B-ENT, 2006, **2**, Suppl. 4, 11-19

Embryology and epidemiology of cleft lip and palate

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Key-words. Cleft lip; cleft palate; embryology; epidemiology; aetiology

Abstract. *Embryology and epidemiology of cleft lip and palate.* Craniofacial anomalies, in particular cleft lip and palate, are major human birth defects with a worldwide frequency of 1 in 700 and substantial clinical impact.
This article reviews the embryology of the face, lip, and palate to enhance the understanding of the pathogenesis of these lesions, with particular attention to the period of susceptibility during gestation, complexity, and the factors that may influence their development. It includes an overview of the prevalence and environmental and genetic causes of cleft lip – with or without cleft palate – and cleft palate.

1. Introduction

Craniofacial birth defects are the fourth most common congenital anomaly in newborns. Cleft lip and palate together represent the most common congenital deformity[1] of the head and the neck.

Oral clefts, including cleft lip (CL), cleft palate (CP), and cleft lip and palate (CLP) constitute a heterogeneous group of non-fatal birth defects known to be multifactorial in origin, in that both genes and environmental factors contribute to their aetiology. Due to both epidemiological and embryological similarities, CL and CLP are usually grouped together as cleft lip with or without cleft palate (CL/P).[2]

We will discuss their developmental pathogenesis, epidemiology and causes.

2. Embryology of cleft lip and palate

Definitions and Classification

A cleft is any opening or division in some part of the anatomy that is not normally open or divided.

Cleft classification is based on embryological development and is defined by the cause and the extent of physical impairment.

Clefts are classified as either non-syndromic or syndromic. In non-syndromic situations, there are no other physical or developmental anomalies besides the CL/P or CP and no known teratogenic exposures that cause CL/P or CP.[3]

CLP is usually non-syndromic: only 10% of all infants with CLP will have an associated syndrome. If the CL occurs without CP, 30% of affected infants will have an identifiable associated syndrome; if the CP occurs without CL, 50% will have an associated syndrome. Syndromic clefts are associated with chromosomal syndromes (more than 350 Mendelian disorders), known teratogenic exposure (alcohol, phenytoin, smoking…) and uncategorised syndromes.[4] Common syndromes involving cleft palate are Apert, Stickler and Treacher-Collins. Van der Woude and Waardenburg syndromes are examples of syndromes with cleft lip with or without cleft palate.

Cleft lip (CL) can occur as a unilateral (on the left or right side) or as a bilateral anomaly. The line of cleft always starts on the lateral part of the upper lip and continues through the philtrum to the alveolus between the lateral incisor and the canine tooth, following the line of incisive suture up to the incisive foramen. The severity of CL varies widely: from a minimal notch located on one side of the lip to the most severe form: a bilateral cleft lip and alveolus that separates the philtrum of the upper lip and premaxilla from the rest of the maxillary arch. When CL extends from the incisive foramen to the palatine suture in the middle of the palate, a cleft lip with cleft palate (CLP) is present. Here also, a wide range of severity may be observed. The cleft line may be interrupted by soft-tissue bridges (skin or mucosa), hard (bone) bridges, or both, constituting an incomplete cleft. This occurs both in unilateral and bilateral CLP.[5]

Cleft palate (CP) is aetiologically and embryologically different from CL/P. Several subtypes of CP can be distinguished

according to severity. The uvula is the location where the minimal form of cleaving of the palate is observed. A more severe form is a cleft of the soft palate. A complete CP is a cleft of the hard palate, soft palate, and uvula.

In a significant proportion of patients, the cleft of the hard palate is covered by mucosa and continues through the soft palate forming a 'submucous' CP.

A cleft anterior to the incisive foramen is also defined as a cleft of the primary palate.

Cleaving posterior to the incisive foramen creates a cleft of the secondary palate.

Understanding the embryology of CL

The developmental pathogenesis of CL has been extensively reviewed.[6-9]

During the first 2 weeks of embryonic life, the human embryo resembles a flat circular plate. In the third week, as the cranial region expands and the neural tube elongates, its shape becomes pear-like.

Specialised neural crest cells derived from the neuro-ectoderm appear as paired columns on the dorsolateral aspect of the neural tube. Despite their ectodermal origin, these neural crest cells make a major contribution to the mesenchyme of the head and neck (ectomesenchyme). The lengthening nervous system results in a flexing of the embryo, bringing the cranial and caudal ends into close proximity. Rapid neural crest cell growth also results in lateral folding.

During the third week of gestation the neural crest cells proliferate and migrate into the fronto-nasal and visceral arch region to form five facial structures or primordia (Figure 1A).

Early in week 4, the five facial primordia develop around the stomodeum (primitive mouth): the fronto-nasal prominence formed by mesenchyme ventral to the forebrain and paired maxillary and mandibular prominences derived from the first branchial arch mesenchyme. The fronto-nasal prominence will form the forehead, nose, and the top of the primitive mouth. The maxillary prominences will form the lateral sides of the stomodeum, and the mandibular prominences will constitute the caudal boundaries.

By the end of week 4, the embryo resembles a horseshoe-shaped cylinder.

Toward the end of the fourth week (Figure 1B), two oval thickenings, the nasal placodes, develop from the ectoderm around the primitive mouth on the lower aspect of the frontonasal prominence. Proliferation of the mesenchymal tissue at the periphery of these ectodermal thickenings produces the medial and lateral nasal prominences. The placodes deepen and sink to form nasal pits (Figures 1C,D,E), which are the precursors of the nose and its structures. The medial nasal prominences and the area above the primitive mouth continue to grow and eventually merge with each other to form the middle part of the upper lip, known as the philtrum.

Rapid growth continues during the fifth and sixth week.

By the end of the sixth and the beginning of the seventh week, rapid proliferation of the maxillary prominences results in the medial nasal prominences merging with each other and the lateral nasal prominences to form the lateral nose and the cheek regions (Figure 1F).

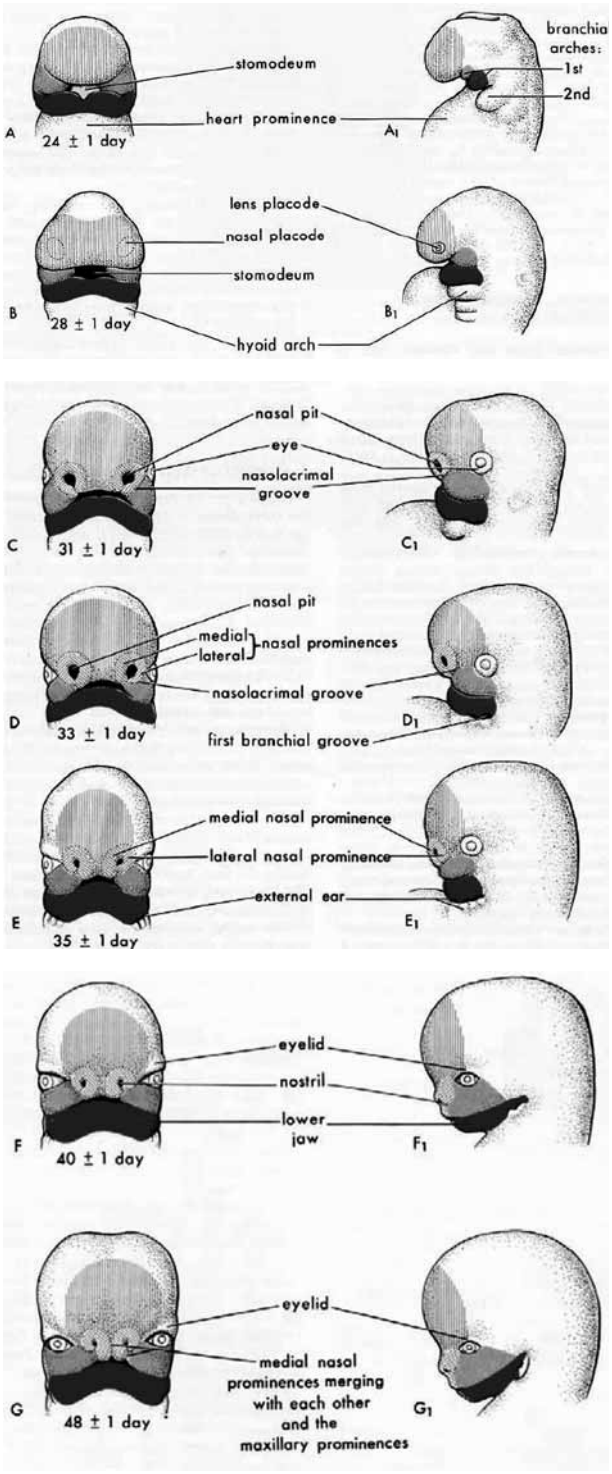
During the eighth week (Figure 1G), the maxillary processes on each side of the mouth grow forward and fuse with the lower edges of the lateral nasal prominences. They extend below the nasal pits to reach and merge with the upper lip's groove, producing a continuous ridge above the mouth that forms the upper lip. Mesodermal tissue migrates from the first branchial arch and reinforces the fused tissues in the developing lip. Normally, this mesodermal tissue assumes a medial position, and the two masses formed by the maxillary prominence will assume lateral positions (Figure 1H).

If this process is delayed, or if one mass is absent, the branchial membrane will pull apart and a CL will ensue (Figure 2). If the maxillary prominence on the affected side fails to merge with the merged nasal prominence, a unilateral cleft will result. If tissues fail to merge on both sides, two grooves are formed, resulting in a bilateral CL.

Understanding the embryology of CP

The developmental pathogenesis of CP has also been extensively reviewed.

The palate begins to form during the fifth week and is not completed until the twelfth week of gestation. The most critical stage is between weeks 6 and 9 (Figures 3A,B,C,D). During this stage, the maxillary prominences merge with the medial nasal prominences beneath the nasal pits, forming a wedge-shaped mass of mesenchymal tissue. As this mass of tissue grows, it separates the



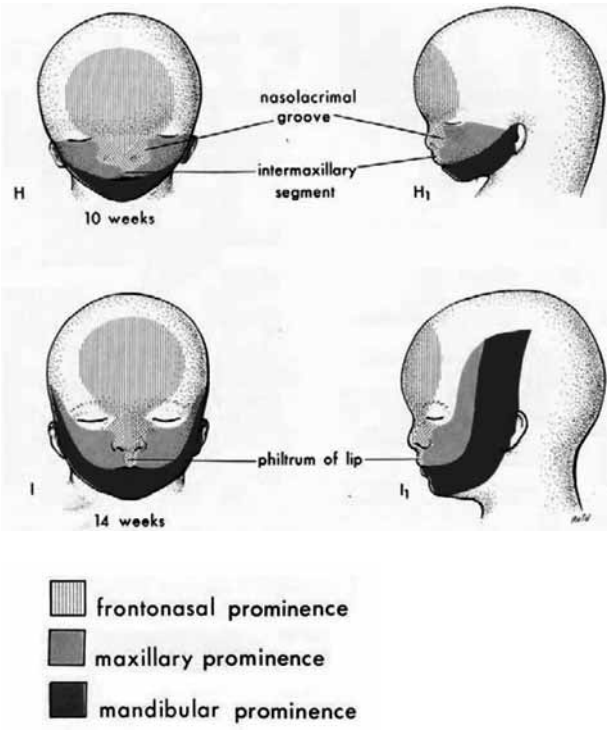


Figure 1
Diagram illustrating progressive stages in the development of the human face. (Modified, with permission, from Moore KL, Persaud TV. *The Developing Human: Clinically Oriented Embryology*. 6th ed. Philadelphia, Saunders; 1998:237-238).

future nostrils from the upper lip and becomes the median palatine process or primary palate (Figures 3E,F). The primary palate is located immediately behind the gum and extends to the incisive foramen.

The secondary palate develops from the paired lateral palatine processes (Figures 4A,B). These shelf-like mesodermal projections arise from the medial aspect of the maxillary prominences and are initially oriented vertically on either side of the developing tongue (Figure 4C). Development of the lower jaw results in a relatively smaller tongue, which moves inferiorly, allowing the palatine shelves to grow towards each other and rotate to a horizontal position during the seventh week of gestation (Figures 4E,F). Once the shelves are elevated to the correct position, there is apoptotic programmed cell death of the medial edges, thinning the epithelium and allowing the tissue from each side to join on the midline in an anterior-to-posterior sequence. During the ninth week, the palatal shelves begin to merge with the free edges of the nasal septum posteriorly. By twelve weeks, fusion is complete and extends from the maxillary and palatine

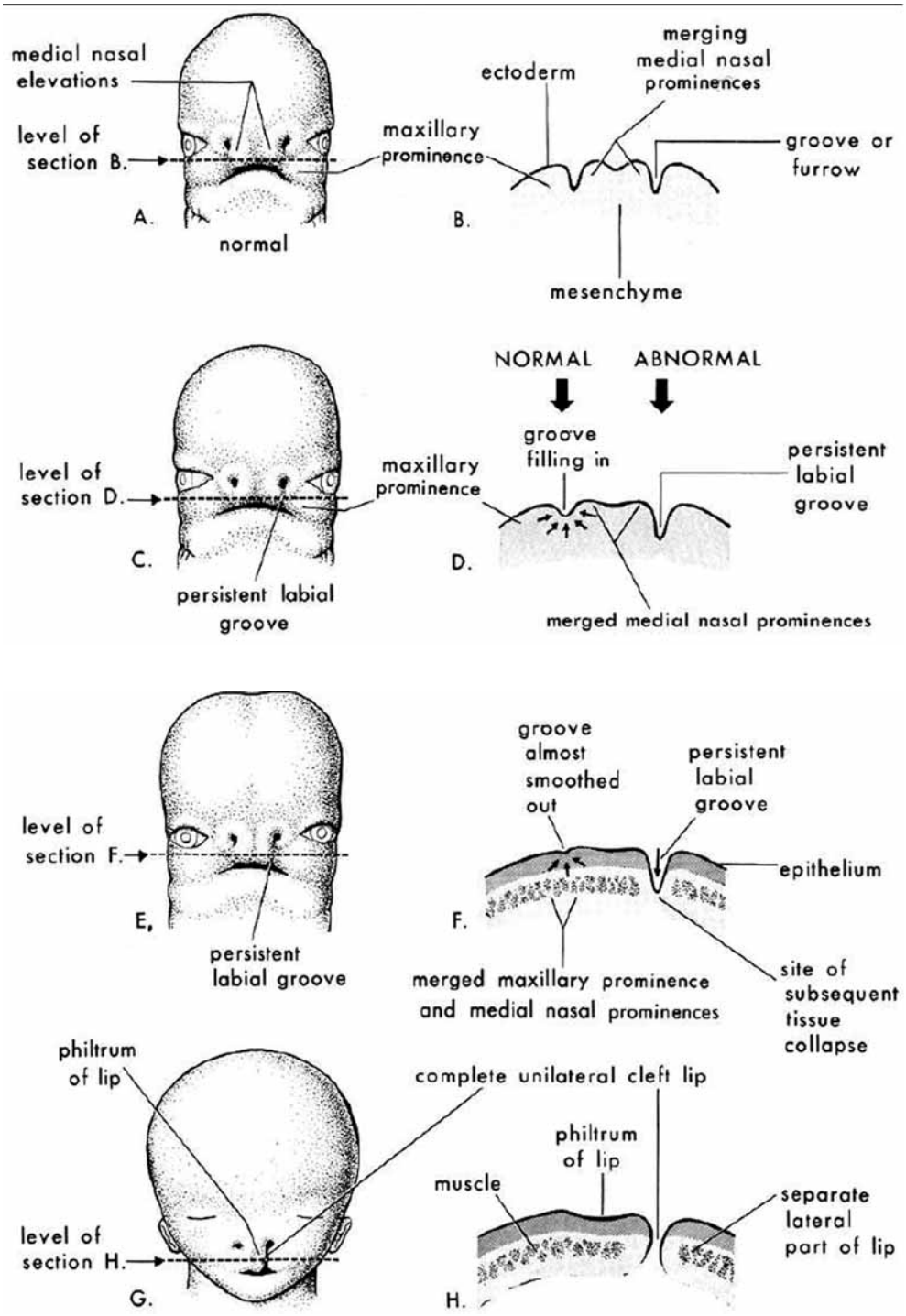


Figure 2
The embryologic origins of a unilateral cleft lip
A. A five-week embryo; B. Horizontal section through the head showing the grooves between the maxillary prominences and the merging medial nasal prominences; C. A six-week embryo with a persistent labial groove on the left side; D. Horizontal section showing the groove gradually filling on the right side as a result of the proliferation of mesenchyme (arrows); E. A seven-week embryo; F. The horizontal section through the head shows how, on the right, the groove between the maxillary and medial prominences has almost disappeared; G. A ten-week foetus with a complete unilateral cleft lip; H. Horizontal section through the head after the stretching of the epithelium and the breakdown of the tissues on the floor of the persistent labial groove on the left, forming a complete unilateral cleft lip. (Modified, with permission, from Moore KL, Persaud TV. *The Developing Human: Clinically Oriented Embryology*. 6th ed. Philadelphia, Saunders; 1998:251).

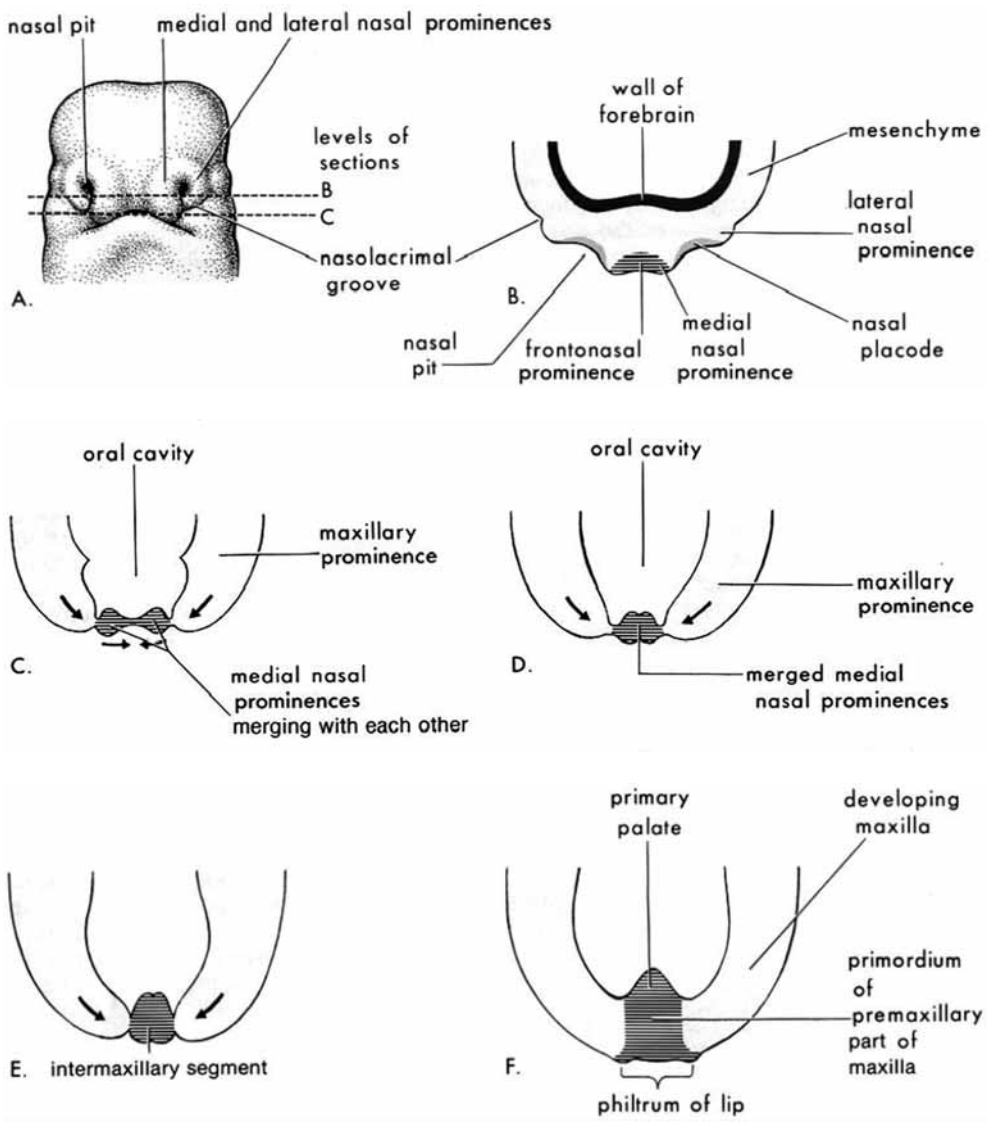


Figure 3
Diagram illustrating progressive stages in the development of the primary palate. (Modified, with permission, from Moore KL, Persaud TV. *The Developing Human: Clinically Oriented Embryology*. 6th ed. Philadelphia, Saunders; 1998).

bones to the palatal shelves, forming the hard palate (Figures 4G,H). The most posterior part that does not ossify becomes the soft palate and the uvula. A CP occurs when this fusion fails.

Embryology in summary

Although CL and CP often occur together, they have different embryologic origins.

Cleft lip results from a failed merging of the maxillary and medial nasal elevations on one or both sides due to the inadequate migration of neural crest cells.

Cleft palate results from the failure of the lateral palatine processes to meet and fuse with each other. This can be the result of 1) defective growth of the palatal shelves, 2) failure of the shelves to rise above the tongue, 3) lack of contact between shelves (excessively wide head), 4) failure to fuse or 5) rupture after fusion of the shelves.

3. Epidemiology of cleft lip and palate

Incidences of CL, CLP, and CP

There is significant racial heterogeneity in the incidence of cleft lip

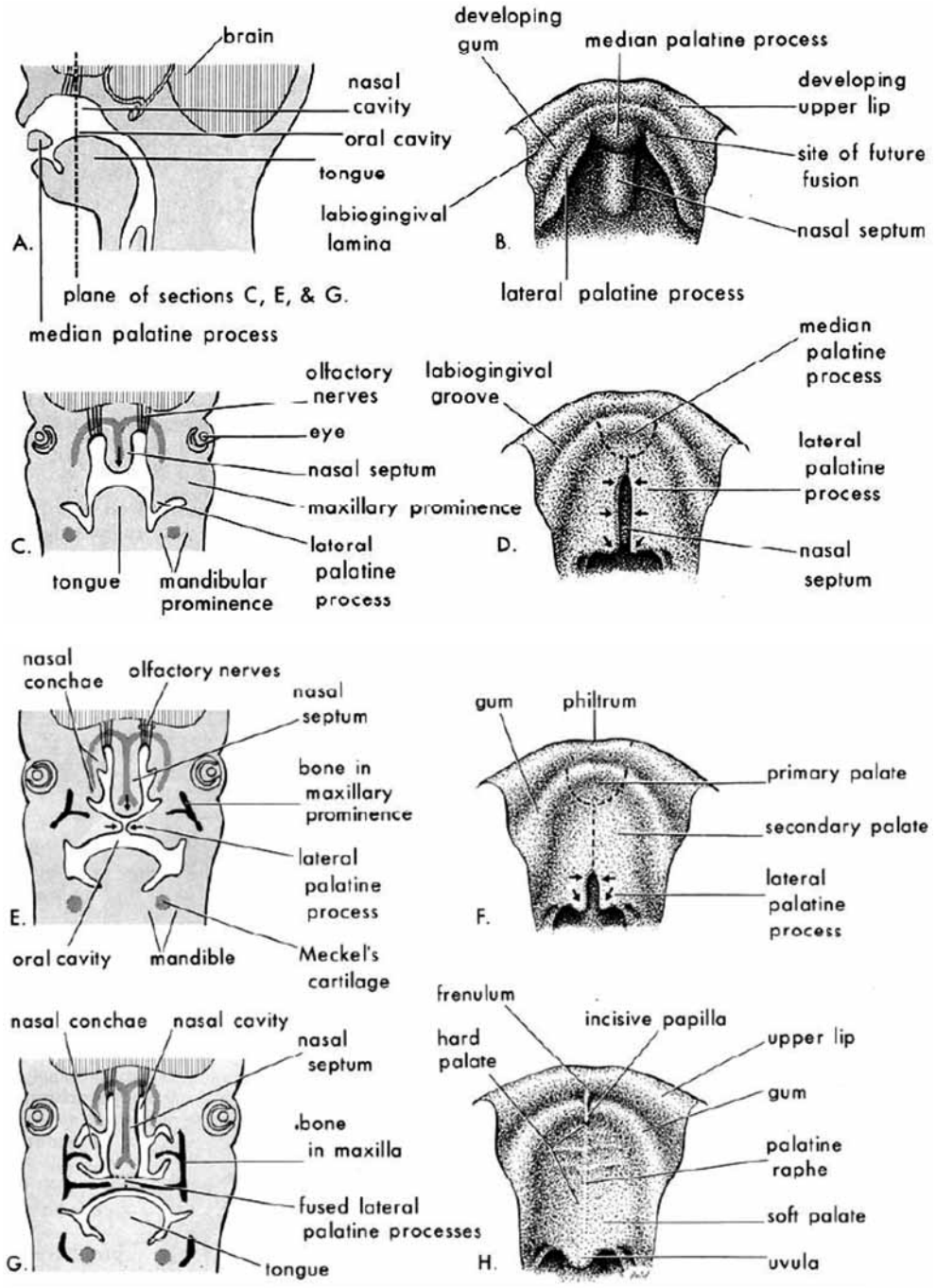


Figure 4
Diagram illustrating progressive stages in the development of the secondary palate. (Modified, with permission, from Moore KL, Persaud TV. *The Developing Human: Clinically Oriented Embryology*. 6th ed. Philadelphia, Saunders; 1998).

with or without cleft palate. The overall incidence of CL/P is 1/700 births. In Caucasians, CL/P occurs in approximately 1 per 1000 live births. The incidence of CL/P is highest among native American Indians (3.6/1000), followed by Asians (2/1000), whites, and blacks (0.3/1000).

The prevalence of CP alone is 0.5 per 1000 live births and does not vary between ethnic groups.

Sex differentiation (male: female ratio) for CL/P is about 2:1, and 1:2 for CP. The secondary palate closes 1 week later in females.

Unilateral clefts occur twice as frequently on the left side as on

the right and are nine times more common than bilateral clefts.

Isolated bilateral CLs are distinctly uncommon, with 86% of bilateral CLs presenting with CPs as well. Unilateral CLs are associated with CP in 68% of cases. Cleft lip increases the likelihood of cleft palate because the tongue gets trapped, preventing it from moving down and therefore increasing the probability of the failure of the shelves to elevate above the tongue.[7,10]

Table 1

Recurrent risk rates for non-syndromic cleft lip with or without cleft palate (CL/P) and for cleft palate (CP)

	Percentage of Predicted Recurrence	
Affected Relatives	**CL/P**	**CP**
None (no family history)	0.1%	0.04%
One sibling	4%	2.5%
One parent	3%	7%
One sibling, one parent	15%	17%
Two siblings	9%	1%
Niece/nephew	1%	
First cousin	0.5%	
Monozygotic twin	40 to 60%	40 to 60%
Dizygotic twin	5%	5%

Inheritance

Fogh-Anderson[11] was the first to describe genetic factors in clefting. Warkany *et al*.[12] reported that environmental components, exposures or deficiencies could cause CL/CP. Many studies since have confirmed these observations. On the basis of these important studies, the inheritance of non-syndromic CL/CP is believed to be multifactorial.

By definition, a condition involves multifactorial inheritance when it is caused by several genetic and environmental factors. Genetic factors create susceptibility to clefts. When environmental factors interact beyond a certain threshold level with a genetically susceptible genotype, a cleft develops in an early stage of development.

Specific risk factors must be considered when counselling for CL/P: the risk increases when 1) the trait is characterised by severe cases, 2) many relatives are affected, 3) the affected persons are close relatives of the person at risk, 4) the gender of the person at risk.

The recurrent risk rates for CL/P and for CP are summarised in Table I. These recurrent risk figures are based on empirical data compatible with a multifactorial model.

In general, the risk for subsequent siblings increases with the severity of the cleft. The other important factor is the gender of the individuals (patient and individual at risk).

When counselling a family with cleft lip and palate it is essential to ascertain whether this is the only malformation (non-syndromic) by examining the patient in minute detail. Then a careful history of both parental families should be taken to ascertain whether in any of the families the clefting is inherited as a sex-linked or an autosomal trait, etc. If all of these types of inheritance have been excluded, the risk figures given in Table 1 apply.

In large series, 21% of patients presented with isolated cleft lip, 46% with cleft lip and palate, and 33% with cleft of the secondary palate only.

Aetiology and prevention

Primary prevention consists of the elimination or avoidance of causal factors, secondary prevention of preventing the birth of infants at high risk.

1) Influences of mechanical forces

A cleft may result if the tongue is not positioned properly because of a smaller chin, as in infants with Pierre Robin sequence.

2) Genetic factors

Overall, migrant groups have rates of CL/P closer to those of their area of origin than to those in the area to which they have moved.[13]

The growth of the detailed structures of the head and face is largely determined genetically, and these processes are known to be dependent on an array of signalling molecules, transcription factors, and growth factors interacting with environmental factors.[14] Many genes have been found to play a role in CL/P and CP aetiology, each possibly contributing to genetic susceptibility through a complex network of gene-gene and gene-environment interactions. The process by which each specific candidate gene interrupts facial development varies. However, they are all involved in effects on the merging process of the prominences by altering a spectrum of signalling molecules, transcription factors, or growth hormones.

Increasingly, studies of genetic polymorphisms are being included in aetiological studies. Some of the investigated gene products are growth factors (e.g., TGFα, TGFβ3), some are transcription factors (e.g., Msx1, SATB2), and some influence the metabolism of xenobiotics (e.g., CYP 1A1, GSTM 1, NAT2), nutrient metabolism (e.g., MTHFR, RARA) or immune responses (5PVRL1, IRF6). The most intensively investigated variants have been of the tumour growth factor alpha (TGFα) and methylenetetrahydrofolate reductase (MTHFR)[15] genes. The results have been inconsistent, as in many other gene-disease associations and related interactions.[16] For example, as found in a recent meta-analysis, while maternal smoking was a consistent risk factor for both CL/P and CP across all studies, the suggestive evidence for gene-environment interaction between the infant's genotype at the Taq1 marker in TGFα and maternal smoking was limited to CP.

3) Environmental factors

Exogenous factors that may increase the risk of CL/P break down into four broad categories: womb environment, external environment, nutrition, and drugs.

Several teratogens are known to increase the risk of CL/P and CP. They include anti-epileptic drugs (phenytoin, valproic acid), thalidomide, dioxins, some pesticides, retinoic acid, maternal cigarette smoking[17] and alcohol use.

Teratogens may contribute to CL/P and CP by disrupting a normal developmental patterning process at a critical stage. Gender differences in the incidence of CP may be related to differences in the timing of palate development. There is a longer window of vulnerability in a female foetus because palatal fusion occurs one week later than in males. Continued research has been focused on identifying whether and how these teratogens interact with specific developmental genes.

Infants exposed to anticonvulsants have a tenfold increased risk of isolated CL. The risks and benefits of specific anticonvulsant regimens must be carefully weighed, balancing the mother's need for treatment and seizure control against the potential teratogenic risk to the foetus.

Retinoic acid and dioxin have been shown to alter the expression of tumour growth factor B3 (TGFB3).

The exposure to four or more alcoholic drinks daily significantly elevated the risk for clefts, especially in those with Msx1 alteration.[18] Alcohol inhibits the migration and differentiation of neural crest cells.

The risk for orofacial clefts as a result of embryonic exposure to tobacco smoke during the first trimester has been found to be related to the level of exposure. Twenty or more cigarettes per day result in a twofold increase whereas less than 20 cigarettes per day resulted in a 1.5-fold increase. Intermittent hypoxia induced by nicotine probably affects facial development. A genetically altered form of TGFA, called α2, may result in an eightfold increase of the risk associated with smoke exposure.[19]

Epidemiological studies indicate that low socioeconomic status (SES) plays a role in clefting. In the Philippines, prevalences of CLP of 2/1000 are reported in indigent populations[20] while parallel studies indicate a prevalence of 1.2/1000 in areas of higher SES. When SES did not change as a result of a geographical move, no change in frequency was noted.[21] This may be related to maternal nutrition.

Maternal nutrition also plays an important role in the prevention of facial clefting. A higher pre-conceptional intake of nutrients predominantly present in fruits and vegetables reduces the risk of offspring affected by orofacial cleft.[22] Daily intake of 400 μg of folic acid, beginning before conception and continuing throughout pregnancy, not only prevents neural tube and abdominal wall defects but also plays a role in the prevention of CL/P and CP.[23] Folic acid intake has been proven to restrict the impact of teratogenic environmental exposure.[24]

No consistent time trends[25] or seasonal patterns[26] in the prevalence at birth of orofacial clefts have been observed.

Both high maternal age and high paternal age were associated with cleft lip with or without cleft palate. Higher paternal age but not maternal age increased the risk of cleft palate only.[27]

Positive associations have been found between maternal obesity in early pregnancy and orofacial clefts in the offspring.[28] The explanation for this association is not known, but a relationship with undetected type-2 diabetes is one possibility.

4. Conclusion

The main objective remains prevention, not correction. Prevention will be conditional on understanding the causes and devising ways to avoid or neutralise them.

At this time, there are strong reasons not to smoke, to refrain from consuming alcohol and to take pre-conceptual and prenatal folic acid since these are effective risk reducers.

Establishing the risk for CL/P and CP in different populations and ethnic groups will help to identify and differentiate between types of teratogenic exposure and genetic predisposition.

Gene-environment research should provide new clues. Identification of those at risk will then lead to selective counselling.

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