Cleft lip and palate

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Clefts of the lip and palate are generally divided into two groups, isolated cleft palate and cleft lip with or without cleft palate, representing a heterogeneous group of disorders affecting the lips and oral cavity. These defects arise in about 1·7 per 1000 liveborn babies, with ethnic and geographic variation. Effects on speech, hearing, appearance, and psychology can lead to longlasting adverse outcomes for health and social integration. Typically, children with these disorders need multidisciplinary care from birth to adulthood and have higher morbidity and mortality throughout life than do unaffected individuals. This Seminar describes embryological developmental processes, epidemiology, known environmental and genetic risk factors, and their interaction. Although access to care has increased in recent years, especially in developing countries, quality of care still varies substantially. Prevention is the ultimate objective for clefts of the lip and palate, and a prerequisite of this aim is to elucidate causes of the disorders. Technological advances and international collaborations have yielded some successes.

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Introduction

Non-syndromic orofacial clefts, which include cleft lip, cleft lip and palate, and cleft palate alone, comprise a range of disorders affecting the lips and oral cavity (figure 1), the causes of which remain largely unknown. Effects on speech, hearing, appearance, and cognition can lead to long-lasting adverse outcomes for health and social integration.

Affected children need multidisciplinary care from birth until adulthood and have higher morbidity and mortality throughout life than do unaffected individuals.[1,2] Findings of studies have shown an increased frequency of structural brain abnormalities[3] and that many children and their families are affected psychologically to some extent.[4] Although rehabilitation is possible with good quality care, orofacial clefts inevitably pose a burden to the individual, the family, and society, with substantial expenditure in terms of health and related services.

Care for children born with these defects generally includes many disciplines—nursing, plastic surgery, maxillofacial surgery, otolaryngology, speech therapy, audiology, counselling, psychology, genetics, orthodontics, and dentistry—but it forms only a part of the clinical load of every area, meaning that care has tended to be fragmented. This fragmentation of care has led to substantial variations in management, which continue to cause controversy. Furthermore, in both developing and developed countries, standards of care for patients with cleft lip, cleft lip and palate, or cleft palate alone remain a cause for concern.[5]

Developmental pathogenesis

Development of the lip and palate entails a complex series of events that require close coordination of programmes for cell migration, growth, differentiation, and apoptosis. Neural crest cells, which delaminate from the neural folds, contribute to and migrate through mesenchymal tissue into the developing craniofacial region where, by the 4th week of human embryonic development, they participate in formation of the frontonasal prominence, the paired maxillary processes, and the paired mandibular processes, which surround the primitive oral cavity. Formation of the nasal placodes (ectodermal thickenings) by the end of the 4th week of embryogenesis divides the lower portion of the frontonasal prominence into paired medial and lateral nasal processes. By the end of the 6th week of development, merging of the medial nasal processes with one another and with the maxillary processes on each side leads to formation of the upper lip and the primary palate. Immediately before completion of these processes, the lateral nasal process has a peak of cell division that renders it susceptible to teratogenic insults, and any disturbance in growth at this critical time can lead to failure of the closure mechanism.[6]

The first sign of overt development of the secondary palate happens during the 6th week of embryogenesis with outgrowth from the maxillary processes of paired palatal shelves, which initially grow vertically down the sides of the developing tongue. During the 7th week of development, the palatal shelves rise to a horizontal position above the tongue and come into contact and fuse

Search strategy and selection criteria

Our search strategy was formulated to identify any meta-analyses and previous systematic reviews in all aspects of orofacial cleft treatment, palatogenesis, and cleft cause and pathogenesis, in addition to all published cohort studies (and where appropriate, comparison groups) and case-control studies. We searched the Cochrane Library, Medline (via PubMed, Internet Grateful Med, OVID, and Knowledgefinder), HealthSTAR, POPLINE, SDILINE, SPACELINE, Embase, OLDMEDLINE, CINAHL, and ASKSAM with a combination of keywords: 'genetics', 'gene-environment interaction', 'risk factors', 'maternal', and 'cleft lip'. A so-called grey literature search was done via the ECHHSR (European Clearing House on Health Systems Reform), and we consulted the UK National Research Register Database to identify any current and unpublished relevant studies. The reference lists and bibliographies of all previous publications were scanned to find any publications not already identified by our electronic search strategy.

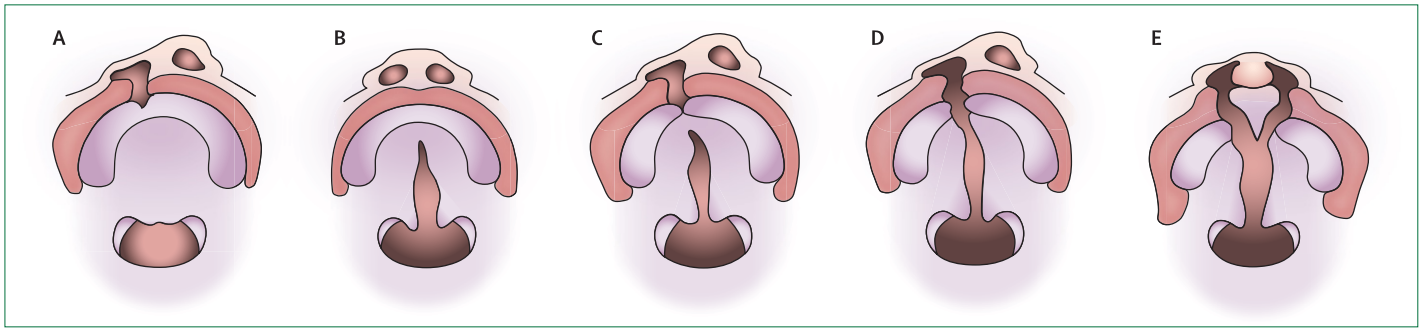

Figure 1: **Non-syndromic orofacial clefts**
(A) Cleft lip and alveolus. (B) Cleft palate. (C) Incomplete unilateral cleft lip and palate. (D) Complete unilateral cleft lip and palate. (E) Complete bilateral cleft lip and palate. Reprinted with permission from: Shaw WC. Orthodontics and occlusal management. Oxford: Butterworth-Heinemann, 1993.

to form a midline epithelial seam, which subsequently degenerates to allow mesenchymal continuity across the palate. The palatal mesenchyme then differentiates into bony and muscular elements that correlate with the position of the hard and soft palate, respectively. In addition to fusing in the midline, the secondary palate fuses with the primary palate and the nasal septum. These fusion processes are complete by the 10th week of embryogenesis; development of the mammalian secondary palate thereby divides the oronasal space into separate oral and nasal cavities, allowing mastication and respiration to take place simultaneously.[6]

Since the lip and primary palate have distinct developmental origins from the secondary palate, clefts of these areas can be subdivided into cleft lip with or without cleft palate and isolated cleft palate in which the lip is not affected. This subdivision is validated by the finding that, under most circumstances, cleft lip with or without cleft palate and isolated cleft palate do not segregate in the same family.[7] Integration of findings of human genetic studies (including positional cloning strategies, parametric-based genetic linkage analysis, non-parametric affected sib-pair approaches, chromosomal analysis, and candidate gene-based association studies) with data of experimental embryological techniques in model organisms has increased our knowledge of both the fundamental mechanisms driving normal facial morphogenesis and how these are disturbed in cleft lip with or without cleft palate and isolated cleft palate.

Mice and chicks have played a central part in dissection of the molecular pathways underlying development of the lip and palate.[8] In both species, development of the lip and primary palate closely parallels that seen in human beings, with facial processes visible at embryonic day (E) 9·5 in mice (stage 19 in chicks) and the upper lip becoming continuous by E11·5 in mice (stage 28 in chicks). Because the embryonic chick face is readily accessible for experimental manipulation and will continue to develop after such interventions in the egg, much of our knowledge of development of the lip and primary palate is derived from analysis of this species. The available evidence, however, suggests that similar, if not identical, mechanisms operate in mice and human beings.

In this context, molecular studies have shown that initiation and outgrowth of the facial processes and specification of their identity is controlled, at least partly, by interaction of fibroblast growth factors, sonic hedgehog (SHH), bone morphogenetic proteins, the homeobox-containing genes *Barx1* and *Msx1*, the distal-less homeobox-containing (*Dlx*) genes, and local retinoic acid gradients.[9–12] By contrast, although fusion events that contribute to formation of the lip and primary palate seem to entail a combination of apoptosis and epithelial-mesenchymal transformation, their molecular control has been studied less extensively. These events are, however, thought to include: SHH; MSX1 and MSX2; and control of signalling by bone morphogenetic proteins and fibroblast growth factors in part by *TP63*—the gene mutated in the allelic disorders ectrodactyly, ectodermal dysplasia, and clefting syndrome and ankyloblepharon, in which cleft lip with or without cleft palate and isolated cleft palate are defining features.[11,13,14]

Conversely, our knowledge of development of the secondary palate has been derived to a much greater extent from analyses of mice, in which morphological events are essentially the same as those happening in human beings, with the palatal shelves initiating from maxillary processes on E12 and growing vertically, lateral to the tongue, on E13.[15] At this stage, each palatal shelf consists of a central core of mesenchyme derived from neural crest cells surrounded by a simple undifferentiated epithelium, comprising a basal layer of cuboidal cells covered by a layer of flattened periderm cells.[16] Molecular control of palatal shelf initiation and vertical growth is thought to entail complex signalling cascades with transcription factors and growth factors and their receptors, including *Osr2*, *Lhx8*, *Msx1*, *Fgf10*, *Fgfr2b*, *Tgfb2*, and *Tgfbr2*.[15] Signalling between the palatal epithelium and mesenchyme is known to have a key role in regulation of palatal growth—eg, fibroblast growth factor 10 (FGF10) signals from the palatal mesenchyme to its receptor FGFR2b, which is expressed in the palatal epithelium. Loss of function of either FGF10 or FGFR2b

causes a reduction in mesenchymal proliferation and an increase in apoptosis, leading to truncation of the palatal shelves.[17] Importantly, activation of FGFR2b by FGF10 is crucial for maintenance of SHH expression in the palatal epithelium: loss of SHH function in this tissue also leads to cleft palate.[17] Signalling between the epithelium and mesenchyme during palatal growth has also been shown between *Msx1*, *Bmp4*, *Shh*, and *Bmp2*; *Msx1* regulates expression of *Bmp2* and *Bmp4* in the mesenchyme and *Shh* and *Bmp4* in medial edge epithelium. In turn, *Shh* stimulates *Bmp2* expression in the mesenchyme, which regulates growth of the palatal shelves.[18] A loss-of-function mutation in *MSX1* has been reported in a patient with cleft lip and palate.[19]

At a precise developmental stage (E14·5), the palatal shelves rapidly move to a horizontal position above the dorsum of the tongue and come into contact. Palatal shelf elevation is thought to be driven by regional accumulation and hydration of glycosoaminoglycans, mainly hyaluronic acid, which provides an intrinsic shelf force, directed by components of the extracellular matrix and local epithelial changes, within a permissive environment provided by differential head growth.[15] Another factor that is important to ensure that the palatal shelves rise correctly is control of competence for oral and palatal shelf adhesion. This mechanism must be regulated precisely so that vertical palatal shelves are adhesion-incompetent while they are in close contact with other structures but once they are raised above the tongue they rapidly acquire adhesion capability if they are not to remain cleft. Control of periderm differentiation by the membrane-bound signalling molecule jagged 2 (JAG2) is important in this process.[20] Another factor central to this process is interferon regulatory factor 6 (IRF6)—the protein encoded by the gene mutated in the allelic disorders van der Woude's syndrome and popliteal pterygium syndrome, which are characterised by varying degrees of cleft lip with or without cleft palate, isolated cleft palate, lower lip pits, hypodontia, and epidermal and genital anomalies.[21–23]

Once the palatal shelves have risen they must adhere and fuse; although only partly characterised, palatal fusion seems to be driven by several cell-adhesion molecules (including nectin 1) and desmosomal components[24,25] and growth factors including transforming growth factor α (TGFA) and epidermal growth factor receptor (EGFR)[26] and members of the transforming growth factor β superfamily—eg, TGFβ3 is essential in these processes. Findings of expression analyses initially indicated that TGFβ3 is expressed specifically in future medial edge epithelium at E13 before palatal shelf elevation and in the medial edge epithelium itself at E14·5, suggesting an important role for this molecule in palatal fusion.[27] This hypothesis is supported by demonstration that ablation of the gene in vivo prevented palatal fusion and that the adverse effect of ablation could be rescued by administration of exogenous TGFβ3.[28,29] Data from subsequent developmental studies have suggested that TGFβ3 might promote palatal fusion via synergistic effects—by stimulating initial adhesion of the palatal shelves, increasing the surface area of the medial edge epithelium through induction of cellular bulges and filopodia, and by promoting degeneration of medial edge epithelium.[29–33] At the molecular level, TGFβ3 has been shown to regulate members of the matrix metalloproteinase family, including TIMP2 and MMP13, which have been implicated in proteolytic degradation of the extracellular matrix.[34] IRF6 is downregulated in the medial edge epithelium of mice with mutations in *Tgfb3* and *Tgfbr2*, which suggests strongly that IRF6 lies downstream of TGFβ3 signalling for the fate of medial edge epithelium.[35,36]

Once the palatal shelves have come into contact and the medial epithelial seam has formed, the seam must degenerate to allow mesenchymal continuity across the palate. Detection of dead or dying epithelial cells together with identification of activated cells positive for caspase 3 and TUNEL (terminal deoxynucleotidyl transferase nick-end labelling) in the disintegrating medial epithelial seam indicates that apoptosis has a key role in seam degeneration.[37] Further evidence for this hypothesis is derived from analysis of palatal development in mice without apoptotic protease-activating factor 1. In these mutant mice, palatal shelf adherence happens normally but the medial epithelial seam does not degenerate.[38] The issue of whether medial epithelial seam cells undergo epithelial-mesenchymal transformation remains controversial, but evidence is emerging that substantial epithelial-mesenchymal transformation does not take place;[36] rather, a subset of medial epithelial seam cells seem to migrate to the oral and nasal surface of the palate where they form triangular areas of epithelial cells.[39] Importantly, if the migration of periderm cells is prevented, these triangular regions fail to form; thus, periderm cells must migrate out of the medial epithelial seam to the epithelial triangular areas to allow fusion to take place. Subsequently, the epithelium on the nasal aspect of the palate differentiates into pseudo-stratified, ciliated columnar cells, and tissue on the oral side changes into stratified, squamous, keratinising cells. Although epithelial differentiation is specified by the underlying mesenchyme,[15] the molecules shaping the fate of the oral and nasal epithelia are unknown.

Descriptive epidemiology

The birth frequency of cleft lip, cleft lip and palate, and cleft palate alone is not known in some parts of the world. In many regions for which information is available, differences in sample source (hospital *vs* population), duration, method of ascertainment, inclusion criteria, and sampling fluctuation restrict comparability.[40] Overall, available findings indicate that orofacial clefts arise in about 1 in 700 livebirths.[41] International data from 57 registries for 1993–98 suggest a variation in prevalence

at birth of cleft lip with or without cleft palate of 3·4–22·9 per 10 000 births, and an even more pronounced variation for isolated cleft palate, with prevalence of 1·3–25·3 per 10 000 births (figure 2).[41] Differences in methods of ascertainment might have a greater effect on isolated cleft palate than on cleft lip with or without cleft palate, because cleft palate is less noticeable externally. Rates of cleft lip with or without cleft palate were high in parts of Latin America and Asia (China, Japan) and low in Israel, South Africa, and southern Europe. Rates of isolated cleft palate were high in Canada and parts of northern Europe and low in parts of Latin America and South Africa. Comparisons between ethnic groups within the USA[42] and the UK,[43] and studies of immigrants to the USA from Japan and China,[42,44] indicate that migrant groups have rates of cleft lip with or without cleft palate closer to those of the area from which they originated than those in the area into which they have moved.

In combined data from European registries for 1995–99, 3·5% of babies with cleft lip with or without cleft palate were stillborn and 9·4% were from terminated pregnancies; respective proportions for isolated cleft palate were 2·4% and 8·1%. No consistent time trends[45] or seasonal patterns[46,47] in prevalence at birth of either defect have been recorded.

Cleft lip with or without cleft palate is most frequent in males, and isolated cleft palate is most typical in females, across various ethnic groups; the sex ratio varies with severity of the cleft,[40] presence of additional malformations, number of affected siblings in a family, ethnic origin, and possibly paternal age.[41] In white populations, the sex ratio for cleft lip with or without cleft palate is about 2:1 (male:female).[40] In Japanese populations, cleft lip and palate shows a significant male excess, but this excess is not seen for cleft lip alone.[48] In white populations, the male excess in cleft lip with or without cleft palate becomes more apparent with increasing severity of cleft and less apparent when more than one sibling is affected in the family.[49,50] By contrast, the male predominance in cleft lip with or without cleft palate is smaller when the infant has malformations of other systems,[41] and findings of one large study suggest predominance in females when the father is age 40 years or older.[51]

Cleft lip with or without cleft palate and isolated cleft palate are associated frequently with other major congenital anomalies. The proportion of individuals with additional anomalies varies greatly between studies but, in general, further defects seem to be more frequent for people with isolated cleft palate than for those with cleft lip with or without cleft palate.[40] Presence of an anomaly of another system might stimulate a detailed clinical examination, leading to detection of mild cleft palate that otherwise might not have been reported had it arisen in isolation. In a study of almost 4000 individuals with isolated cleft palate in Europe, 55% of cases were isolated, 18% were recorded in association with other anomalies, and 27% were noted as part of recognised syndromes.[52] For cleft lip with or without cleft palate, in a report of more than 5000 patients, 71% of cases were isolated and 29% were seen in association with other anomalies.[53] Adoption of a standardised classification of clefts, such as that suggested by Tolarova and Cervenka,[54] would be helpful.

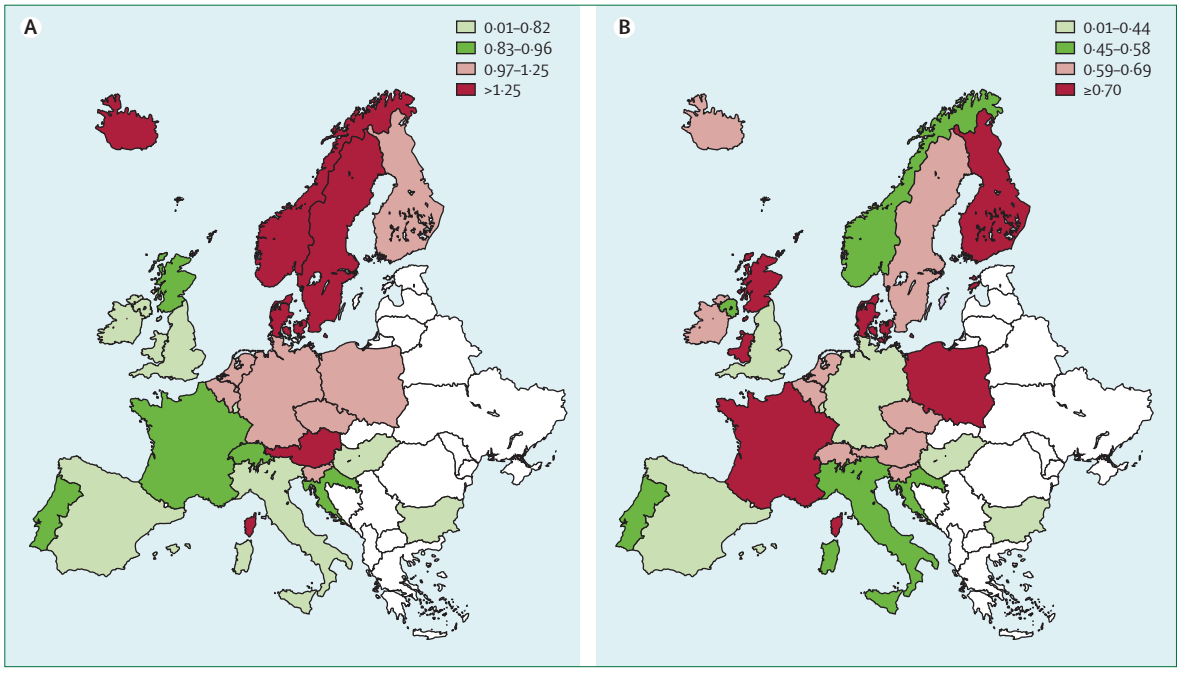

Figure 2: **European birth prevalence per 1000 livebirths of non-syndromic cleft lip and palate**
(A) Cleft lip with or without cleft palate. (B) Isolated cleft palate. Reprinted with permission of the Eurocran project (http://www.eurocran.org).

Consistent associations between orofacial clefts and socioeconomic status have not been established,[55] which could be attributable to differences in measurement and classification of socioeconomic status, differential participation in case-control studies, and variations in inclusion criteria for cases. However, many of the world's most deprived populations do not have surveillance systems for birth defects, and the perception that prevalence at birth is high in some of these regions is not evidence based. The WHO International Collaborative Research on Craniofacial Anomalies project is currently addressing gaps in birth defects surveillance, particularly in developing countries.

Lifestyle and environmental risk factors

Epidemiological and experimental data suggest that environmental risk factors might be important in cleft lip and palate, and maternal exposure to tobacco smoke, alcohol, poor nutrition, viral infection, medicinal drugs, and teratogens in the workplace and at home in early pregnancy have all been investigated. This work is reinforced by the finding that pregnancy planning confers protection.[56,57]

Maternal smoking during pregnancy has been linked consistently with increased risk of both cleft lip with or without cleft palate and isolated cleft palate, with a population-attributable risk as high as 20% (figure 3).[58,59] This association might be underestimated because passive exposure to smoke has not been assessed in most studies. Maternal alcohol use is a well known cause of fetal alcohol syndrome; however, the role of alcohol in isolated orofacial clefts is less certain, with positive associations reported in some studies[60–62] but not others.[63,64] Social and dietary contexts of alcohol consumption are varied and complex and can include modifying or confounding effects of nutrition, smoking, stress,[65] or drug use.

Findings of observational studies suggest a role for maternal nutrition in orofacial clefts, even though assessments of dietary intake or biochemical measures of nutritional status are challenging and generally are not available in many impoverished populations with the highest rates of orofacial clefts. In future studies, measurement of exposure should be enhanced and harmonised across studies, data pooled, and full account made for potential confounding.

In most studies, maternal use of multivitamin supplements in early pregnancy has been linked to decreased risk of orofacial clefts; in a meta-analysis,[66] multivitamin use was associated with a 25% reduction in birth prevalence of orofacial clefts. Data suggest a possible interaction between maternal hyperthermia during pregnancy and use of vitamin supplements, such that supplementation diminishes the increased risk for orofacial clefts associated with hyperthermia.[67] To ascertain from this work which nutrients are protective is difficult, and whether other healthy behaviours of multivitamin users confound these results is unknown.

Previous trials to investigate maternal multivitamin supplementation for prevention of orofacial clefts have been inadequate because of small sample sizes and insufficient data to allow evaluation of results.[68,69] In a Hungarian trial of multivitamins for primary prevention of birth defects the rate of neural-tube defects was significantly lowered, but the study was too small to ascertain whether multivitamins prevented orofacial clefts.[70] The control group received trace elements, including zinc, which could be protective against cleft lip, cleft lip and palate, and cleft palate alone, therefore possibly obscuring a treatment effect. In another randomised controlled trial, in which women choosing to take folic acid supplements before or during pregnancy were randomly allocated either high-dose (2·5 mg) or low-dose (1·0 mg) folic acid,[71] prevalence of orofacial clefts was higher in the high-dose group than in the low-dose group.

Folate deficiency causes clefts in animals,[72] and folate antagonists are associated with increased risk of orofacial clefts in people.[73] The role of dietary or supplemental intake of folic acid in human cleft disorders is uncertain. In North America, where fortification of grains with folic acid has been mandatory since the late 1990s, some evidence suggests a decline in prevalence at birth of cleft lip with or without cleft palate,[74,75] but this outcome has not been recorded in Australia, where fortification was voluntary.[76] For all clefts combined, a decrease was seen in the USA[77] but not in Canada[78] or Chile.[79] Findings of case-control studies of multivitamin supplements containing folic acid,[80–85] maternal dietary folate intake,[81,84,86] and red cell and plasma folate[87–90] are inconsistent.

Raised mean serum concentrations of homocysteine (determined partly by folate status) in mothers of infants with cleft lip, cleft lip and palate, or cleft palate alone have been reported.[87,88] Vitamin B6 (pyridoxine and related compounds) is also a cofactor in homocysteine metabolism and reduces the occurrence of these clefts in animals.[91] Biomarkers of poor vitamin B6 status were associated with increased risk of orofacial clefts in the Netherlands[87] and the Philippines.[89] Vitamin B6 deficiency is typical in populations with high intakes of polished rice in Asia, and these groups also seem to have high rates of cleft lip, cleft lip and palate, and cleft palate alone.[89]

Zinc is important in fetal development, and deficiency of this nutrient causes isolated cleft palate and other malformations in animals.[92] Mothers of children with cleft lip, cleft lip and palate, or cleft palate alone in the Netherlands had lower concentrations of zinc in erythrocytes than did mothers of children without clefts, and similar differences were noted between children with and without these defects.[93] In the Philippines, zinc deficiency is widespread, and high maternal amounts of zinc in plasma were associated with low risk of orofacial clefts with a dose-response relation.[94]

For the **WHO International Collaborative Research on Craniofacial Anomalies project** see http://www.who.int/genomics/anomalies/cfaproject

Other nutrients that could play a part in development of orofacial clefts include riboflavin[95] and vitamin A.[96,97] Fetal exposure to retinoid drugs can result in severe craniofacial anomalies,[98] but the relevance of this finding to dietary exposure to vitamin A is uncertain.

Maternal occupational exposure to organic solvents[99] and parental exposure to agricultural chemicals[100,101] have been associated inconsistently with cleft lip, cleft lip and palate, and cleft palate alone. Anticonvulsant drugs, notably diazepam, phenytoin, and phenobarbital,[102–104] increase risk of these anomalies. Positive associations with maternal corticosteroid use in pregnancy have been reported.[105] Such findings must be interpreted cautiously because of possible publication bias.

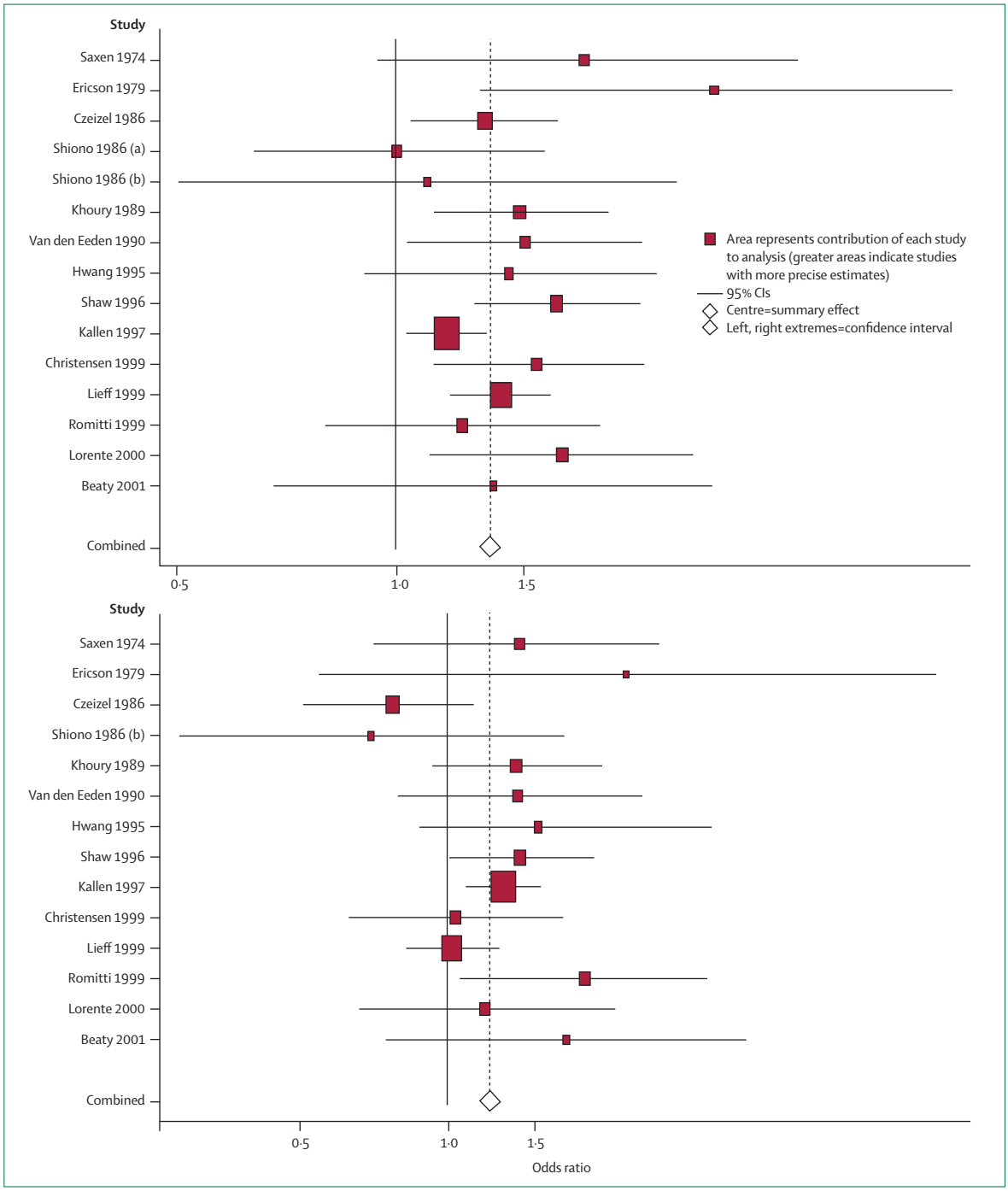

Figure 3: **Forest plots of maternal smoking and cleft lip with or without cleft palate (upper) and isolated cleft palate (lower)**
Reprinted from reference 58, with permission of the World Health Organization.

Interferon regulatory transcription factors are activated after viral infection. Association of *IRF6* with clefts raises the possibility that viral infection in the first trimester of pregnancy might enhance risk of a cleft.[106]

Genetic factors

Cleft lip with or without cleft palate is listed as a feature of more than 200 specific genetic syndromes, and isolated cleft palate is recorded as a component of more than 400 such disorders.[107] The proportion of orofacial clefts associated with specific syndromes is between 5% and 7%.[108] If specific genetic disorders are excluded, the recurrence risk to siblings is greater than that predicted by familial aggregation of environmental risk factors.[109] Concordance rates for cleft lip, cleft lip and palate, and cleft palate alone are higher in monozygotic twin pairs than in dizygotic pairs.[110] The familial clustering and concordance recorded in twins with cleft lip with or without cleft palate and isolated cleft palate is specific for each defect, and therefore the anomalies are thought to have heterogeneous causes.[111–113] Predominance of left-sided clefting and the male excess of cleft lip with or without cleft palate[40] also suggest the importance of genetic susceptibility. Findings of segregation analyses indicate that the number of genes implicated is likely to be fairly small: three or four major loci were reported in an analysis of data from the west of Scotland,[114] and two to 14 loci were recorded by analysis of familial datasets from England.[115] The patterns might differ according to ascertainment, environmental contribution, and population gene-pool effect.[116,117]

Findings of linkage studies have suggested various loci could have a causal role in cleft lip and palate,[118,119] including regions on chromosomes 1, 2, 4, 6, 14, 17, and 19 (*MTHFR*, *TGFA*, D4S175, *F13A1*, *TGFB3*, D17S250, and *APOC2*), with putative loci suggested at 2q32–q35 and 9q21–q33. Inconsistency of results could indicate the small size of studies or genetic heterogeneity.

Various genetic polymorphisms have been investigated in population-based association studies. Some gene products studied are growth factors (eg, TGFA, TGFβ3), transcription factors (eg, MSX1, IRF6, TBX22), or factors that play a part in xenobiotic metabolism (eg, CYP1A1, GSTM1 [glutathione S-transferase μ1], NAT2 [N-acetyltransferase 2]), nutrient metabolism (eg, MTHFR [methylenetetrahydrofolate reductase], RARA [retinoic acid receptor α]), or immune response (eg, PVRL1, IRF6). The most intensively investigated variants have been of the *TGFA*[120–122] and *MTHFR*[66,123,124] genes. Data have been inconsistent, indicating the challenges of researching gene-disease associations and related interactions.[125]

The gene *IRF6*, which has a causal association with van der Woude's syndrome, is also linked strongly to the isolated form of clefting.[126] This finding has been replicated in many different populations and ethnic groups (figure 4).[127–130] Variants of genes linked to syndromic forms of cleft lip with or without cleft palate that have a mendelian mode of inheritance can also produce phenocopies of non-syndromic clefts.[5] This observation suggests that a strategy of choosing variants of genes associated with syndromic forms of cleft lip with or without cleft palate as candidates for investigations into the cause of non-syndromic clefts could be productive. Other examples of mendelian-inherited syndromes and related genes that, if mutated, could result in or modify the expression of cleft lip with or without cleft palate include Kallmann's syndrome (*FGFR1*),[131] ectrodactyly, ectodermal dysplasia, and clefting syndrome (*TP63*),[132,133] X-linked clefting and ankyloglossia (*TBX22*),[134] Gorlin's syndrome (*PTCH1*),[135] and Margarita Island ectodermal dysplasia (*PVRL1* [heterozygous]).[136]

Although discovery of the genetic cause of van der Woude's or popliteal pterygium syndromes will have no immediate therapeutic benefit, advantages for diagnosis are instant, and this knowledge will be potentially useful in genetic counselling. If one gene mutation, which can be identified by prenatal diagnosis, causes cleft lip, cleft lip and palate, or cleft palate alone in a proportion of people, identification of individuals at high risk of having children with the same defect will be possible.

Fitzpatrick and colleagues[137] have studied rare, apparently balanced, chromosomal rearrangements associated with isolated cleft palate and have identified *SATB2* as an important gene in development of the human secondary palate. This group of researchers has identified several other chromosomal aberrations that strongly suggest misregulation of *SOX9* in Pierre Robin sequence. Jakobsen and co-workers[138] reported that the genes *PVRL1* (chromosome 11) and *GAD1* (chromosome 2) might also contribute to the cause of Pierre-Robin sequence. Genome-wide association is emerging as a powerful technique in polygenic diseases, and is expected to play a part in discovery of the genetic cause of orofacial clefts in the future.[139]

Gene-environment interaction

Investigation of gene-environment interaction is important for several reasons. First, estimates of the main effects of genes or environment could be biased if interaction is not taken into account.[140] Second, our understanding of cause and pathogenesis is enhanced by such studies. Finally, findings of interaction work can inform decisions about public health strategies.

With respect to cleft lip and palate, many potential interactions have been tested. Genes and risk factors investigated in such studies include: *TGFA* and smoking[141–143] and vitamin supplements;[144] *TGFB3* and smoking and alcohol;[60,145,146] *MSX1* and smoking and alcohol;[60,146,147] polymorphisms affecting xenobiotic metabolism (eg, *EPHX1* [epoxy hydrolase], *GSTM1*, *GSTT1*, *NAT1*, *NAT2*, or *CYP1A1*) and smoking,[148–150] occupational exposures,[98] and maternal medicinal drug use;[151] *RARA*

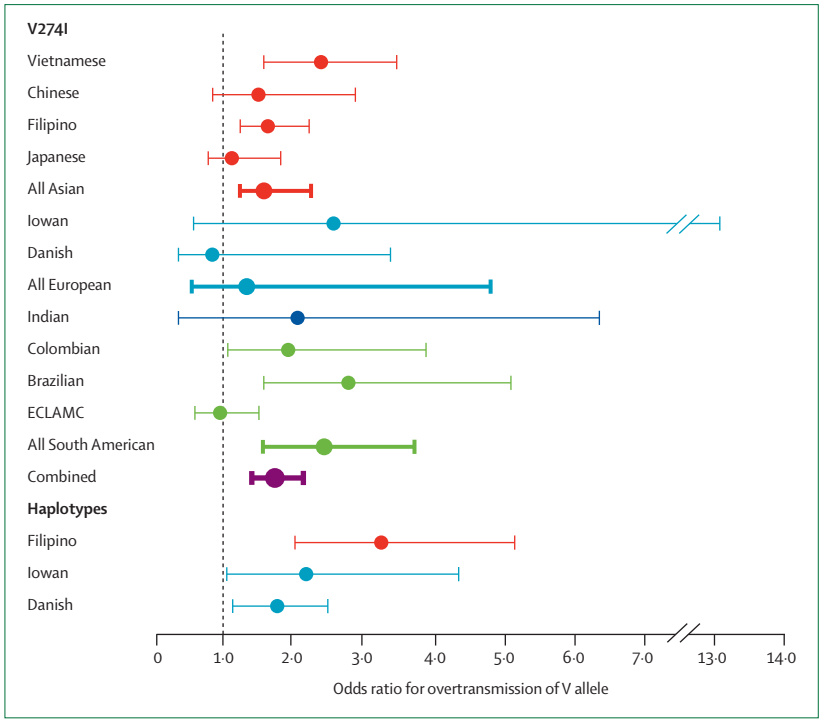

Figure 4: **Overtransmission of polymorphisms at *IRF6* locus**
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polymorphisms and maternal intake of vitamin A;[96] and polymorphisms affecting folate metabolism (eg, *MTHFR*, *RFC1*) and maternal folate intake.[60,88,90,152–154]

Findings on interactions have been inconclusive. Reasons for uncertainty include: low statistical power to detect or exclude interaction; differences between studies in the individuals who have been genotyped (eg, mother alone or with infant); research confined to populations in a few industrialised countries; and non-existent or unreported replication work. Establishment of a collaborative group has been proposed, through the WHO International Collaborative Research on Craniofacial Anomalies project, to undertake meta-analyses and pooled analyses of studies of relations between craniofacial anomalies and putative genetic polymorphisms. Furthermore, gene variants are usually considered one at a time, whereas, a priori, variants of many genes might be expected to modulate the effects of an exposure.[155]

For the **Eurocran project** see http://www.eurocran.org

Clinical management

Services and treatment protocols for management of children with cleft lip and palate can differ remarkably within and between developed countries. In Europe, a networking initiative funded by the European Union in the late 1990s reached consensus on a set of recommendations for cleft care delivery, which were subsequently adopted by WHO.[5] However, findings of a network survey indicated that these guidelines were seldom matched in practice.[156]

The absence of a sound evidence base for selection of treatment protocols was shown by a striking diversity of practices across Europe for surgical care of just one cleft subtype—unilateral complete cleft of lip, alveolus, and palate. Of 201 teams doing primary surgical repair for this defect type, 194 different protocols were being practised. Even though 86 (43%) groups closed the lip at the first operation and the hard and soft palate together at the second, 17 possible sequences of operation to close the cleft were being used. One operation was needed to completely close the cleft in ten protocols (5%), two were needed in 144 (71%), three operations were used in 43 (22%), and four were needed in four protocols (2%). Around half used presurgical orthopaedic techniques with mostly passive plates and some teams also used a plate to assist with feeding.

These uncertainties in treatment indicate the paucity of published randomised trials of cleft care.[5] Such studies present particular challenges for planning and recruitment in comparison of surgical techniques, because trial protocols must take account of the surgical learning curve. However, several well-planned, large-scale, surgical randomised controlled trials are now in follow-up periods (figure 5). So far, only a brief systematic review of cleft care has been published,[157] as has a systematic review of prevalence of dental caries in children with clefts.[158]

Reliability of prenatal ultrasonographic diagnosis has been increasing, although sensitivity is still low, particularly for cleft palate.[159,160] The rate of termination of pregnancy because of presence of a cleft varies between countries, but it remains generally low.[161] Genetic testing in the future could enhance sensitivity and specificity of prenatal diagnosis for syndromic and non-syndromic orofacial clefts.

Service organisation, inequality of care, and treatment uncertainty are widespread issues,[5,41] and scarce resources put basic surgical treatment beyond the reach of thousands of children in developing countries. Accordingly, WHO have highlighted the need for effective international collaboration on strategies to enhance clinical care, through interaction of regional cooperatives such as the Eurocran project. Several research priorities were noted by WHO, including: surgical repair of different orofacial cleft subtypes; surgical methods for correction of velopharyngeal insufficiency; methods for management of perioperative pain, swelling, and infection; and nursing. Clinical trials of these issues would need to include sufficient numbers of patients to be of adequate power. Other multidisciplinary studies of cleft care might include: use of prophylactic ventilation tubes (grommets) for middle-ear disease; presurgical orthopaedic techniques; methods to achieve optimum feeding before and after surgery; and different approaches to speech therapy. In developing countries, trials need to address affordable surgical, anaesthetic, and nursing care.

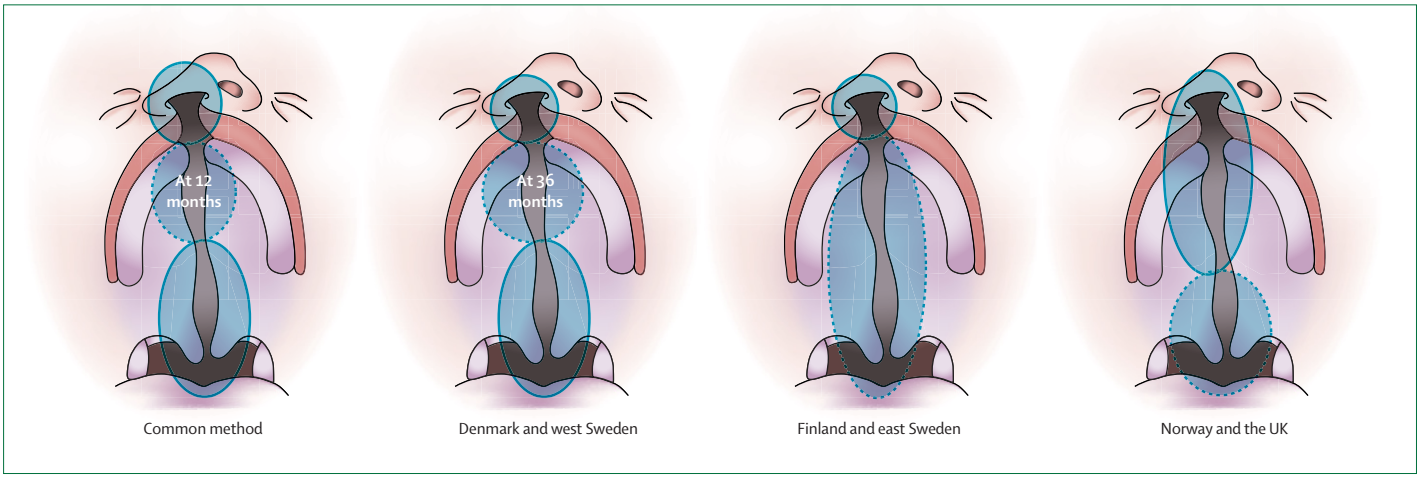

Figure 5: **Techniques used for surgical repair of complete unilateral cleft lip and palate**
Dotted and full circles indicate parts of the cleft that are repaired at different times in various randomised surgical protocols. When there are two full circles, these repairs were completed during the same surgical procedure. Reprinted with permission of the Eurocran project (http://www.eurocran.org).

International adoption of guidelines for provision of clinical services and for maintenance and analysis of minimum clinical records of cleft care is desirable to hasten cohort studies across centres. Various registries of clinical outcomes have emerged and are working independently. Efforts should be made to harmonise these initiatives.

For rare interventions, prospective registries should be established to accelerate collaborative monitoring and critical appraisal, equivalent to phase I trials. Relevant topics would be craniosynostosis surgery, ear reconstruction, distraction osteogenesis for hemifacial macrosomia and other skeletal variations, midface surgery in craniofacial dysostosis, and correction of hypertelorism.

Another urgent issue is the need to create collaborative groups (or to enhance networking of existing groups) to develop and standardise outcome measures. Work on psychological and quality-of-life measures and economic outcomes is needed especially urgently. Collaboration between clinicians and laboratory-based scientists is also essential, not only to describe phenotype much more sensitively than has been done hitherto but also to augment knowledge translation from bench to bedside. Such collaboration has not yet happened in the description and ascertainment of the importance of microforms. Findings of many orofacial clefting studies in various populations have shown that parental craniofacial phenotype is distinctive when compared with that of the non-cleft population.[162] Additional so-called microforms in orbicularis oris morphology and activity,[163] dermatoglyphics,[164] non-right-handedness,[165] anomalies of the cervical spine,[166] and tooth dysmorphology[167] have also been reported. Genotype-phenotype correlation research in this area could yield important information on risk factors.

In large parts of the world, routine public health services cannot afford treatment for cleft lip and palate. Other solutions, incorporating various amounts of charitable and non-governmental support, include high-volume indigenous centres of excellence, contracts between non-governmental organisations and local hospitals, and volunteer short-term surgical missions. WHO recommends promotion of dialogue between different non-governmental organisations to develop agreed codes of practice and adopt the most appropriate forms of aid for local circumstances, with emphasis on support that favours indigenous long-term solutions.

Primary prevention of orofacial clefts

Identification of modifiable risk factors for oral clefts is the first step towards primary prevention. Such preventive efforts might entail manipulation of maternal lifestyle, improved diet, use of multivitamin and mineral supplements, avoidance of certain drugs and medicines, and general awareness of social, occupational, and residential risk factors. The proportion of clefts attributable to maternal smoking in populations with a high prevalence of smoking in women of reproductive age was estimated at 22%.[168] However, the link with smoking was not even mentioned in international reports on smoking and health.[5,169,170] Tobacco use is rapidly increasing in women of reproductive age in many countries because they are targeted actively by tobacco marketing campaigns.[169,171] Pictures of children's faces have been used to establish some of the world's largest medical charity organisations devoted to surgical repair of orofacial clefts. A similar approach might prove effective in public health campaigns to reduce tobacco use by women.[5]

Multivitamin and mineral supplements are associated consistently with reduced risk of cleft lip, cleft lip and palate, and cleft palate alone. However, adverse effects of long-term use of supplements containing antioxidant vitamins have been reported;[172] therefore, clarification of

the specific nutrients and minerals that account for this apparent inverse association is important.

Clinical trials will ultimately be needed to test nutritional hypotheses for prevention of orofacial clefts. A US-Brazilian collaborative randomised controlled trial has been implemented to address whether high-dose folic acid supplementation is more effective than a lower dose to prevent recurrence of non-syndromic cleft lip with or without cleft palate. To be definitive, however, trials will need to be large and—for reasons of efficiency and public health effect—a range of reproductive outcomes should be examined simultaneously. The next reasonable step for research into orofacial clefts might be observational studies of nutrients and food groups, genes, and metabolism to narrow the range of candidate nutrients.

Conclusions

Large, multicentre, collaborative studies[125] are needed to elucidate both environmental (including lifestyle) and genetic risk factors for cleft lip and palate and interactions between them. Exposure measurement is challenging; cleft lip, cleft lip and palate, or cleft palate alone should be encouraged as an endpoint in cohort studies of reproductive outcome, and exposure assessment needs to be harmonised in such studies. The Public Population Project in Genomics is an international consortium to promote collaboration between researchers in population genomics and is an initiative that would help to harmonise data from large-scale, prospective, cohort studies, helping to enhance comparability of studies feeding in to pooled analyses of gene-environment interaction. Similarly, collaborations are needed to elucidate better the issues surrounding management of orofacial clefts, to establish equipoise between different options, to undertake randomised controlled trials and other evaluations of interventions, and to facilitate knowledge translation.

For the **Public Population Project in Genomics** see http://p3gconsortium.org/

Conflicts of interest

We declare that we have no conflicts of interest.

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