be treated and should be increased in heavy growth and on large plants to ensure thorough coverage. The spray volume should also be increased as the size of treated crops increases throughout the growing season. As a guide for mature crops:

Bananas, tobacco, vines, other row crops 200 to 1100 L/ha

Pome, stone fruit 1100 to 4000 L/ha

Citrus 4000 to 8000 L/ha

Aerial application: May be applied by agricultural aircraft. Use at least 30 to 50 L spray mixture/ha.

Spray timing

Treatment should begin prior to disease infection and continue until the threat of disease passes.

Repeated applications may be required to protect new growth. Reduce the spray interval when weather conditions favour disease development.

Fungicide Resistance Warning GROUP FUNGICIDE

INNOVA MANCOZEB 750 Fungicide is a member of the multi site activity group of fungicides. For fungicide resistance management the product is a Group Y fungicide. Some naturally occurring individual fungi resistant to INNOVA MANCOZEB 750 and other Group Y fungicides may exist through normal genetic variability in any fungal population. The resistant individuals can eventually dominate the fungi population if these fungicides are used repeatedly. These resistant fungi will not be controlled by INNOVA MANCOZEB 750 or other Group Y fungicides, thus resulting in a reduction in efficacy and possible yield loss.

Since the occurrence of resistant fungi is difficult to detect prior to use, Syngenta Crop Protection Pty Ltd accepts no liability for any losses that may result from the failure of INNOVA MANCOZEB 750 to control resistant fungi.

PROTECTION OF LIVESTOCK

DO NOT graze treated turf/grass or feed turf/grass clippings from any treated area to poultry or livestock.

PROTECTION OF WILDLIFE, FISH, CRUSTACEANS, ENVIRONMENT AND OTHERS

Toxic to fish.

Drift and runoff from treated areas may be hazardous to aquatic organisms in neighbouring areas.

DO NOT contaminate streams, rivers or waterways with the chemical or used containers.

DO NOT re-use containers.

STORAGE AND DISPOSAL

Store in the closed, original container in a dry, cool, well ventilated area out of direct sunlight.

Shake bag contents into spray tank until the bag is empty. DO NOT dispose of undiluted chemicals on site. Puncture or shred and bury empty bags in a local authority landfill. If no landfill is available, bury the containers below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty bags and product should not be burnt.

SAFETY DIRECTIONS

May irritate the eyes and skin. Repeated exposure may cause allergic disorders. Avoid contact with eyes and skin. DO NOT inhale dust.

When opening the container and preparing spray, wear:

- **cotton overalls, over normal clothing, buttoned to the neck and wrist**
- **washable hat**
- **elbow length PVC gloves**
- **goggles**
- **disposable dust mask covering mouth and nose**

When using the prepared spray wear:

- **cotton overalls, over normal clothing, buttoned to the neck and wrist**
- **washable hat**
- **elbow length PVC gloves**

Wash hands after use. After each day's use, wash gloves, goggles and contaminated clothing.

FIRST AID

If poisoning occurs, contact a doctor or Poisons Information Centre. Phone 131 126. Avoid giving

alcohol.

MATERIAL SAFETY DATA SHEET

If additional hazard information is required refer to the Material Safety Data Sheet. For a copy phone 1800 067 108 or visit our website at www.syngenta.com.au

MANUFACTURER'S WARRANTY AND EXCLUSION OF LIABILITY

Syngenta has no control over storage, handling and manner of use of this product. Where this material is

not stored, handled or used correctly and in accordance with directions, no express or implied representations or warranties concerning this product (other than non-excludable statutory warranties) will

apply. Syngenta accepts no liability for any loss or damage arising from incorrect storage, handling or use.

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are registered trademarks of a Syngenta Group Company
* Registered trademark
APVMA Approval No: 61356/25/0906 IMa0806
Batch No
e of Manufacture
Expiry Date 2 years from date
of manufacture
UN-3077
ENVIRONMENTALLY HAZARDOUS
SUBSTANCE, SOLID, N.O.S. (MANCOZEB)
MARINE POLLUTANT
PACKING GROUP III
HAZCHEM 2X
MARINE POLLUTANT
In a transport emergency dial 000, Police or
Fire BrigadeFor specialist advice in an emergency only, call 1800 033 111 (24 hours)

Appendix 7: Manganese

(http://www.umm.edu/altmed/articles/manganese-000314.htm)

DESCRIPTION

Manganese is an essential trace mineral in animal nutrition and is believed to be an essential trace mineral in human nutrition, as well. Manganese is a metallic element with atomic number 25 and an atomic weight of 54.94 daltons. Its chemical symbol is Mn. Manganese exists in the oxidation states Mn^{2+} or Mn(II) and Mn^{3+} or Mn(III) under physiological conditions.

Dietary manganese-deficiency in animals results in a wide variety of structural and physiological defects, including growth retardation, skeletal and cartilage malformations, impaired reproductive function, congenital ataxia due to abnormal inner ear development, optic nerve abnormalities, impaired insulin metabolism and abnormal glucose tolerance, alterations in lipoprotein metabolism and an impaired oxidant defense system.

Manganese deficiency states have not been well documented in humans. There is one report of a man maintained for four months on a manganese-deficient diet and also given magnesium-containing antacids. The symptoms which occurred included a decrease in serum cholesterol, depressed growth of hair and nails, scaly dermatitis, weight loss, reddening of his black hair and beard and impaired blood clotting. He responded to a diet containing manganese. In another report, men fed a low-manganese diet manifested low serum cholesterol levels and dermatitis. Short-term manganese supplementation did not reverse these symptoms.

In still another report, young women fed a manganese-poor diet were found to have mildly abnormal glucose tolerance and increased menstrual losses of manganese, calcium, iron and total hemoglobin. Finally a child on long-term total parenteral nutrition (TPN) lacking manganese manifested bone demineralization and impaired growth that were corrected by supplementation with manganese.

Manganese is the preferred metal cofactor for glycosyltransferases. Glycosyltransferases are important in the synthesis of glycoproteins and glycosaminoglycans (GAGs or mucopolysaccharides). Glycoproteins are involved in the synthesis of myelin and the clotting factors, among other things. Manganese-containing metalloenzymes include manganese superoxide dismutase, the principal antioxidant enzyme of mitochondria, arginase, pyruvate carboxylase and glutamine synthetase.

The richest dietary sources of manganese include whole grains, nuts, leafy vegetables and teas. Manganese is concentrated in the bran of grains which is removed during processing. Mean intakes of manganese worldwide range from 0.52 to 10.8 milligrams daily.

ACTIONS AND PHARMACOLOGY

ACTIONS

Manganese may have antioxidant activity. Manganese has putative anti-osteoporotic and anti-arthritic activities.

MECHANISM OF ACTION

Manganese ions have been found to scavenge hydroxyl and superoxide radicals. The mechanism of binding of manganese ions to these reactive oxygen species is not known. Manganese is a crucial component of the

metalloenzyme manganese superoxide dismutase (MnSOD). MnSOD is found in mitochondria and is the principal constituent of the mitochondrial oxidant defense system. Rats and mice fed manganese-deficient diets are found to have reduced MnSOD activity in heart muscle and nervous tissue. They also have mitochondrial abnormalities and pathological changes in these tissues. The pathological changes are thought to result from oxidative damage due to the decreased activity of MnSOD which normally would protect against this damage.

Dietary manganese deficiency results in skeletal and cartilage malformations in animals and in one human report. It is thought that this is due to decreased activity of the manganese-dependent glycosyltransferases which, among other things, are involved in the synthesis of glycosaminoglycans or GAGs. GAGs are crucial for healthy cartilage and bone. However, there is as yet only very preliminary evidence that supplemental manganese has any effect on the promotion of bone or cartilage formation in humans who are not manganese-deficient. One study reported that manganese when taken in combination with calcium, copper and zinc may improve bone mineral density in postmenopausal women with osteoporosis.

PHARMACOKINETICS

There is scant information on the pharmacokinetics of manganese in humans. The efficiency of absorption (fractional absorption) of ingested manganese appears to be low, about 5%. Absorption efficiency appears to decrease as dietary intake of manganese increases. It increases with low dietary intake of manganese. Absorption appears to occur throughout the small intestine and appears to occur by both active-transport and passive diffusion mechanisms. Manganese ions are transported via the portal circulation to the liver. In what forms manganese is transported to the liver—bound to albumin, alpha$_2$-macroglobulin, hydrated manganese complexes, etc.—is also unclear. A fraction of manganese is taken up by hepatocytes and a fraction is transported by the systemic circulation to the various tissues of the body. Some manganese is bound to the plasma protein transferrin, but there also appear to be other carriers that transport manganese in the systemic circulation. Manganese is found principally in the mitochondria of cells. Absorbed manganese is excreted primarily via the biliary route. Very little manganese is excreted in the urine.

INDICATIONS AND USAGE

Apart from its uses in rare overt deficiency disorders, manganese might have some efficacy in osteoporosis and osteoarthritis as well as in some with premenstrual syndrome (PMS). Evidence for these benefits is preliminary.

RESEARCH SUMMARY

Manganese supplementation, in combination with calcium, zinc and copper, showed some efficacy in postmenopausal osteoporosis. Manganese ascorbate, in combination with glucosamine hydrochloride and chondroitin sulfate, was helpful in treating knee osteoarthritis pain in a recent randomized, double-blind, placebo-controlled pilot study. Followup on these studies is needed. Similarly, there is an isolated study needing followup that suggested some possible benefit from manganese in alleviating some PMS symptoms, including anxiety, depression, irritability and mood swings.

CONTRAINDICATIONS, PRECAUTIONS, ADVERSE REACTIONS

CONTRAINDICATIONS

Manganese supplements are contraindicated in those with liver failure. Some patients with end-stage liver disease have been found to accumulate manganese in their basal ganglia. It is thought that manganese may play a role in the

hepatic encephalopathy in those with liver failure. Manganese is eliminated primarily through the bile, and hepatic dysfunction leads to depressed manganese excretion.

Manganese supplements are contraindicated in those hypersensitive to any component of a manganese-containing supplement.

PRECAUTIONS

Pregnant women and nursing mothers should avoid intakes of manganese above the upper limit of the estimated safe and adequate daily dietary intake (ESSADI). The ESSADI for those 11 years and older is 2.0 to 5.0 milligrams daily.

ADVERSE REACTIONS

Oral manganese supplements are generally well tolerated. Oral manganese, however, may be neurotoxic in those with liver failure. Manganese is primarily eliminated via the biliary route, and hepatic dysfunction leads to depressed manganese excretion. Manganese may accumulate in the basal ganglia of those with liver failure and may exacerbate hepatic encephalopathy and/or cause Parkinson's disease-like symptoms.

Manganese is toxic under certain conditions. Hepatic failure was discussed above. Mine workers exposed to high concentrations of manganese dust develop what is known in the mining villages of northern Chile, where this disorder has been found, as "locura manganica" or manganese madness. In later stages of this disease, symptoms similar to those of Parkinson's disease are observed. Levodopa is the treatment of the later stages of manganese madness.

There are a few reports of manganese intoxication occurring in those on long-term total parenteral nutrition (TPN) who developed parkinsonism which was treated with levodopa.

INTERACTIONS

DRUGS

Antacids: Magnesium-containing antacids, such as aluminum hydroxide/magnesium hydroxide, aluminum hydroxide/magnesium carbonate and aluminum hydroxide/magnesium trisilicate, may decrease the absorption of manganese if taken concomitantly.

Laxatives: Magnesium-containing laxatives may decrease the absorption of manganese if taken concomitantly.

Tetracycline: Tetracycline may reduce the absorption of manganese if taken concomitantly.

NUTRITIONAL SUPPLEMENTS

Calcium: Calcium supplements may decrease the absorption of manganese if taken concomitantly.

Iron: Non-heme iron supplements may reduce the absorption of manganese if taken concomitantly.

Magnesium: Magnesium supplements may decrease the absorption of manganese if taken concomitantly.

FOODS

Concomitant intake of manganese with foods rich in phytic acid (unleavened bread, raw beans, seeds, nuts and grains and soy isolates) or oxalic acid (spinach, sweet potatoes, rhubarb and beans) may depress the absorption of manganese.

DOSAGE AND ADMINISTRATION

There are several forms of supplementary manganese, including manganese gluconate, manganese sulfate, manganese ascorbate and manganese amino acid chelates. Manganese is available as a stand-alone supplement and also in combination products. One combination product used for bone/joint health contains chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate.

Typical supplemental intake of manganese ranges from 2 to 5 milligrams daily.

The Food and Nutrition Board of the U.S. National Academy of Sciences has recommended the following estimated safe and adequate daily dietary intake (ESADDI) values for manganese:

Age (years)	ESADDI (milligrams)
0 to 0.5	0.3 to 0.6
0.5 to 1	0.6 to 1.0
1 to 3	1.0 to 1.5
4 to 6	1.5 to 2.0
7 to 10	2.0 to 3.0
11 to 18	2.0 to 5.0
Adults	2.0 to 5.0

Up to 10 milligrams daily of manganese is considered safe.

LITERATURE.

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Appendix 8: Participant Information Sheet

Southern Cross University

Participant Information Sheet

My name is Graeme Wallace. I am studying at Southern Cross University (SCU) and completing a PhD. As part of this study, I am conducting research to try to identify possible causes of Cleft Lip and Palate in children. My Supervisor at SCU is Dr Tini Gruner. She is available at the following contact numbers.

Dr Tini Gruner
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Southern Cross University
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NSW 2480
Australia
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Fax: (02) 6620 3307
Mobile: 0416 175 153
e-mail: tini.gruner@scu.edu.au

Volunteers

The volunteers we are seeking are mothers of children born with a non-syndromic cleft lip and/or palate (CLP) whose child having this condition is not more than two years old. We understand that you have volunteered to be part of this program and that your child is under the age of two.

Project Outline

At this stage we hope that by talking to you and others we may be able determine the best course to follow for a detailed research study. Clefting has in the past been described by researchers as a multifactorial problem which in essence has meant that nobody knows why this occurs.

Most research has been carried out on surgical procedures and speech therapy. A small amount of work has been done trying to determine the cause(s) of the problem but most of this work has been conducted in animals. Some family studies have been carried out, but these have been restricted to mother/father/child relationships. While this research has suggested that a family connection may exist this has not been verified by other research.

I would like to conduct an interview with you based on a questionnaire which, once collated with others, may provide information that could expand our knowledge of the subject.

For your information a copy of the questionnaire is attached. The interview will take approximately 30 minutes. I would like to conduct this interview at a time and place convenient to you.

I must point out that your involvement is on a purely voluntary basis and you are free to withdraw at any time should you not wish to proceed.

Any information provided by you, no matter what form this takes, would be returned to you if you subsequently felt that you did not want it included as part of this research.

We assure you that your involvement as a participant in this program does not involve any risk to you at all, with all information provided being confidential, and none will be disclosed to third parties. Should papers be published using data gained from this study all the information will be of an anonymous nature, and none of your personal details will be disclosed.

If you wish to receive any feedback in relation to the research program and its progress please contact me at the address which is listed below.

The ethical aspects of this study have been submitted to and considered by the Southern Cross University Human Research Ethics Committee – EC00137, and the approval number is ECN 06-155.

If you have any complaint or reservations about any ethical aspect of your participation in this research you may contact the Committee through the Ethics Complaints Officer, Ms Sue Kelly, (telephone (02) 6626-9139 or fax (02) 6626 9145, email: sue.kelly@scu.edu.au

Any complaint you make will be treated in confidence and investigated, and

you will be informed of the outcome.

My contact details are:

Graeme H. Wallace

Unit 1, 13 Elizabeth Street

Doncaster East

Victoria 3109

Australia

Telephone: 61 3 9848 7890 (Office)

61 3 9848 3514 (Private)

Email: graemew@bigpond.net.au

Thank you for your participation.

Graeme Wallace

Appendix 9: Participant Consent Form

Southern Cross University

Cleft Lip and Palate Research Program – Participant Agreement form

We are conducting a research program to try and determine the cause of Cleft Lip and Palate and invite you to help us with this study. At this stage the cause is unknown but we hope that by talking to you and others we might get an idea where to begin the search for the solution to this problem.

All the information will be kept confidential throughout the research program and your name will not be disclosed in any reporting which is undertaken as part, or at the conclusion, of the study. Should you at any time wish to withdraw the information you have provided we would willingly return all relevant data.

It is necessary that your agreement to this program is without pressure from us and is done of your own free will. To confirm this we would appreciate it if you could sign this form below.

I……. of the following address

……..

freely and willingly agree to participate in this research program to try and identify the cause of Cleft Lip and Palate occurring in children.

I further agree to the results of any analysis of the information provided being used as part of the research paper when written and trust that the outcome of the research will benefit other members of the community.

Signed…… Date…….

Print Name……

Witness …… Date……

Print Name……. Title ……

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Email: jacinta.arellano@scu.edu.au

Appendix 10: Questionnaire

Southern Cross University

Non-Syndromic Cleft Lip and Palate Study Questionnaire

In this questionnaire, we ask general questions about your health at the time of the birth, the foods you eat and information about yourself, your family and those of the father and his family. The information that we collect is kept confidential and all future reference to you is as a Code Number. No personal information is passed on to a third party.

Personal Details

Mrs./Miss/Ms. Surname: …….

Given Names: …….

Address

……

……

……

Post Code ……..

Email Address …….

Contact Phone Numbers (optional)

Work…… Home ……

After Hours …… Mobile……

Do you currently work? ☐ Yes ☐ No

If Yes; what is your occupation? ……

If No; what was your previous occupation ? ……

How long were you employed in this field? ……..

What is the father's occupation? ……

How long has he been employed in this field?

What is your age? …… What is the father's age? …….

How many children do you have and what are their ages? (Please circle sex of child)

Child 1 Age…….. M / F

Child 2 Age…….. M / F

Child 3 Age…….. M / F

Child 4 Age…….. M / F

Which child has a CL/P? ……

Did the child with the CL/P have: (please circle)

Unilateral Cleft Lip

Bilateral Cleft Lip

Unilateral Cleft Lip with Cleft Palate

Bilateral Cleft Lip with Cleft Palate

Cleft Palate only

With this child did you have an Ultrasound? ☐Yes ☐No

At what time in the pregnancy was this done? ☐ 12 Weeks ☐ 20 Weeks

☐ Other time; please state ……..

Was the problem diagnosed during the Ultrasound ☐Yes ☐No

Did any of your other children have medical issues? ☐Yes ☐No

If Yes; please describe:

……

……

What is your height? ……Ft……..inches. Or…….metres

What was your weight prior to the pregnancy with the child that was born with CL/P?

…… kg.

Do you smoke at present? ☐Yes ☐No

If Yes; how many cigarettes per day? …… ☐

How long have you been smoking? …….

Have you smoked in the past? ☐Yes ☐No

If Yes; how many cigarettes per day? . ☐

How long ago did you quit? …… For how many years did you smoke?……

Does the child's father smoke? ☐Yes ☐No

If Yes: How many cigarettes per day? ☐

Were you a regular smoker prior to being pregnant? ☐Yes ☐No

Were you or the father smoking during the pregnancy of the child with the CLP?

☐ Yes ☐ No

Have you had any of the following medical conditions have you had diagnosed by a doctor? (please tick)

- ☐ High blood pressure
- ☐ Heart disease
- ☐ Angina
- ☐ Stroke
- ☐ Diabetes
- ☐ Insulin resistance
- ☐ Overweight
- ☐ Migraine
- ☐ Arthritis

☐ Cancer (specify type) …….

☐ Kidney disease

☐ Liver Disease

☐ Asthma

☐ Gout

☐ Hepatitis B or C

☐ HIV

☐ Hypothyroid

☐ Hyperthyroid

☐ Eczema

☐ Allergies - Food

……

- Drugs …….

- Environmental toxins

…….

☐ None of the above

List any medications taken regularly or during the pregnancy when the child was born with the CL/P.

Medication	Dose	How Often?
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….

Do you follow or have you followed a special diet? (Please tick)

☐ Vegan

☐ Vegetarian

☐ Weight Loss - Low Calorie

- Low Carbohydrate
- Low fat
- High Protein

☐ Other

Briefly list foods that you do not eat:

…….

…….

…….

…….

…….

Do you crave certain foods? If so please list.

……..

……..

……..

……..

Did you take any supplements (such as, vitamins, minerals, herbs) either before or during the pregnancy

☐ Yes ☐ No Before Pregnancy ☐ During ☐

If Yes; please list:

Supplement	**Type**	**Dose**	**How Often?**	**Taken Before,**

	(Vitamin = V) (Mineral = M) (Herb = H) (Other = O)			**or during Pregnancy, or both?**

Did you drink alcohol during the pregnancy? ☐ Yes ☐ No

If Yes; what quantity and how often? ……

Family Genealogy

Did you have a CL/P? ☐ Yes ☐ No

If Yes; describe type: …….

Did the father have a CL/P?

☐ Yes ☐ No

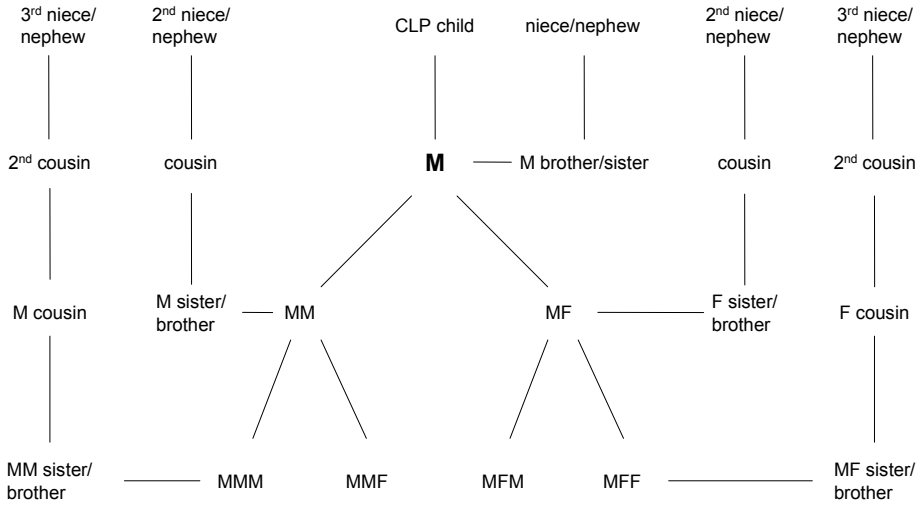
If Yes; describe type:

Is (are) there case(s) of clefting in other members of the family? ☐ Yes ☐ No

If Yes; please circle in the charts below the relationship between you/the father and the person where a cleft occurred, and indicate beside that whether it was one of the following:

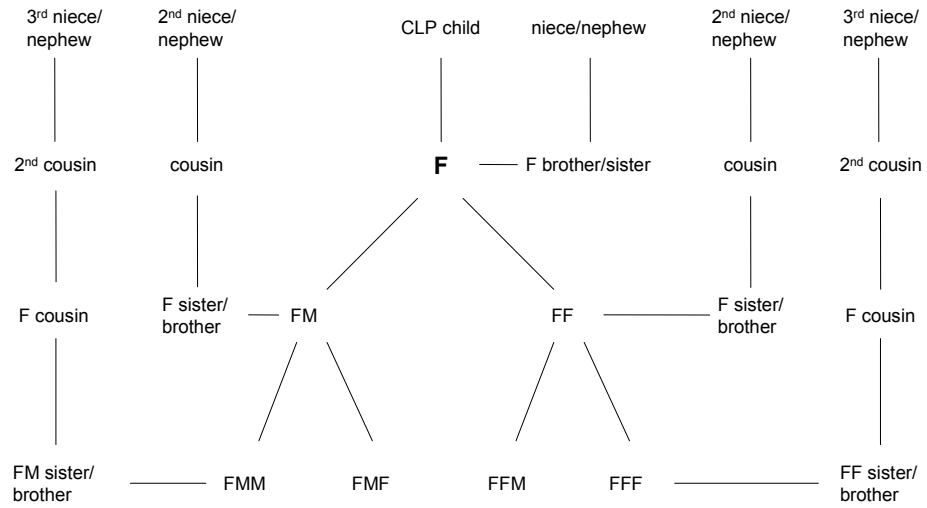
1. Cleft Lip – Unilateral – CL(U)
2. Cleft Lip – Bilateral – CL(B)
3. Cleft Lip, Unilateral and Palate – CL(U)P
4. Cleft Lip, Bilateral and Palate – CL(B)P
5. Cleft Palate only – CP
6. Syndromic CL and/or P – CLP(S). In this case please state which syndrome in words. …….

Relationship via mother's family



M = mother, F = father

Relationship via father's family



M = mother, F = father

Are you aware of any medical condition that appears to be inherent in your family?

☐ Yes ☐ No

If Yes; describe type: …….

Are you aware of any medical condition that appears to be inherent in the father's family?

☐ Yes ☐ No

If Yes; describe type: …….

Additional Information

Is there any further information that you believe may be relevant to the study?

☐ Yes ☐ No

If so please outline the details:

…….

…….

Supplementary Questions

What was the birth weight of this child? Kg. lbs. oz.

Did you carry this child to full term? ☐Yes ☐No

If "No". What was the duration? Weeks

How would you describe yourself:

☐ Easy going

☐ Worries sometimes

☐ Worries often

☐ Continually worries about the future

In the period one month prior to conception to two months after conception was there any event that occurred in your life, or your families, that caused you anxiety or stress above the normal stresses of life?

☐ Yes ☐ No

If Yes; please describe:

……

……

Appendix 11: Participant Information Sheet and Consent Form Stage 5.

INFORMATION SHEET

The Aetiology of non-Syndromic Cleft Lip and Palate

Introduction

My name is **Graeme Wallace** and I am conducting research as part of a Doctor of Philosophy Degree at Southern Cross University. My qualifications are in the fields of Chemistry and Nutritional Medicine. Thank you for agreeing to take part in this project.

What is this research about?

We are investigating potential causes of Cleft Lip and Palate (CLP) in children, including family history, nutritional deficiencies, environmental toxins and stress levels prior to and during pregnancy by studying one group of mothers whose unborn child has been diagnosed with CLP and another group of mothers whose child has not been diagnosed with CLP.

Oxygen is an essential element in the maintenance of life; however during cellular metabolism damaging products called free radicals are produced. These elements are unstable and highly reactive structures that can interact with important cellular components or even the cell membrane. Oxidative stress describes the steady state of oxidative damage in the cell due to reactive oxygen species such as free radicals. These may have a nutritional basis and develop from metabolic reactions including energy production, or from sources outside the body such as exposure to cigarette smoke, environmental pollutants or ionising radiation.

This project aims to investigate potential causes of CLP by determining whether oxidative stress can be linked with altered genetic expression in the fetus by exploring the possibility that oxidative stress is a result of a nutritional deficiency, the presence of an environmental toxin or whether it is a result of physical or emotional stress.

Who is conducting the study?

The study is being conducted by Mr Graeme Wallace and is supervised by Dr Tini Gruner who is a Naturopath and Lecturer at Southern Cross University in Lismore NSW. Laboratory samples will be analysed by Gribbles Pathology.

What am I required to do?

Firstly we ask you to complete a questionnaire that will provide us with information relating to various aspects of your nutritional, medical, environmental and social history. The questionnaire could take about an hour of your time to complete. Any information we obtain from you that personally identifies you will be kept in secure storage, accessible only to the study team. You will be allocated a unique number and all data will be analysed according to this number, not your name. Data will be reported in aggregate form – that is, no data will be reported that even identifies your unique number. If the information gathered in the consent form or questionnaire causes you any distress we can arrange for you to see a counsellor. The cost of seeing the counsellor would not be covered by the study.

We need to collect a blood sample, a 24 hour urine sample and a hair sample from you.

Twenty four hour urine test

You will be supplied with a collection bottle for this test, and you should only use this bottle for the collection. It is important that you drink your usual amount of fluids each day. When you get up in the morning pass your first specimen into the toilet, do not collect this specimen. During the day collect all urine using a clean plastic container and pour into the collection bottle. Store the bottle in a cool place and rinse the plastic container after use. When you get up the next morning (at the same time as the day before) pass urine into the plastic container and add it to the collection bottle. Write the time and date on the bottle.

Deliver the urine specimen to the nearest Gribbles Pathology Unit or nominated collection centre. It would be best to coordinate your visit to the pathology unit and have your blood taken at the same time.

Blood test

We need to test your blood for various study parameters such as, a full blood count, liver function, urinary electrolytes, iron studies, lipid levels, thyroid function, vitamin and mineral levels, antioxidant levels, and other relevant measurements. These tests will be fasting tests, so you should not eat anything for 12 hours before the clinic, but you can drink water, but not tea or coffee or orange juice. The tests will be conducted at Gribbles Pathology and you will need to make arrangements with a Gribbles Pathology Unit nearest your home regarding the time of your blood test (The nearest collection centre to you will be advised by the researcher). We will be happy to supply you with copies of the results of these tests.

Hair analysis

We will provide you with a kit for this test and this will contain instructions on what you need to do and where to take the hair sample. The amount of hair required will be minimal and will not cause you any cosmetic concerns,

Costs

The cost of the blood, urine and hair analysis will be met by the study team. If you have transport difficulties which will prevent you attending the Pathology Unit please inform the study team.

When you have your baby

We would like to ring you after you have your baby to see how you and your baby are, and whether you had a boy or a girl and the baby's weight and height. If you prefer not to have this contact please tell us before you sign the consent form.

Are there any risks involved in the study?

Blood collection

You will probably already have had a blood test, however, because the testing we want to have done is more comprehensive than normal we need you to provide samples for this. The

standard method of blood collection which no doubt you are fully aware of, will involve placing a standard needle into a vein of your arm. This needle will remain in your arm for about 1 minute, as the necessary blood (approximately 20mL) is collected. Occasionally, when blood is extracted, patients experience minor bruising around the vein. Pressure will be applied on the area for a period of five minutes after the blood has been taken and this will lessen the possibility of bruising. There is also a slight risk of infection related to the use of needles. The method of blood collection will be carried out according to the 'Universal Precautions' guidelines set out in the NHMRC publication "Infection control in the Health Care Setting". Only sterile needles will be used. Personnel engaged in blood collection are accredited phlebotomists, who are fully aware of the regulations and protocols concerning blood borne pathogens and prevention of infection. The risk of HIV in these procedures is minimal, as all blood collections will be performed according to the protocols outlined by Universal Precautions. Personnel working with the blood in the laboratory use the same precautions. All blood and urine collected will be analysed by Gribbles Pathology.

The responsibility of the researcher.

Any information that is obtained in connection with this study, and that can be identified with you, the participant, will remain confidential and will be disclosed only with your permission. We will also undertake to notify your doctor if any of the clinical or blood assessment results reveal a health problem. If you do not have a doctor we will arrange for you to see a physician of your choosing. Once the results have been analysed we will write and inform you of the study outcomes.

The responsibility of the participant.

Your responsibility is to fully disclose information that could affect your personal safety. You also need to fully disclose information that may affect the results of the research.

What happens if I decide I want to pull out of the study?

You are free to withdraw participation from the study at any time. However we ask that you let us know your decision. If you do decide to withdraw from the study all of your data would be destroyed if you ask us to do this.

How can I make further enquires about this study?

If you have any questions or concerns about this study you can contact any one of the following people:

Dr Tini Gruner
School of Health & Human Sciences
Department of Natural and Complementary Medicine
Southern Cross University
PO Box 157
Lismore
NSW 2480
Australia
Telephone: (02) 6620.3349
Email: tini.gruner@scu.edu.au

Mr Graeme H. Wallace
Unit 1, 13 Elizabeth Street
(P.O. Box 4130)
Doncaster East
Victoria 3109
Australia
Telephone: (03) 9848 7890
Mobile: 0418 248 983
Email: graemew@bigpond.net.au

This research has been approved by the Southern Cross University Human Research Ethics Committee. The approval number is: ECN-10-004. If you have any concerns about this project, please contact, in writing in the first instance:

Ms Sue Kelly
Ethics Complaints Officer/HREC

Southern Cross University

PO Box 157

Lismore NSW 2480

Tel: (02) 6626 9139

Email: sue.kelly@scu.edu.au

CONSENT FORM

CONFIDENTIAL

(This consent form is based on the National Statement on Ethical Conduct in Human Research)

The Aetiology of Non-Syndromic Cleft Lip and Palate

NOTE: This consent form will remain with the Southern Cross University researcher for their records.

Tick the box that applies, sign and date and return in the stamped, and addressed envelope supplied.

I agree to take part in the Southern Cross University research project specified above. Yes ☐ No ☐

I understand that my participation is voluntary. Yes ☐ No ☐

I have been provided with information at my level of comprehension about the purpose, methods, demands, risks, inconveniences and possible outcomes of this research. I understand this information. Yes ☐ No ☐

I agree to be interviewed by the researcher if that is required. Yes ☐ No ☐

I agree to make myself available for a further interview if required. Yes ☐ No ☐

I agree to complete the questionnaire asking me about the background to my pregnancy and any nutritional and health aspects. Yes ☐ No ☐

I agree to attend a Pathology collection facility in my local area to provide a sample of blood and urine to be analysed by a recognized commercial laboratory at no cost to myself. Yes ☐ No ☐

I understand that I can choose not to participate in part or all of this research at any time, without consequence. Yes ☐ No ☐

I understand that any information that may identify me will be de-identified at the time of analysis of any data. Therefore, I, or any information I have provided cannot be linked to my person

(*Privacy Act 1988 Cth*) Yes ☐ No ☐

I understand that neither my name nor any identifying information will be disclosed or published. Yes ☐ No ☐

I understand that all information gathered in this research is confidential. It is kept securely and confidentially for no more than 5 years at the University after which time it will be destroyed Yes ☐ No ☐

I am aware that I can contact the Supervisor or other researchers at any time with any queries. Yes ☐ No ☐

I understand that the ethical aspects of this research have been approved by the SCU Human Research Ethics Committee. Approval # ECN-07-141 Yes ☐ No ☐

If I have concerns about the **ethical conduct** of this research, I understand that I can contact the SCU Ethics Complaints Officer. All inquiries are confidential and should be in writing, in the first instance, to the following: Yes ☐ No ☐

Ms Sue Kelly

Graduate Research College

Secretary, HREC

Southern Cross University

Po Box 157

Lismore NSW 2480

Email: sue.kelly@scu.edu.au

Tel: (02) 6626 9139

Participant's name:

I would like to receive my results and I would like to receive any published material about the outcomes of this research. Yes ☐ No ☐

Participant's address for correspondence:

Participant's signature:

Contact: Tel: Email:

Witness Name (If possible):

Witness Signature:

Date:

Researcher's Signature:

Appendix 12: Questionnaire, Stage 5

Non-Syndromic Cleft Lip and Palate Study Questionnaire

In this questionnaire, we ask general questions about your health at the time of conception and early pregnancy, the foods you eat and information about yourself, your family and those of your partner and family. The information that we collect is kept confidential and all future reference to you is as a Contact Number. No personal information is passed onto a third party.

Personal Details

Mrs./Miss/Ms. Surname: …….

Given Names: ……

Address

……

……

……

Post Code ……..

Email Address …….

Contact Phone Numbers (optional)

Work…… Home ……

After Hours …… Mobile……

Please note that this page will be removed from the rest of the questionnaire and filed separately as soon as the form is received to ensure that your privacy is maintained. The data will be given a code so that should the researcher need to contact you again, your information can be retrieved by him. No other person will have the possibility of linking the data back to you.

1. **Do you currently work (paid employment or self employed) ?**

☐ Yes ☐ No

If Yes; what is your occupation? ……

If No; what was your previous occupation ? ……

How long were you employed in this field? ……..

2. **Does your partner work (paid employment or self employed) ?**

☐ Yes ☐ No

If Yes; what is his occupation? ……

If No; what was his previous occupation ? ……

How long was he employed in this field? ……..

3. **How would you define your socioeconomic status?**

☐ Poor

☐ Comfortable

☐ Above average

☐ Well off

4. **What is your age? ……What is your partner's age? …….**

5. How many children do you have and what are their ages? (Please circle sex of child)

Child 1 Age…….. M / F

Child 2 Age…….. M / F

Child 3 Age…….. M / F

Child 4 Age…….. M / F

6. How many weeks pregnant are you? ……

What is the due date for the birth of this child? ……..

Was this a natural conception or IVF? ☐ Natural ☐ IVF

7. If your child has a cleft was the problem diagnosed during the Ultrasound

☐ Yes ☐No

At what time in the pregnancy was this detected? ……

8. Did any of your other children have medical issues?

If Yes; please describe:

……

……

9. What is your height? ……Ft……..inches. or…….metres

10. What was your weight prior to the pregnancy with this child ?

…… kg.

11. Do you smoke at present?

☐Yes ☐No

If Yes; how many cigarettes per day? ……

How long have you been smoking? …….

Have you smoked in the past?

☐Yes ☐No

If Yes; how many cigarettes per day? .

How long ago did you quit? …… For how many years did you smoke?……

12. Does your partner smoke?

☐Yes ☐No

If Yes: How many cigarettes per day?

13. Were you a regular smoker prior to being pregnant?

☐Yes ☐No

In which year did you stop smoking? …….

14. Were you or your partner smoking during the pregnancy of this

☐ Yes ☐ No

15. Which of the following medical conditions have you had diagnosed by a doctor?
(please circle)

High blood pressure

Heart disease

Angina

Stroke

Diabetes

Insulin resistance

Overweight

Migraine

Arthritis

Cancer (specify type) …….

Kidney disease

Liver Disease

Asthma

Gout

Hepatitis B or C

HIV

Hypothyroid

Hyperthyroid

Eczema

Allergies - Food ……..

- Drugs …….

- Environmental toxins ……

None of the above

16. Have you at any time had a miscarriage?

☐ Yes ☐ No

If yes, please supply details of when this occurred and what its relationship was to the timing of this current pregnancy.

……

……

……

17. List any medications taken regularly before or during the pregnancy

Medication	Dose	How Often?
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….
……	…….	…….

18. Do you follow or have you followed a special diet? (Please circle)

Vegan

Vegetarian

Weight Loss - Low Calorie

- Low Carbohydrate
- Low fat
- High Protein

Other

19. Briefly list foods that you do not eat:

……

……

……

……

……

20. Do you crave certain foods? If so please list.

……..

……..

……..

……..

21. Did you take any supplements (such as, vitamins, minerals, herbs) either before or during the pregnancy?

☐ Yes ☐ No Before Pregnancy ☐ During ☐

If Yes; please list:

Supplement	**Type** (Vitamin = V) (Mineral = M) (Herb = H) (Other = O)	**Dose**	**How Often?**

22. Did you drink alcohol during the pregnancy?

☐ Yes ☐ No

If Yes; what quantity and how often? ……

Family Genealogy

23. Did you have a CL/P?

☐ Yes ☐ No

If Yes; describe type: …….

24. Did your partner have a CL/P?

☐ Yes ☐ No

If Yes; describe type: …….

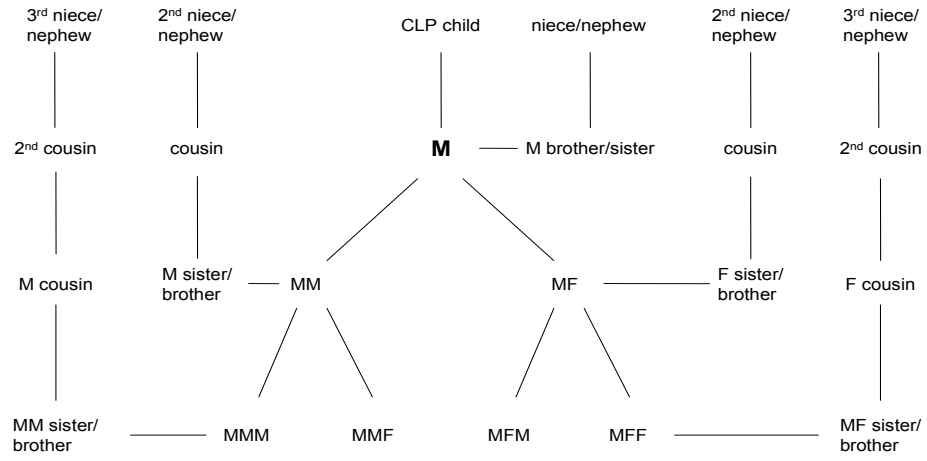
25. Is (are) there case(s) of clefting in other members of the family?

☐ Yes ☐ No

If Yes; please circle in the charts below the relationship to your child of that person where a cleft occurred, and indicate beside that whether it was one of the following:

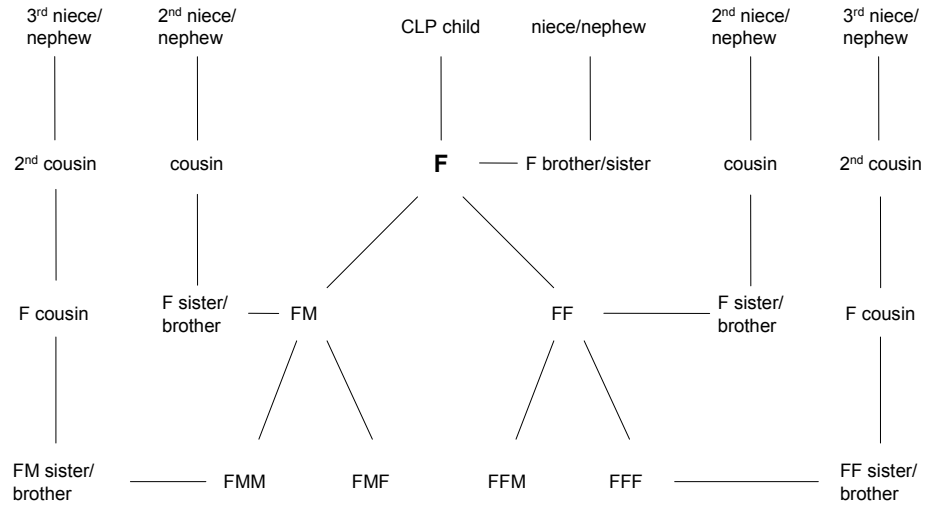
7. Cleft Lip – Unilateral – CL(U)
8. Cleft Lip – Bilateral – CL(B)
9. Cleft Lip, Unilateral and Palate – CL(U)P
10. Cleft Lip, Bilateral and Palate – CL(B)P
11. Cleft Palate only – CP
12. Syndromic CL and/or P – CLP(S). In this case please state which syndrome in words. …….

26. Relationship via Mother's family



M = mother, F = father

27. Relationship via father's family



M = mother, F = father

28. Are you aware of any medical condition that appears to be inherent in your family?

☐ Yes ☐ No

If Yes; describe type: …….

29. Are you aware of any medical condition that appears to be inherent in your partner's family?

☐ Yes ☐ No

If Yes; describe type: …….

30. How would you describe yourself ? :

☐ Easy going

☐ Worries sometimes

☐ Worries often

☐ Continually worry

31. In the period up to three months prior to the pregnancy and into the first trimester did anything occur in your life or in the lives of those around you that may have increased the level of stress in your life above the normal day to day activities?

☐ Yes ☐ No

If Yes; Please describe:

……

……

……

32. Is there any further information that you believe may be relevant to the study?

☐ Yes ☐ No

If Yes; please outline the details:

……

……

……

……

May I contact you after the birth of your baby to gather a few more details which are outlined in the following questions.

☐ Yes ☐ No

33. What was the term of the pregnancy? ……..Weeks

34. What was the weight of the child? ……Kg.

or …….lbs. ……..oz.

35. What was the sex of the child M F

36. Did the child have a cleft? ☐ Yes ☐ No

If Yes please tick the appropriate box below:

☐ Unilateral Cleft Lip

☐ Bilateral Cleft Lip

☐ Unilateral Cleft Lip with Cleft Palate

☐ Bilateral Cleft Lip with Cleft Palate

☐ Cleft Palate only

37. Did the child have any other birth anomalies or medical conditions. If yes please describe below:

……
……
……

Thankyou for taking the time to complete the questionnaire. I will call you when I have received the questionnaire back. Any queries you may have can be discussed then or you may wish to call me if you have any questions on any of the issues raised. (Tel: 03 9848 7890 or 0418 248 983). You are welcome to make a copy of this questionnaire for your own records.

Appendix 13: Independent Statistical Analysis

Mann-Whitney U Test comparing means between Control and CLEFT

P-value < 0.05 (highlighted red) indicates the mean of that mineral for CLEFT is significantly different* than Controls. for others (i.e. P-value > 0.05) the means may still be different, but they are NOT considered statistically significant.

* at 5% significance level.

Table A13.1 Blood and urine analysis

Variables	Control (mean)	CLEFT (mean)	P.value
Age	34.50	31.17	0.183
Child.position.in.family	1.85	1.17	0.036
Weeks.pregN/Ant.at.testing	19.98	23.75	0.146
Weight.prior.pregN/Ancy	66.56	57.27	0.141
Height	163.81	161.33	0.452
BMI	24.93	21.88	0.385
Haematology: Whole Blood			
Haemoglobin	114.77	121.20	0.320
RBC	3.88	3.94	0.830
PCV	0.37	0.37	0.674
MCV	93.65	94.40	0.647
WCC	8.76	9.34	0.591
Neutro	6.48	6.90	0.879
Lymph	1.68	1.68	0.927
Mono	0.42	0.68	0.143
Eosin	N/A	N/A	N/A
Baso	N/A	N/A	N/A
Platelets	247.35	221.25	0.428
ESR	19.80	15.00	0.372
General Chemistry – Serum			
Sodium	137.00	138.00	0.137
Potassium	3.99	3.98	0.806
Chloride	104.54	105.40	0.462
BicarboN/Ate	26.19	26.00	0.869
Anion.Gap	10.00	10.67	0.510
Urea	3.03	3.42	0.138
Creatinine	50.88	46.40	0.282
eGFR	N/A	N/A	N/A
Urate	0.21	0.21	0.808
Calcium	2.21	2.16	0.282

Corr..Ca.	2.30	2.27	0.332
Magnesium	0.84	0.60	0.286
Phosphate	1.21	1.10	0.096
T..Protein	63.77	61.60	0.109
Albumin	33.46	32.80	0.705
Globulin	27.86	28.33	1.000
ALP	59.96	70.60	0.295
Bilirubin	8.65	5.40	0.039
GGT	10.38	8.40	0.466
AST	18.27	21.60	0.404
ALT	18.27	23.00	0.871
LD	158.83	139.67	0.195

General Chemistry: Serum /Plasma

Glucose.Random	4.17	4.26	0.880

Endocrinology Department : Serum

RBC.Folate	N/A	N/A	N/A
Insulin..Fasting.	7.20	6.00	0.783

Endocrinology Department : Serum/Plasma/Urine

pl.cortisol..rndm	563.92	742.60	0.007

Lipid Studies : Serum

Total.Chol.	5.64	6.68	0.162
HDL.Chol.	1.97	2.14	0.573
LDL.Chol.	3.02	3.66	0.302
Triglyceride	1.52	1.90	0.517
LDL.HDL.ratio	1.53	1.82	0.514
Chol.HDL.ratio	2.99	3.28	0.661

Toxicology: Red Cells

Zinc	226.50	226.50	N/A

Iron Studies: Serum/Plasma

Serum.Iron	18.76	16.24	0.290
Transferrin	3.34	3.57	0.488
Saturation	24.58	19.60	0.298
Ferritin	41.67	35.60	0.644

General Chemistry: Serum

Homocysteine	6.14	5.52	0.344
Vitamin.A	1.35	1.17	0.090
Vitamin.E..Total.Tocopherol.	35.86	46.02	0.030
Active.B12	100.33	100.33	N/A
Total.B12	288.74	248.60	0.631

Copper	32.22	31.96	0.893
Zinc.1	10.83	11.86	0.236
Manganese	0.76	0.17	1.000
Selenium	2.30	1.30	1.000
Chromium	18.00	18.00	N/A

Special Chemistry: 24 Hr. Urine

Volume	1907.42	1927.20	0.470
Coll.Period.	24.00	24.00	N/A
Creatinine.1	5.56	5.40	0.751
Creat..Excret.	9.83	10.16	0.707
Sodium.1	74.00	78.20	0.764
Sodium.Excret.	131.48	146.20	0.308
Potassium.1	40.22	39.20	0.764
Potassium.Excret.	72.48	75.60	0.928
pH	6.30	7.40	0.134
Selenium.Excretion	N/A	N/A	N/A

Sample 2: 24 Hr. Urine

Volume.1	1987.38	1817.00	1.000
Coll.Period..1	24.00	24.00	N/A
Creatinine.2	5.48	5.90	0.787
Creat..Excret..1	9.69	10.64	0.851
Calcium.1	3.63	3.36	0.685
Calcium.Excret.	6.89	6.18	0.925
Magnesium.1	1.95	2.20	0.800
Magnesium.2	2.90	4.50	1.000
Chromium.1	N/A	N/A	N/A
Zinc.2	4.17	5.00	0.262
Manganese.1	N/A	N/A	N/A
Manganese.excretion	N/A	N/A	N/A
Copper.1	N/A	N/A	N/A
Iodine	144.39	124.23	0.523
Selenium.1	N/A	N/A	N/A
Cobalt	25.00	25.00	N/A

Amino Acid Profile

Alanine	326.82	378.25	0.226
Arginine	36.35	51.00	0.193
ASA..3..Gaba	0.00	0.00	N/A
Aspargine	73.88	101.00	0.038
CYSTA..allo.iso.leucine	0.00	0.00	N/A
Cysteine	14.93	13.75	0.960

Citrulline	15.82	18.25	0.365
Glutamic.Acid	90.82	94.25	0.928
Glutamine	396.56	426.50	0.344
Glycine	207.59	213.00	0.754
Histidine	78.65	103.50	0.081
HCYS.ASA..2	0.00	0.00	N/A
Iso.Leucine	45.53	48.50	0.686
Leucine	102.41	112.75	0.501
Lysine	173.06	201.25	0.117
Methionine	17.59	21.50	0.043
Ornithine	56.29	61.75	0.654
Phenylalanine	53.71	67.00	0.107
Proline	104.38	118.00	0.276
Serine	95.35	122.75	0.151
Taurine	102.24	138.00	0.446
Threothine	163.82	199.25	0.073
Tyrosine	42.82	56.25	0.151
Valine	168.65	184.25	0.347

Table A13.2 Hair analysis

Mineral	Control (mean)	CLEFT (mean)	P.value
Calcium	64.96	122.83	0.288
Magnesium	7.58	10.52	0.322
Sodium	15.65	3.67	0.197
Potassium	6.15	2.17	0.183
Copper	4.56	2.90	0.699
Zinc	16.35	22.33	0.015
Phosphorus	12.23	12.17	0.863
Iron	0.72	1.07	0.364
Manganese	0.09	0.04	0.612
Chromium	0.05	0.04	0.406
Selenium	0.06	0.10	0.306
Boron	0.28	0.23	0.530
Cobalt	0.01	0.01	0.605
Molybdenum	0.00	0.00	0.804
Sulphur	4163.08	4067.50	0.556
Germanium	0.01	0.01	0.806
Barium	0.13	0.16	0.611
Bismuth	0.29	0.01	0.762

Rubidium	0.01	0.00	0.322
Lithium	0.00	0.00	0.125
Nickel	0.03	0.06	0.051
Platinum	0.00	0.00	N/A
Thallium	0.00	0.00	N/A
VaN/Adium	0.01	0.00	0.169
Strontium	0.18	0.53	0.147
Tin	0.08	0.07	0.697
Titanium	0.14	0.12	1.000
Tungsten	0.00	0.00	N/A
Zirconium	0.03	0.02	0.680
Antimony	N/A	N/A	N/A

Appendix 14: Information Sheet and Consent Form Stage 6

INFORMATION SHEET

The Aetiology of non-Syndromic Cleft Lip and Palate

Introduction

My name is **Graeme Wallace** and I am conducting research as part of a Doctor of Philosophy Degree at Southern Cross University. My qualifications are in the fields of Chemistry and Nutritional Medicine. We invite you to take part in this project which we hope may provide further understanding regarding the occurrence of Cleft Lip and Palate in children.

What is this research about?

We are investigating potential causes of Cleft Lip and Palate (CLP) in children, including family history, nutritional deficiencies, environmental toxins and stress levels prior to and during pregnancy by studying one group of mothers whose child has been born with a CLP and another group of mothers whose child had no anomalies.

Diet has an impact on every part of our life. It provides the nutrients that we need to produce the energy required for us to undertake our daily tasks. Obviously due to differing circumstances or location within a country the dietary intake of each individual varies. Such variations can impact on different people in different ways.

This project aims to investigate potential causes of CLP by determining the nutritional status of a mother who has had a child with a CLP to see if specific nutrients can be linked to altered genetic expression in the fetus

Who is conducting the study?

The study is being conducted by Mr Graeme Wallace and is supervised by Dr Tini Gruner who is a Naturopath and Nutritionist and Lecturer at Southern Cross University in Lismore NSW.

Dr Jacinta Arellano and Associate Professor Claire Roberts are co-supervisors in this study. We are supported in the Philippines by members of Operation Rainbow Australia Limited, Interplast, the local hospital staff where surgery is taking place, and the generous support of the local Rotary members and Freemasons.

What am I required to do?

Firstly we ask you to answer some questions so that the local staff member can complete a questionnaire on your behalf that will provide us with information relating to various aspects of your nutritional, medical, environmental and social history. The interview should take about ten minutes of your time to complete. Any information we obtain from you that personally identifies you will be kept in secure storage, accessible only to the study team. You will be allocated a unique number and all data will be analysed according to this number, not your name. Data will be reported collectively, that is, no data will be reported that even identifies your unique number.

Secondly, we would appreciate it if we could take a small sample of your hair.

The responsibility of the researcher

Any information that is obtained in connection with this study, and that can be identified with you as the participant, will remain confidential and will be disclosed only with your permission.

The responsibility of the participant

Your responsibility is to fully disclose information that could affect your personal safety. You also need to fully disclose information that may affect the results of the research.

What happens if I decide I want to pull out of the study?

You are free to withdraw your participation from the study at any time. However, we ask that you let us know your decision. If you do decide to withdraw from the study all of your data will be destroyed at your request.

How can I make further enquires about this study?

If you have any questions or concerns about this study you can contact any one of the following people:

Mr Graeme H. Wallace

Unit 1, 13 Elizabeth Street

(P.O. Box 4130)

Doncaster East

Victoria 3109

Australia

Telephone: 61 3 9848 7890

Mobile: 61 418 248 983

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This research has been approved by the Southern Cross University Human Research Ethics Committee. The approval number is: ECN-. If you have any concerns about this project, please contact, in writing in the first instance:

Ms Sue Kelly

Ethics Complaints Officer/HREC

Division of Research

Southern Cross University

PO Box 157

Lismore, NSW 2480

Tel: 61 2 6626 9139

Email: sue.kelly@scu.edu.au

CONSENT FORM

CONFIDENTIAL

(This consent form is based on the National Statement on Ethical Conduct in Human Research)

The Aetiology of Non-Syndromic Cleft Lip and Palate

NOTE: This consent form will remain with the Southern Cross University researcher for their records.

Tick the box that applies, sign and date and return in the stamped and addressed envelope supplied.

I agree to take part in the Southern Cross University research project specified above. Yes ☐ No ☐

I understand that my participation is voluntary. Yes ☐ No ☐

I have been provided with information at my level of comprehension about the purpose, methods, demands, risks, inconveniences and possible outcomes of this research. I understand this information. Yes ☐ No ☐

I agree to be interviewed by the researcher if that is required. Yes ☐ No ☐

I agree to make myself available for a further interview if required. Yes ☐ No ☐

I agree to complete the questionnaire asking me about the background to my pregnancy and any nutritional and health aspects. Yes ☐ No ☐

I understand that I can choose <u>not</u> to participate in part or all of this research at any time, without consequence. Yes ☐ No ☐

I understand that any information that may identify me will be <u>de-identified</u> at the time of analysis of any data. Therefore, I, or any information I have provided, cannot be linked to my person. (*Privacy Act 1988 Cth*) Yes ☐ No ☐

I understand that neither my name nor any identifying information will be <u>disclosed or published.</u> Yes ☐ No ☐

I understand that all information gathered in this research is confidential. It is kept securely and confidentially for five years at the University after which time it will be destroyed. Yes ☐ No ☐

I am aware that I can contact the researcher or supervisor at any time with any queries. Yes ☐ No ☐

I understand that the ethical aspects of this research have been approved by the SCU Human Research Ethics Committee. Approval # ECN- Yes ☐ No ☐

If I have concerns about the ethical conduct of this research, I understand that I can contact the SCU Ethics Complaints Officer. Yes ☐ No ☐

I would like to receive my results and I would like to receive any published material about the outcomes of this research. Yes ☐ No ☐

If Yes:

Participant's name:

Participant's address for correspondence:

Participant's signature:

Contact: Tel: Email:

Witness Name

Witness Signature:

Date:

Researcher's Signature:

Appendix 15: Questionnaire Stage 6

Non-Syndromic Cleft Lip and Palate Study Questionnaire

In this questionnaire, we ask general questions about your health at the time of conception and early pregnancy, the foods you eat and information about yourself, your family and those of your partner and family. The information that we collect is kept confidential and all future reference to you is as a Contact Number. No personal information is passed onto a third party.

Personal Details

Mrs/Miss/Ms Surname: …….

Given Names: ……..…….

Address

……

……

……

Post Code ……..

Email Address …….

Contact Phone Numbers (optional)

Work…… Home ……

After Hours …… Mobile……

Please note that this page will be removed from the rest of the questionnaire and filed separately as soon as the form is received to ensure that your privacy is maintained. The data will be given a code so that should the researcher need to contact you again, your information can be retrieved by him. No other person will have the possibility of linking the data back to you.

1. **Do you currently work (paid employment or self-employed)?**

☐ Yes ☐ No

If Yes, what is your occupation? …….....

If No, what was your previous occupation? …….....…

How long were you employed in this field? …….……..

2. **Does your partner work (paid employment or self-employed)?**

☐ Yes ☐ No

If Yes, what is his occupation? …….....……

If No, what was his previous occupation? …….....……

How long was he employed in this field? ……....……..

3. **How would you define your socioeconomic status?**

☐ Poor

☐ Comfortable

☐ Above average

☐ Well off

4. **What is your age? ……What is your partner's age? …….**

5. **How many children do you have and what are their ages? (Please circle sex of child)**

Child 1 Age…….. M / F Child 5 Age…….. M / F

Child 2 Age…….. M / F Child 6 Age…….. M / F

Child 3 Age…….. M / F Child 7 Age…….. M / F

Child 4 Age…….. M / F Child 8 Age…….. M / F

6. Did any of these children have a cleft? If so please nominate which ones and the type of cleft below:

……

……

7. **Did any of your children have other medical issues?**

☐ Yes ☐ No

If Yes, please describe:

……..……

……..……

8. What was the length of your last pregnancy? …….Weeks

9. How many weeks since your last child was born? ……...Weeks

10. What is your height? ……Ft……..inches or…….metres

11. What is your weight? …… kg

12. Do you smoke at present?

☐Yes ☐No

If Yes, how many cigarettes per day? ……

How long have you been smoking? …….....…….

Have you smoked in the past?

☐Yes ☐No

If Yes, how many cigarettes per day? .☐

How long ago did you quit? …….....

For how many years did you smoke?…….....

13. Were you a regular smoker prior to being pregnant?

☐Yes ☐No

In which year did you stop smoking? …….....

14. Does your partner smoke?

☐Yes ☐No

If Yes: How many cigarettes per day? ☐

15. Were you or your partner smoking during the pregnancy with the last or cleft child? ☐ Yes ☐ No

16. **Which of the following medical conditions have you had diagnosed by a doctor?** (please tick)

☐ High blood pressure

☐ Heart disease

☐ Angina

☐ Stroke

☐ Diabetes

☐ Insulin resistance

☐ Overweight

☐ Migraine

☐ Arthritis

☐ Cancer (specify type) …….....

☐ Kidney disease

☐ Liver Disease

☐ Asthma

☐ Gout

☐ Hepatitis B or C

☐ HIV

☐ Hypothyroid

☐ Hyperthyroid

☐ Eczema

☐ Allergies - Food …….....

- Drugs …….....

- Environmental toxins …….....

None of the above

17. Have you at any time had a miscarriage?

☐ Yes ☐ No

If yes, please supply details of when this occurred and what its relationship was to the timing of the pregnancy with the cleft child.

……..

……..……

……..

18. List any medications taken regularly before or during the last pregnancy

Medication	Dose	How often?
……	…….	…….....
……	…….	…….....
……	…….	…….....
……	…….	…….....
……	…….	…….....
……	…….	…….....

19. Do you follow or have you followed a special diet? (Please tick)

☐ Vegan

☐ Vegetarian

☐ Weight Loss
- Low Calorie ☐
- Low Carbohydrate ☐
- Low fat ☐
- High Protein ☐

☐ Other

20. Briefly list foods that you do not eat:

……..

……..

……..

……..

……..

21. Did you crave certain foods prior to or during the pregnancy with the last child or cleft child? If so please list.

……..

……..

……..

……..

22. **Did you take any supplements (such as, vitamins, minerals, herbs) either before or during the pregnancy?**

☐ Yes ☐ No Before ☐ During ☐

If Yes, please list:

Supplement	Type*	Dose	How Often?

*Vitamins, minerals, herbs etc

23. Did you drink alcohol during the pregnancy?

☐ Yes ☐ No

If Yes; what quantity, what type, and how often?

…….....

24. Have you made any significant changes to your diet since your last child was born? If so please indicate below:

……
……

Family Genealogy

25. Did you have a CL/P?

☐ Yes ☐ No

If Yes, describe type:

…….....

26. **Did your partner have a CL/P?**

☐ Yes ☐ No

If Yes, describe type:

…….....

27. Is (are) there case(s) of clefting in other members of the family?

☐ Yes ☐ No

If Yes, please indicate in the space below:

…….……

…….

28. **Are you aware of any medical condition that appears to be inherent in your family?**

☐ Yes ☐ No

If Yes, describe type:

…….....

29. Are you aware of any medical condition that appears to be inherent in your partner's family?

☐ Yes ☐ No

If Yes, describe type:

…….....

30. How would you describe yourself?

☐ Easy going

☐ Worries sometimes

☐ Worries often

☐ Continually worry

31. In the period up to three months prior to this pregnancy and into the first trimester did anything occur in your life or in the lives of those around you that may have increased the level of stress in your life above the normal day to day activities?

☐ Yes ☐ No

If Yes, please describe:

……

……

……

32. Is there any further information that you believe may be relevant to the study?

☐ Yes ☐ No

If Yes, please outline the details:

……

……

……

Taking a Hair Sample

Sample Type

Scalp hair is the only source recommended for analysis. Pubic and other body hair should only be used as a last resort if scalp hair is not available.

Note: Pubic and other body hair is only recommended for confirmation of elevated toxic metals found in the scalp hair and/or to rule out external contamination of the scalp hair.

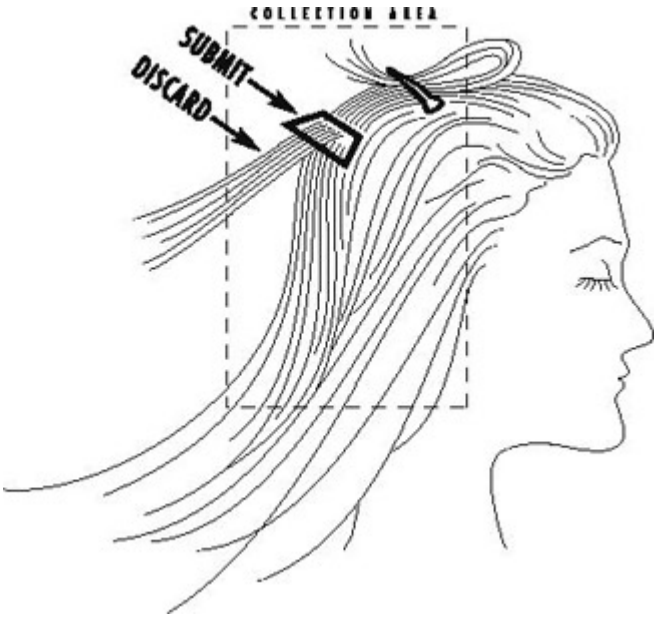
Preparation

The portion of hair to be collected should be untreated, i.e. not permed, dyed or bleached. If all of the hair has been chemically treated, wait until sufficient new virgin growth has emerged to allow collection. The hair should also be free of all gels, oils and hair creams prior to sample collection. For those individuals environmentally and/or occupationally exposed to external contaminants, (welding, mining, etc...) special care should be taken to limit exposure between washing of the hair and the collection of the sampled hair.

Location

Each collected sample should be taken in small portions from at least four to five different locations of the scalp. The recommended areas for collection are the nape of the neck, posterior vertex and posterior temporal regions.

Note: All reference range correlations, dietary recommendations and interpretive report content assumes that the complete hair specimen originated solely from the above defined regions of the scalp.



Sample Length

High grade stainless steel scissors or thinning shears should be used to cut the hair as close to the scalp as possible. The length of the collected hair should not exceed be approximately 4-5 cm if the woman's child is 3 months or younger, 5-6 cm if the child is 6 months, and 6-7 com if the child is 6-12 months old. Where a woman has short hair this may not be possible and so

the sample should be as near as possible to the appropriate length. The proximal portion (that part closest to the root) should be retained and the excess discarded.

Note: The proximal portion is reflective of the most recent metabolic activity.

Weight

The weight requested for a hair specimen is a minimum of 125 milligrams (0.125 Gram). Use of the Hair Weight Scale Cards and this will insure the collection of sufficient sample weight. However, if a Hair Weight Scale is not available, one full teaspoon should approximate the weight requirement.

Packaging

Upon cutting the sample, the hair should be placed directly into a clean hair specimen envelope normally provided by the laboratory and then sealed with the glue flap only. Do not use plastic bags in place of the standard paper envelopes to hold the hair specimen. In addition, do not use staples, paper clips, adhesive tape, aluminium foil or other metal and paper material of any kind to seal, secure or wrap the hair envelope and/or the hair specimen contained within.

Appendix 17: Interclinical Test Methods (HTMA)

A brief synopsis

InterClinical laboratories works with Trace Elements Inc, a licensed and certified clinical laboratory.

Upon arrival at the laboratory facility, each hair sample is weight checked with high sensitivity balance equipment.

The sample is then based in a test tube, a reagent is added and the sample is hydrolysed into liquid form by a uniform temperature-controlled microwave digestion (CEM Mars 5 Plus) technique.

Once prepared in this manner, the hydrolysed sample undergoes ICP-Mass Spectrometry (Sciex Elan 6100 and 9000 models) to achieve elemental readings in part per million (ppm) and part per billion (ppb) readings.

Detailed Description

Each hair sample has been analysed by a licensed and certified clinical laboratory that undergoes regular inspections with the Clinical Laboratory Division of the Department of Health and Human Services, HCFA.

ICP-Mass Spectrometry (Sciex Elan 6100 and 9000 models) is used for all trace element determinations. These multiple systems are capable of easily handling a high volume of specimens in applications such as HTMA where limits of detection requirements are in the part-per-million (ppm) and low part-per-billion (ppb) range

All testing is performed in a laboratory clean room environment. The clean room utilizes HEPA filtration systems ensuring that air quality and temperatures are isolated in order to protect equipment and processed specimens from potential contamination

Additionally, the most advanced high-volume and uniform temperature-controlled microwave digestion (CEM Mars 5 Plus) technique is utilised. Microwave digestion is the method of choice for speed, reduced contamination, complete digestions, and retention of analytes to ensure precise results

Each patient result from the laboratory is based upon an hourly National Institute of Standards and Technology (NIST) traceable standard curve, a rigorous quality control validation for every 24 specimens and is compared to a representative reference range derived from the like analysis of an international collection of normal and "healthy" subjects.

The laboratory also performs routine spiked sample recoveries, daily split specimen analysis and voluntarily participates in various inter-laboratory test comparison (TC) surveys.

High sensitivity balances used by the laboratory for calibration/QC check standards and specimen weighing are calibrated with weight sets traceable to NIST. All stock standards used for daily calibration and Quality Control are prepared by a leading ISO 9001 certified laboratory.

All standard material is sourced from NIST standard reference material. Further, the laboratory uses 18 megohm double-deionized water, sterile polypropylene wet-digestion and sampling test tubes, acid-leached, triple-rinsed miscellaneous glassware and plastic ware. All glassware when used is Class A.

Laboratory management utilizes state-of-the art proprietary data management program that features automated quality control (AQC) software to assist the chief technologist and laboratory director in validating all QC test results and individual specimen test results prior to release for eventual report processing.

The laboratory successfully participates in an on-going proficiency testing program with Le Centre de Toxicologie du Quebec, which offers urine, blood and hair tissue elemental testing involving clinical laboratories that utilize high resolution instrumentation in North America and Europe.

Quality Control

The following is a brief description of the quality control materials and solutions the laboratory utilizes in each daily analytical run. This sequential format does not represent other daily and routine QC procedures that are performed by technologists in the laboratory prior to each analysis.

- Calibration Blank
- Calibration Standard 1
- Calibration Standard 2
- Calibration Standard 3
- Initial Calibration Check Standard--Low Level (ICCS)
- Initial Calibration Check Standard--High Level (ICCS)
- Laboratory Reagent Blank (LRB)
- Pooled Hair Check Solution (PHCS)
- Split Hair Specimen (SHS)
- Pooled Hair Check Material (PHCM)
- Certified Reference Material--Hair (CRM-H)
- Patient Specimen 1
- Patient Specimen 2
- Patient Specimen 3
- Patient Specimen 4
- Patient Specimen 5
- Patient Specimen 6
- Patient Specimen 7
- Patient Specimen 8
- Patient Specimen 9
- Patient Specimen 10
- Patient Specimen 11

- Patient Specimen 12
- Continuing Calibration Check Standard (CCCS)
- Continuing Calibration Blank (CCB)
- Patient Specimen 13
- Patient Specimen 14
- Patient Specimen 15
- Patient Specimen 16
- Patient Specimen 17
- Patient Specimen 18
- Patient Specimen 19
- Patient Specimen 20
- Patient Specimen 21
- Patient Specimen 22
- Patient Specimen 23
- Patient Specimen 24
- End Calibration Check Standard--Low Level (ECCS)
- End Calibration Check Standard--High Level (ECCS)

Initial Calibration Check Standard -- Low Level (ICCS)

Initial Calibration Check Standard -- High Level (ICCS)

Solutions (two levels) prepared in the same manner as the calibration standards and used to verify the calibration curve before analysis of patient specimens and QC samples begin.

Laboratory Reagent Blank (LRB)

Used to evaluate all potential contaminants/interferences in the reagents, laboratory environment and apparatus within the test method.

Pooled Hair Check Solution (PHCS)

Homogenous solution of pooled hair that has been pre-digested using standard method digestion. Used to indicate day-to-day and within-run precision associated with instrument calibration.

Split Hair Specimen (SHS)

Second analysis of a random patient specimen taken from the previous day's run. Using a submitted patient sample to indicate day-to-day laboratory precision associated with the entire test method; sample preparation, digestion and instrument analysis.

Pooled Hair Check Material (PHCM)

Homogenous pooled hair that is exposed to the same laboratory environment, reagents and apparatus as the patient specimens throughout the entire test method. Used to indicate day-to-day laboratory precision associated with sample preparation, digestion and calibration.

Certified Reference Material --Hair (CRM-H)

Certified Hair Reference Material, obtained from National Institute for Environmental Studies, Japan. Used to indicate day-to-day accuracy and precision of the test method; including reagents, sample prep, digestion and analysis.

Continuing Calibration Check Standard (CCCS)

Solution prepared in the same manner as the calibration standards and analysed in the middle of each subset of patient specimens. Used to verify the previously established calibration curve and confirms the accurate analyte quantitation for all patient specimens occurring after the initial calibration check standards.

Continuing Calibration Blank (CCB)

A solution prepared in the same manner as the calibration blank then analysed after the CCCS to show any contamination or carryover.

End Calibration Check Standard -- Low Level (ICCS)

End Calibration Check Standard -- High Level (ECCS)

Solutions (two levels) prepared in the same manner as the calibration standards and analysed at the end of each subset of patient specimens. Used to verify the previously established calibration curve and confirms the accurate analyte quantitation for all patient specimens occurring after the continuing calibration check standards (CCCS).

Test results for all of the above Quality Control materials/solutions and patient specimens are analysed in detail by the AQC lab software and then reviewed by the Chief Chemist and Laboratory Director for compliance to strict quality control limits. Failure to meet the QC criteria requires that the complete analytical process is repeated until all QC data is within acceptable limits. No data is released from the laboratory until such time.

Appendix 18: Women and Birth Publication

"Non-syndromic cleft lip and palate: Could stress be a causal factor?"

Women and Birth (2011) 24, 40–46

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/wombi

Non-syndromic cleft lip and palate: Could stress be a causal factor?

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Received 28 June 2010; received in revised form 11 August 2010; accepted 11 August 2010

KEYWORDS
Cleft lip;
Cleft palate;
Stress;
Oxidative stress

Abstract The aetiology of non-syndromic cleft lip and palate has as yet not been clearly defined. Familial relationships, environmental toxins and nutritional status have all been considered without conclusive results, although in some studies a potential link between non-syndromic cleft lip and palate and any one or more of these factors has been proposed.

Elevated stress, particularly an extended term of traumatic stress, can lead to oxidative damage at the cellular level via hypothalmus–pituitary–adrenal (HPA) axis dysregulation, high cortisol and cytokine production. The effect of this hormonal shift is to re-direct the blood supply to the mother's muscles, thereby reducing the supply to the placenta, causing a potential nutritional deficiency which may then result in a genetic alteration in the foetus.

Mothers with a child aged two years or younger who had been born with a cleft, who were members of CleftPals, a family support group, volunteered to be participants in this qualitative study. The research first called for a survey to be completed by the mother and this was then followed by an interview conducted by the researcher. The study involved families living in the three eastern States of Australia.

The results suggest that physical and/or emotional stress may well be implicated in clefting. While little work has been done in considering stress as a causal factor, the existing literature suggests, as does this study, that elevated stress levels at, or soon after, conception appear to affect foetal development.

Introduction

Three forms of oral defects have been considered in this study: cleft lip (CL), cleft palate (CP) and cleft lip and palate (CLP). Throughout this paper the letters 'U' will be used for unilateral and 'B' for bilateral and the abbreviation CLP will be used as a generic term unless stated otherwise.

The aetiology of non-syndromic CLP, a state where either the lip or palate or both have not completely formed, has to date not been adequately determined. Most researchers suggest that the cause is multifactorial.

Non-syndromic clefting is distinct from syndromic clefting in that the clefts to the lip and/or palate are the only anomalies, while in syndromic clefting the cleft occurs with

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1871-5192/$ – see front matter
doi:10.1016/j.wombi.2010.08.001

other well-documented abnormalities, such as respiratory or cardiac anomalies. Syndromes that are associated with clefting include Van der Woude, Teacher-Collins and Pierre Robin as well as several others. In these cases the clefting is generally the lesser of the concerns.

The author has been a Director of a charity called Operation Rainbow Australia Limited for nearly 20 years. This organization sends medical teams to third world countries to provide surgical support to children with facial anomalies who are living in poverty. The question to the surgeons has always been: 'why not find the cause rather than repair the result?' The answer has always been: 'surgeons do what surgeons do best – operate'.

As this was not an acceptable answer a research program was established. This paper covers one segment of a much larger study into the aetiology of CLP, the question being 'what causes this birth anomaly?'

At an early stage in this investigation stress immediately before or at an early stage in the pregnancy appeared to be involved as a risk factor which may be related to these anomalies.

Literature review

CLP has been recognized for many centuries. The earliest known reported reference to clefting comes from China[1] where it is claimed that in AD 390 an unknown surgeon successfully closed a cleft on an 18 year old girl, 'Wei Yang-Chi', who later became the Governor General of several provinces.

More rigorous research into CLP appears to have started early in the 20th century.[2] No clear linkages have been established that point to definitive causative factors. Most researchers suggest that the cause is multifactorial, and indeed this may well be the case. Research papers covering genetics, familial associations, nutrition, drug use by the mother and environmental toxins have been canvassed to determine whether there could be any connection which may lead to a better understanding of the nature of this problem.

Incidence

CLP, CL or CP is not something that has just appeared in our society, nor is it confined to an individual group within a nation or socioeconomic sector. The birth prevalence of CLP was reported to be 1 per 1000 among Caucasian populations in England.[3] In Asian populations, the reported prevalence ranges from 1.11 to as high as 2.06 per 1000.[4–7]

In Australia the records are kept on a State by State basis, with the quality of the recording varying greatly. No differentiation is made between syndromic and non-syndromic forms of clefting. Using Queensland and Victorian data, the incidence is 1.5 and 1.8 per 1000, respectively. These statistics[8] relate to 697,513 births in Queensland and 795,323 in Victoria over a five-year period.

The researcher visited the Philippines at the start of this research project as part of a surgical mission, as the incidence of clefting in that country was reportedly much higher than in other countries. The researcher found that the frequency of CLP at a national level has as yet not been determined as the Government does not keep a record of such birth defects. One study in that country[9] analysed the hospital records of 47,969 newborns over six sites between 1989 and 1996 and found that the incidence was 1.94 per 1000 for all clefting, with the higher incidence of 2.3 per 1000 for a second child also having a cleft.

In the Philippines a total of 1,640,698 births were recorded in 2002. Sixty seven percent of these births occurred in the home and only 28% of the children were born in hospitals. The Philippine Health statistics do not indicate where the remaining 5% were born but presumably the person completing the birth record omitted to fill in that section indicating place of birth. Of the total births only 67% were attended by medical professionals and some of these professionals may have been merely health workers. This means that the research carried out by Murray et al.[9] was skewed towards those of a higher socioeconomic level who could afford to have their children born in a hospital.

Doctors working in the Philippine Hospital (Quezon City) indicated that it was their opinion that clefting was a problem of the poor, suggesting that the incidence of CLP among the general population was much higher than that recorded by the hospitals. The link between low socioeconomic status and stress has been well documented.[10,11] The authors indicate that this group has a higher allostatic load due to less coping skills, poorer lifestyle choices and in most cases less freedom to choose their desired outcomes. The poor in the Philippines in general are housed in shanty type accommodation and exist on not much more than two handfuls of rice per day. This then does not constitute a balanced diet which, when combined with the environment that they are exposed to on a daily basis clearly leaves them open to disease and other medical problems.

Stress

Initially this research project aimed to consider other possible causal factors of CLP, such as: the mother's alcohol and drug use, smoking habits and nutritional supplementation taken prior to and during the pregnancy. However, even at an early stage in the study stress seemed to be an issue and this led to considering other work that had been done linking stress to clefting.

Stress levels affect the metabolism and thus the environment in which the cells reproduce and develop. If stress levels are low or of short duration, physiology and cell development and replication can return to normal, while continuously high stress levels lead to possible abnormal development or cell necrosis.[12] The development and function of foetal tissue and organs is directly proportional to the amount of blood they receive. When passing through the placenta, the hormones of a mother experiencing stress will profoundly alter the distribution of blood flow in her foetus and change the character of her developing child's physiology.[13,14]

A study in Czechoslovakia[15] retrospectively analysed the stress levels of mothers who gave birth to children with clefts. They separated the children into two groups: (1) children with bilateral cleft lip and palate (B/CLP), and (2) children with all other types of clefts. The finding which they describe as "the most impressive"[15] part of their study showed that in group 1, 47.1% of mothers "were severely disturbed with depressive and anxiety states. Family conflicts, alcoholism of the father,

death or disease of close relatives were common findings."[15] In group 2 the figure was 35.7%.

A recent study carried out by researchers in Basrah, Iraq[16] shows that the incidence of clefting in the period 2003–2005 had doubled compared to 1996–1998. The study cites increased stress levels, poor access to adequate nutrition and an increase in environmental toxins as possible causal factors.

These two studies[15,16] suggest that there may be an association between stress of the mother during pregnancy and occurrence of CLP. The authors suggest that if a cleft occurs it would seem evident that a genetic alteration must have taken place. The increase in incidence of clefting in the Iraqi population within a short period of time suggests that there are external forces at work leading to the genetic alteration. In the Basrah study it was suggested that these external forces could be stress due to the war, nutritional deficiencies or environmental toxins.

Methodology

Families who had a child two years or younger with a cleft were included in the study. Cases that were clearly syndromic in nature were excluded. All families were associated with the family support group called 'Cleft Pals' through which contact was made with the mother. Cleft Pals, which operates in all states of Australia, agreed to indicate to their members that research was to be carried out and asked for volunteers to participate.

Only families living in those States of Australia (Queensland, New South Wales and Victoria) with the highest population were included to reduce the travel costs should it be necessary for face-to-face interviews. This also ensured that the families lived in different environmental conditions. Considering that environmental toxins could be involved in clefting, as some researchers have suggested,[17–19] it was essential to ensure that families included in the study came from different geographic locations so that any localized toxin exposure did not skew the results.

The study, carried out in the years 2007–2008, was questionnaire-based and covered areas that may affect the health of the developing foetus. A number of physical and lifestyle questions, such as whether the parents smoked, the mother consumed alcohol during pregnancy, whether she had taken any medication or nutritional supplements, and details of her age at conception, height and weight were included in the questionnaire. She was also asked for the medical history of both parents, whether either had had a cleft at birth, and whether there was any history of clefting in the family. The mother was not asked whether conception had been via IVF but where it is recorded the mother had considered this to be a stressful process. After completion of the questionnaire the parents (often just the mothers) were contacted by the researcher to verify the data that had been submitted.

Only demographic data and the responses to questions relating to the mother's stress levels and/or events that occurred around the time of conception were considered for inclusion in this research paper. As stress levels vary, as do the coping skills of those experiencing this stress, it was thought that the type and degree of clefting may be directly proportional to the level of perceived stress. In order to determine if this was the case the responses were classified into three groups according to the level of perceived stress.

The Questionnaire

The part of the questionnaire relating to mental/emotional stress that the mother experienced at or around the time of conception contained the following questions:

Question 1.

How would you describe yourself?

- Easy going
- Worries sometimes
- Worries often
- Continually worries about the future

Question 2.

In the period one month prior to conception to two months after conception, was there any event that occurred in your life or your family that caused you anxiety or stress above the normal stresses of life?

☐ Yes ☐ No

If yes please describe:

Each questionnaire received was followed up by communication with the mother either face-to-face or by phone to clarify the data submitted.

Ethics

The research project was approved by the Human Research Ethics Committee at Southern Cross University (HREC # ECN-05-163). The authors did not approach any of the participants directly to seek their inclusion. The study was outlined to the family support group CleftPals who circulated the information to their members. Those wishing to participate who fell within the study parameters then contacted the researchers after receiving an information package.

Results

Forty-seven families responded, representing 48 children. Two children were included from one family: the first born with a U/CL and the second with a CLP. One family had twins, one being born with a U/CL and the other with no anomaly.

Four of the children had syndromic clefts and so those families were excluded. This left 43 families and 44 children in the study.

The results are summarized in Tables 1 and 2.

Mother's age at conception of the child

The mean age of the mothers at conception giving birth to a child with CLP was 31.7 (SD = 5.83) years. The ages ranged from 21 to 44 years.

Position of child in family

Forty-nine percent of the CLP children were first born, and 51% were the second child to be born in the family. None of the mothers had more than two children.

Table 1 Stress analysis.

Degree of stress	Stress type	Stressors
Group 1: Traumatic stress	Employment	Redundancy, bullying, resigning due to stress, partner redundancy
	Family	Family arguments, leaving family, family separation, death in family, entered into marriage, relationship breakdown, left husband, husband did not want child, husband's use of alcohol
	Pregnancy	IVF, twins diagnosed, sex of baby not wanted, difficulty to conceive
	Psychological	Suffering depression, obsessive compulsive disorder
Group 2: Elevated stress	Relocation	Leaving husband, town, or country
	Employment	Relationship with employer, work overload
	Family	Stressed from first child, fatigue, death in family
	Pregnancy	Difficulty conceiving
	Psychological	Continual worry, concern about health of foetus
	Relocation	Moved house
	Physical	Major haemorrhage, illness

Sex of child with cleft
Eighty one percent of the children with clefts were male.

Types of clefts
Twenty-one percent of children had a unilateral CL only and 18.5% had a CP as the only anomaly. Seven percent had a B/CLP and 53.5% had a U/CLP.

Ultrasound testing did not detect any of the single CP anomalies, and it did not detect four of the CLP abnormalities, two of these being B/CLPs. However, it did detect all other anomalies no later than 20 weeks into the pregnancy.

BMI and weight of mother at conception
The average body mass index (BMI) of the mother ranged from 18.4 to 34.3, with a mean of 24.1 (SD = 3.47). The mother's weight at conception ranged from 47.0 kg to 104.0 kg with a mean of 67.0 kg (SD = 12.15).

Length of pregnancy
Sixty percent of mothers carried the child to full term and 4.6% beyond full term by 10 days to 3 weeks. The mothers who did not carry to full term gave birth between 34 and 39 weeks (35.4%).

Birth weight
The mean birth weight was 3.37 kg (SD = 0.65) with a range of 1.2 kg to 4.94 kg.

Mental/emotional stress
In 16 of the 43 families (37.2%) the mothers indicated that at or around the time of conception their lives were highly stressful. In a further 15 cases (34.9%) the mothers indicated stress or anxiety but of a much milder nature. There was no possible way of quantifying the level of stress as experience of stress is very subjective. However, some level of stress or

Table 2 Summary of results.

Demographics	Group 1: Traumatic stress	Group 2: Elevated stress	Group 3: No stress
	Mean ± SD	Mean ± SD	Mean ± SD
Mother's age at birth	33.8 ± 5.46	32.2 ± 5.70	30.4 ± 6.32
Mother's weight at conception	70.9 ± 14.98	64.9 ± 8.80	67.5 ± 11.61
BMI	25.8 ± 3.49	23.1 ± 3.10	24.5 ± 3.47
Child's birth weight	3.6 ± 0.38	3.3 ± 0.69	3.5 ± 0.84
Sex of child			
Male	14	12	10
Female	2	3	3
Position of child in family			
First child	7	9	6
Second child	9	6	7
IVF conceptions	1	3	0
Cleft type			
U/CL	3	2	3
CP	5	1	2
U/CLP	6	11	8
B/CLP	2	1	0
Total clefts	16	15	13

anxiety was evident at or around the conception period in 72% of cases (n = 31).

Individual responses

Obviously the researchers were not able to determine quantitatively the level of stress or the coping skill of the participant but relied purely on the mother's perception regarding degree of trauma and therefore stress. Obviously what is traumatic to one individual may in fact be a minor irritation to another. The category and types of stressors, as indicated by the mothers' responses to question 2 in the questionnaire, were thematically analysed and are listed in Table 1.

The following responses by the mothers to open-ended questions are examples of the issues that were highlighted by respondents.

Traumatic stress: 'Extremely stressful job involving bullying, finally leaving job. Also at the time tension and arguing with the family. I removed myself from the family and now do not visit or phone'.

Elevated stress: 'Completely fatigued with second child. Prone to worry and get stressed. Very tired and exhausted'.

Grouped results

While stress, as defined by the women themselves, seemed to be the major risk factor involved in this study the results were further grouped to determine whether any of the other items, e.g. BMI, might also be linked to CLP. The results were first separated by stress level and then the various demographics listed for each group.

The decision regarding where to group each mother was based on the mother's own definition of the level of stress that occurred.

Group 1 included cases where a specific traumatic event had occurred.

Group 2 included cases where elevated stress was noted.

Group 3 included cases where the mother indicated that there was no particular highly stressful incident and that she coped well with day-to-day stress.

The results of this grouping are set out in Table 2.

Discussion

The average birth age for Australian mothers was 27.3 years in 1985 and 30.7 years in 2005, an increase of 3.4 years.[8] The birth age of the study group of 31.7 years is slightly higher than the mean age group of mothers giving birth in 2005, and allowing for the trend upwards is probably much closer to the age group for 2008.

Considering that there was even distribution of clefts between the first and second born child it suggests that clefting is quite random and that external factors are much more likely to trigger a genetic response. However, what could have changed for the mothers between the first and second pregnancy are factors related to their lifestyle, marital situation, nutritional status and/or stress levels. If pregnancies were in close succession the mother may not have been able to recover fully from the nutrient drain to the first child. We did not ask whether there had been a partner change since the birth of the first child where the child with the cleft was the second born. Obviously this should be done in future research.

The results show that the majority of the children born with a cleft are male. Statistics published for Victoria support this.[20] No reason to date has been established to understand this phenomenon. It does suggest, however, that the genetic alteration(s) that result in CLP are associated with the X Chromosome. The proposition is that as a male has only one X chromosome; any interference in that area may be more damaging to the foetus than a similar shift in the female, where two X chromosomes are present. Recent research[21] has linked the X chromosome gene TBX22 with isolated CP. As there are a number of T-Box genes it is possible that either TBX22, or one closely related to it, could be involved in the other anomalies.

The absence of a direct link of the child with the cleft to past generations tends to suggest that clefting is not hereditary and probably therefore not a genetic trait. Even if there is a genetic trait, it would seem that for the gene to be expressed some external factor needs to trigger the genetic response, such as oxidative gene damage.[22]

As clefting has occurred by 12 weeks' gestation[23] it suggests that perhaps the first ultrasound should be undertaken between 12 and 20 weeks, as opposed to just one at 20 weeks. Should an ultrasound be taken at both 12 and say 18 weeks, doctors could be more confident about the absence of facial anomalies, with the exception of an isolated cleft palate.

In Australia it is quite normal for an expectant mother to have an ultrasound at 12 and 20 weeks. The question of safety in having ultrasonography has been considered[24] and the conclusion of the World Health Organization was that the use of this technique during pregnancy appears to be safe. The meta-analysis which was used to consider the safety of this procedure included 6716 citations and 19 from secondary sources. It also included 61 publications reporting data from 41 different studies: 16 controlled trials, 13 cohort and 12 case-control studies. The authors are therefore not opposed to the judicious use of this technique if it can potentially be helpful to the mother and the baby. Early detection of a cleft in a foetus allows the parents to seek information on the special needs this child will require when born.

The authors considered whether body weight may play a role in clefting. A normal range of BMI for women could be considered to be between 18.5 and 24.9.[25] The women in this study tended to be at the top end of this range, with the variation in weight in this study being quite wide. BMI alone could not be considered as having influenced the results. While obesity has been considered to be a risk factor by other researchers[26,27] these results do not support that hypothesis. Certainly within the sample group some women were overweight at conception but this cannot be taken as a generalization. The average BMI of the sample group also supports this fact. Therefore, other factors may have been implicated, although to be certain that insulin resistance is not involved serum insulin analyses would be required. Insulin resistance is the forerunner to type 2 diabetes which in turn is associated with obesity and is associated with an inflammatory state of the body.[28] Inflammation causes oxidative stress and has the potential to harm genes.[29]

The individual responses showed that the mothers in Group 1 experienced quite traumatic events around conception or in the first few weeks of the pregnancy. The level of stress is difficult to quantify as each individual copes in different ways. Indeed, even some of the events experienced by Group 2 could be traumatic in the eyes of the person and yet when viewed by

others be considered as normal events in everyday life. We can only say that the women themselves considered these as stressful events.

The women in group 3 had children with clefts yet emotional stress did not seem to be a risk factor. Considering that stress for instance also derives from nutrient depletion or imbalance, toxins or inflammation this could still articulate into oxidative damage on a cellular level. As other researchers have stated, clefting could be multifactorial with other risk factors being prevalent, which to date, both others and we have not been able to isolate. Research is currently under way to explore this further.

Stress and pregnancy

Oxidative damage can result from nutritional deficiencies and/or the presence of environmental toxins.[22,29] However, physical and emotional stress, both of which result in altered cortisol levels, could also be involved. Such stressors in themselves can lead to oxidative damage at the cellular level via hypothalmus–pituitary–adrenal (HPA) axis dysregulation, high cortisol and cytokine production.[30] High cortisol levels have been linked to abdominal adiposity, insulin resistance and metabolic syndrome,[31] all of which may be involved in oxidative damage.

Stress levels affect the environment in which the cells reproduce and develop. If stress levels are low or of short duration, cell development may be impeded but can return to normal, while continuously elevated stress levels lead to possible abnormal development or cell necrosis.[12] The development of foetal tissue and organs is directly proportional to the amount of blood they receive, and hence their function. When passing through the placenta, the hormones of a mother experiencing stress may profoundly alter the distribution of blood flow in her foetus which could potentially lead to changes in the physiology of her developing child.[13,14]

Stress may be involved in a myriad of other disorders, and relaxation methods such as meditation are often recommended during treatment in order to minimize stress. In this study we recognize that stress opens up a large field of endeavour but we restricted our investigation to the role of emotional stress in the occurrence of CLP. Should the stress theory prove correct then future studies could be more widespread and look at the effect elevated stress has on other first trimester issues.

Conclusion

Of all of the criteria assessed, mental/emotional stress appears to be a likely risk factor in the occurrence of CLP. To date this has only been considered by a very small number of other researchers.

As stress is experienced subjectively and reactions to the same stressor are dependent on the coping skills and resources of the individual, there was no possible way to quantify the degree of stress other than assess it qualitatively. Certainly it does indicate that in any future studies attempts must be made to determine the level of stress, how this affects the biochemistry of the pregnant woman, and what effect that may have on the developing foetus. This could be assessed via stress questionnaires, and from both blood and urine testing during the pregnancy. Oxidative stress can be measured quantitatively using samples of either blood or urine. Further studies are currently under way.

Acknowledgements

This study could not have been undertaken without the assistance and co-operation of the members of the family support group CleftPals. Their continuing work to provide physical and emotional support to families with a cleft child cannot be underestimated and their eagerness to support the study was greatly appreciated.

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Appendix 19: Mann-Whitney Analysis of the Philippine Data

Variable	Control (mean)	CLEFT (mean)	W	P.value
Age	27.31	31.72	203	0.022
Cleft.Child.position.in.family	0.00	3.60	0	0.000
Number.of.children.in.family	2.54	3.60	210.5	0.027
Weight.prior.pregnancy	48.35	54.74	216	0.040
Height	1.53	1.52	357.5	0.542
BMI	20.54	23.56	168	0.003
Calcium	268.08	204.08	428.5	0.052
Magnesium	14.47	23.69	211	0.032
Sodium	30.58	51.00	322	0.962
Potassium	24.35	24.24	382	0.286
Copper	0.98	0.99	300	0.643
Zinc	39.31	45.84	363	0.480
Phosphorus	12.69	12.80	313.5	0.834
Iron	2.14	2.54	390	0.223
Manganese	0.10	0.59	260.5	0.228
Chromium	0.04	0.04	345	0.679
Selenium	0.07	0.07	416	0.080
Boron	0.12	0.14	267	0.278
Cobalt	0.00	0.01	278	0.373
Molybdenum	0.01	0.37	310	0.781
Sulphur	3939.12	3810.40	437.5	0.035
Germanium	0.00808	0.00740	504	0.000
Barium	0.11	0.15	248.5	0.151
Bismuth	0.00	0.00	349	0.484
Rubidium	0.03	0.05	321.5	0.955
Lithium	0.00	0.01	299	0.153
Nickel	0.04	0.05	345	0.710
Platinum	0.00	0.00	325	NaN
Thallium	0.00	0.00	337.5	0.347
Vanadium	0.02	0.05	224	0.058
Strontium	0.79	1.16	224	0.058
Tin	0.02	0.04	299.5	0.604
Titanium	0.17	0.07	310	0.778
Tungsten	0.01	0.00	361	0.195
Zirconium	0.01	0.01	284.5	0.144
Uranium	0.00	0.00	380	0.302
Arsenic	0.01	0.02	222	0.053
Berylium	0.00	0.00	337.5	0.347

Mercury	0.07	0.05	436	0.033
Cadmium	0.00	0.02	321	0.946
Lead	0.16	0.15	356.5	0.499
Aluminium	1.09	1.23	321	0.947

Mann-Whitney U Test (comparing means between Control and CLEFT)

P-value < 0.05 (highlighted red) indicates the mean of that mineral for CLEFT is significantly different* than Controls. for others (i.e. P-value > 0.05) the means may still be different, but they are NOT considered statistically significant.

***** at 5% significance level**

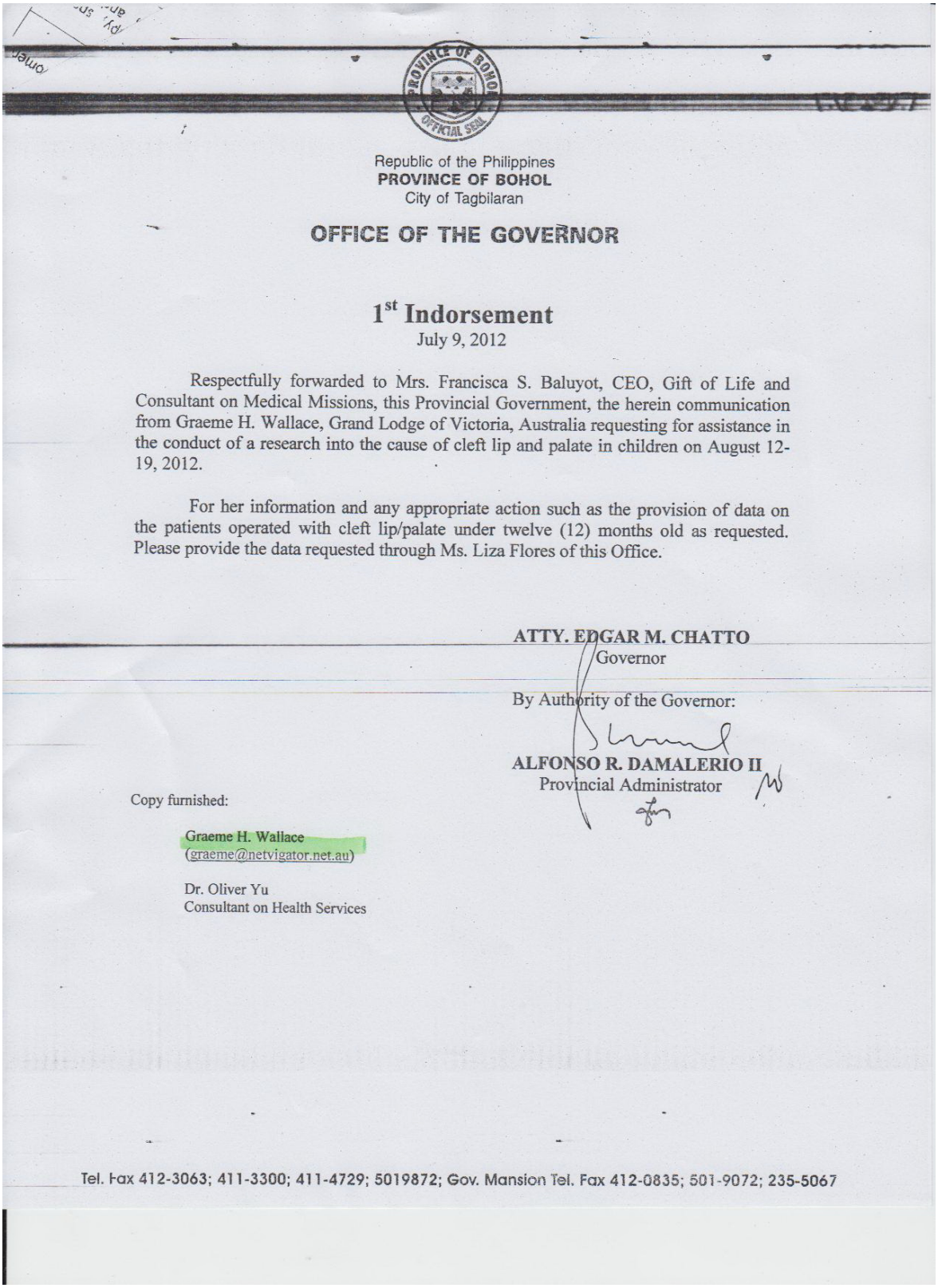
Variable	Control %	CLEFT %	X	P.value
Smoking	3.85%	20.00%	1.8367	0.175
Partner.smoking	3.85%	0.00%	3E-31	1.000
Alcohol	19.23%	12.00%	0.1054	0.745
Medical.Issues	0.00%	4.00%	0.0004	0.984
Medication	100.00%	88.00%	1.5018	0.220
Supplements	3.85%	0.00%	3E-31	1.000
Miscarriage	0.00%	4.00%	0.0004	0.984
Stress	7.69%	40.00%	5.7069	0.017

Test on the Proportions (comparing proportions of "Yes" between Control and CLEFT)

P-value < 0.05 (highlighted red) indicates the mean of that mineral for CLEFT is significantly different* than Controls. for others (i.e. P-value > 0.05) the means may still be different, but they are NOT considered statistically significant.

***** at 5% significance level**

Appendix 20: Letter of Endorsement from the Governor of Bohol, Philippines.



Republic of the Philippines
PROVINCE OF BOHOL
City of Tagbilaran

OFFICE OF THE GOVERNOR

1st Indorsement
July 9, 2012

Respectfully forwarded to Mrs. Francisca S. Baluyot, CEO, Gift of Life and Consultant on Medical Missions, this Provincial Government, the herein communication from Graeme H. Wallace, Grand Lodge of Victoria, Australia requesting for assistance in the conduct of a research into the cause of cleft lip and palate in children on August 12-19, 2012.

For her information and any appropriate action such as the provision of data on the patients operated with cleft lip/palate under twelve (12) months old as requested. Please provide the data requested through Ms. Liza Flores of this Office.

ATTY. EDGAR M. CHATTO
Governor

By Authority of the Governor:

ALFONSO R. DAMALERIO II
Provincial Administrator

Copy furnished:

Graeme H. Wallace
(graeme@netvigator.net.au)

Dr. Oliver Yu
Consultant on Health Services

Tel. Fax 412-3063; 411-3300; 411-4729; 5019872; Gov. Mansion Tel. Fax 412-0835; 501-9072; 235-5067

Appendix 21: Mann-Whitney Analysis of the Australian Data

Variable	Control (mean)	CLEFT (mean)	W	P.value
Age	32.9	32.18	58.5	0.831554022
Cleft.Child.position.in.family	1.5	2.27	6	0.330281253
Number.of.children.in.family	2.1	2.27	54	0.967668087
Age.of.Child.with.cleft.in.weeks	17.8	19.27	47.5	0.621725759
Weight.prior.pregnancy	68.1	65.45	64	0.548039034
Height	1.657	1.64	62	0.64672844
BMI	24.76	24.35	55	1
Calcium	94.6	68.81818182	66	0.467855158
Magnesium	11.17	6.290909091	70	0.306912061
Sodium	6	3.818181818	61.5	0.664448266
Potassium	2.8	4.363636364	61	0.687225602
Copper	3.43	4.427272727	54	0.971904014
Zinc	25.5	17.81818182	66	0.456881598
Phosphorus	13.1	12.09090909	69.5	0.294148777
Iron	0.71	0.627272727	66	0.45193308
Manganese	0.0717	0.051818182	61.5	0.672052501
Chromium	0.04	0.034545455	73.5	0.160293017
Selenium	0.038	0.048181818	38.5	0.248828323
Boron	0.212	0.233636364	48	0.646513908
Cobalt	0.0022	0.007	44	0.412384859
Molybdenum	0.0026	0.002818182	50.5	0.766044657
Sulphur	4091.2	4181.363636	49.5	0.724599605
Germanium	0.0058	0.004272727	75	0.156030158
Barium	0.1083	0.074545455	64.5	0.525158776
Bismuth	0.0228	0.013	58	0.825338718
Rubidium	0.00344	0.006345455	68.5	0.359808636
Lithium	0.001	0.001090909	50	0.390828231
Nickel	0.021	0.020909091	69.5	0.295034723
Platinum	0.001	0.001	55	NA
Thallium	0.0005	0.0005	55	NA
Vanadium	0.0028	0.002818182	51	0.795310974
Strontium	0.309	0.191818182	68.5	0.358870178
Tin	0.018	0.035454545	55.5	1
Titanium	0.078	0.074545455	53	0.914283174
Tungsten	0.001	0.001454545	50	0.390828231
Zirconium	0.02	0.030909091	50	0.671536811

Uranium	0.00065	0.000854545	51	0.741544507
Arsenic	0.0033	0.002909091	73.5	0.173558801
Berylium	0.001	0.001	55	NA
Mercury	0.057	0.042	62.5	0.614853402
Cadmium	0.0109	0.001272727	64.5	0.396393677
Lead	0.11	0.1	60.5	0.340355742
Aluminium	0.478	0.563636364	46	0.542441293

Variable	**Control %**	**CLEFT %**	**X**	**P.value**
Smoking	0.00%	9.09%	2.60E-32	1
Partner.smoking	0.00%	18.18%	0.453409091	0.500720462
Alcohol	70.00%	63.64%	2.77E-31	1
Medication	30.00%	72.73%	2.312169421	0.128364783
Special.Diet	10.00%	0.00%	0.002386364	0.9610385
Family.CLP	0.00%	9.09%	2.60E-32	1
Stress	36.36%	70.00%	1.2188	0.270

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