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Review Article

CLEFT LIP AND PALATE; A COMPREHENSIVE REVIEW

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ABSTRACT

Congenital cleft-Lip and cleft palate has been the subject of many genetic studies, but until recently there has been no consensus as to their modes of inheritance. In fact, claims have been made for just about every genetic mechanism one can think of. Recently, however, evidence has been accumulating that favors a multifactorial basis for these malformations.

Cleft lip and palate (CLP) are birth defects that affect the upper lip and the roof of the mouth. CLP has a multifactorial etiology, comprising both genetic and environmental factors. In this review we discuss the recent data on the etiology of cleft lip and palate.

The etiology of CLP seems complex, with genetics playing a major role. Several genes causing syndromic CLP have been discovered. Three of them—T-box transcription factor-22 (*TBX22*), poliovirus receptor-like-1 (*PVRL1*), and interferon regulatory factor-6 (*IRF6*)—are responsible for causing X-linked cleft palate, cleft lip/palate–ectodermal dysplasia syndrome, and Van der Woude and popliteal pterygium syndromes, respectively; they are also implicated in nonsyndromic CLP. The nature and functions of these genes vary widely, illustrating the high vulnerability within the craniofacial developmental pathways. The etiological complexity of nonsyndromic cleft lip and palate is also exemplified by the large number of candidate genes and loci. To conclude, although the etiology of nonsyndromic CLP is still largely unknown, mutations in candidate genes have been identified in a small proportion of cases. Determining the relative risk of CLP on the basis of genetic background and environmental influence (including smoking, alcohol use, and dietary factors) will be useful for genetic counseling and the development of future preventive measures.

The purpose of the present paper is to present the etiology of cleft lip and cleft palate both the genetic and the environmental factors. It is suggested that the genetic basis for diverse kinds of common or uncommon congenital malformations may very well be homogeneous, whilst, at the same, the environmental basis is heterogeneous.

Keywords: *Cleft Lip, Cleft Palate, Etiology, Genetic, Multifactorial*

INTRODUCTION

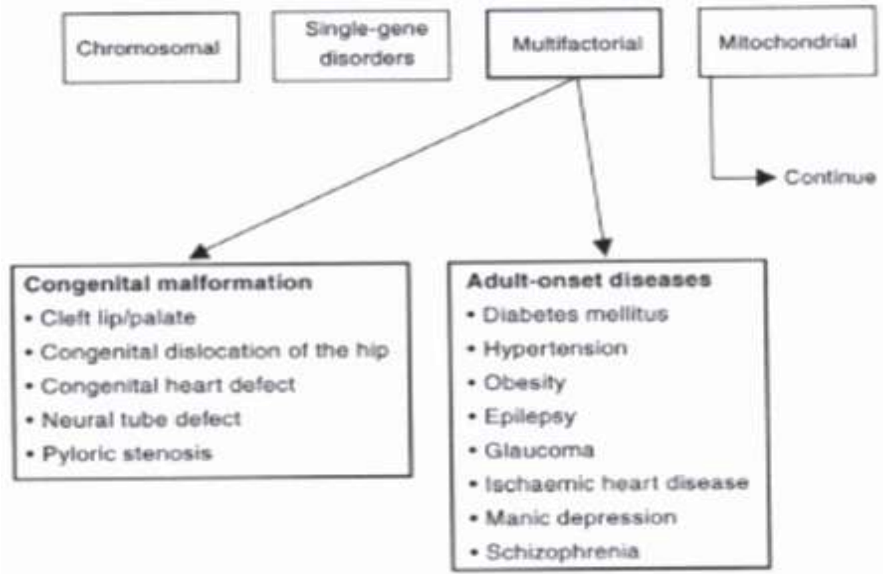
A short review of the normal embryonic development of the facial primordia is necessary before reviewing the factors that may interfere with this development leading to clefts of the lip and the palate.

In the developing embryo migration of cell masses, fusion of facial processes and the differentiation of tissues are three important events that lead eventually to an adult appearance. The pattern of development as well as cells respond to environmental signals. Since both factors are present and interact, it is difficult to ascertain the exact role of each of them. Table 1.

The facial primordia (a series of small buds of tissue that forms around the primitive mouth) are made up mainly of neural crest cells that originate from the cranial crest (Ferguson, 1988). Neural crest cells migrate to the primitive oral cavity where, in association with ectodermal cells, form the maxillary processes. Palatal shelves from these processes arise at embryonic day 45 in humans. An intrinsic force, mainly produced by the accumulation and hydration of hyaluronic acid-1, is progressively generated within the palatal shelves and reaches a threshold level which exceeds the force of resistance factors (e.g. tongue). Synthesis and hydration of hyaluronic acid by palatal mesenchyme is stimulated by epidermal growth factor and transforming growth factor beta. The erectile shelf elevating force is partly directed by bundles of type I collagen which runs down the center of the vertical shelf from its base to its tip. Moreover the epithelial covering and associated basement membrane of the palatal shelf exhibit

differential traction, which serve to constrain and direct the swelling osmotic force. In addition the palatal mesenchymal cells are themselves contractile and secrete various neurotransmitters that affect both mesenchymal cell contractility and glycosaminoglycan dehydration and therefore play a role in palate morphogenesis (Ferguson, 1988). Figure 1.

Table 1: Multifactorial Disorders - Cleft Palate



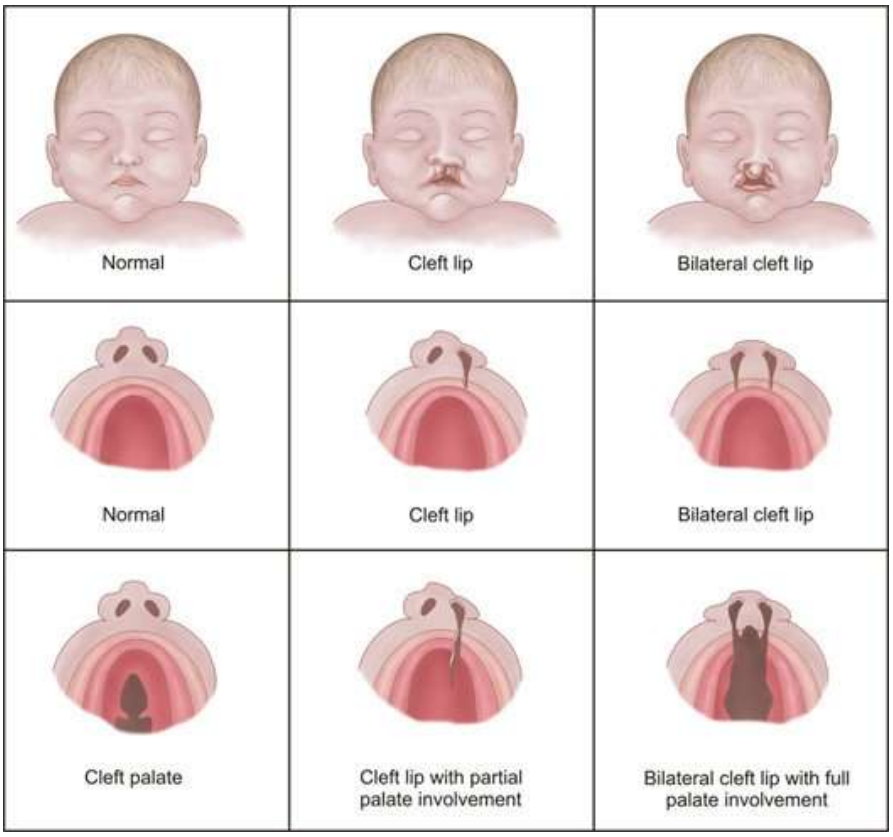


Figure 1: Orofacial Clefts

At this precise developmental stage the shelves rapidly elevate to a horizontal position above the dorsum of the tongue. Self elevation probably occurs within minutes or hours. The medial edge epithelia of the approximating palatal shelves fuse with each other developing cell adhesion molecules and desmosomes to form a midline epithelial seam. The epithelial seam starts to thin by expansion in palatal height and epithelial cell migration onto the oral and nasal aspects of the palate (Ferguson, 1988) and then degenerates establishing mesenchyme continuity across the intact horizontal palate. Medial edge epithelial cells cease DNA synthesis 24-36 hours prior to shelf contact and this is referred to as programmed cell death (PCD). The basement membrane on each side of the epithelial seam remains intact even when it has completely thinned. Epithelial-mesenchymal recombination experiments have demonstrated that epithelial differentiation is specified by the mesenchyme and that medial edge epithelial cell death is a murder by the underlying mesenchyme rather than an intrinsic epithelial suicide (Ferguson, 1988). The ways in which mesenchyme could signal epithelial differentiation is either through extracellular matrix molecules (i.e. collagen molecules), through soluble factors (i.e. growth factors), direct cell-to cell contact, or combinations of all of the above. The actual period of fusion of the mesenchymal shelves may be just a matter of minutes, but complications in events leading up to and during fusion will result in a palatal clefting of varying severity (Ferguson, 1988; Moore *et al.*, 1988).

Seam disruption also occurs by migration of a large number of epithelial seam cells (perhaps 50%) into the palatal mesenchyme (Ferguson, 1988). These fragments very quickly become indistinguishable from other palatal mesenchyme cells. The epithelia on the nasal aspect of the palate differentiate into pseudostratified ciliated columnar cells whilst that on the oral aspect of the palate differentiates into stratified squamous non-keratinized cells. Osteogenic blastemata for the palatal processes of the maxillary and palatine bones differentiate in the mesenchyme of the hard palate while several myogenic blastemata develop in the soft palate (Moore *et al.*, 1988; Sulik *et al.*, 1988).

During the period of shelf elevation, there is almost no growth in head width but constant growth in head height. This establishes a conductive orofacial environment that permits the expanding palatal shelves to occupy a position above the dorsum of the tongue (Sulik *et al.*, 1988).

In human embryos palatal shelves elevate simultaneously on day 43 (22-24 mm CRL), and the palate is closed by 55 days (33-37 mm CRL). The mesenchymal fusion is complete by 60 days (45-46 mm CRL)-12 (Ferguson, 1988; Moore *et al.*, 1988; Sulik *et al.*, 1988; Melnick, 1986). Fig.2

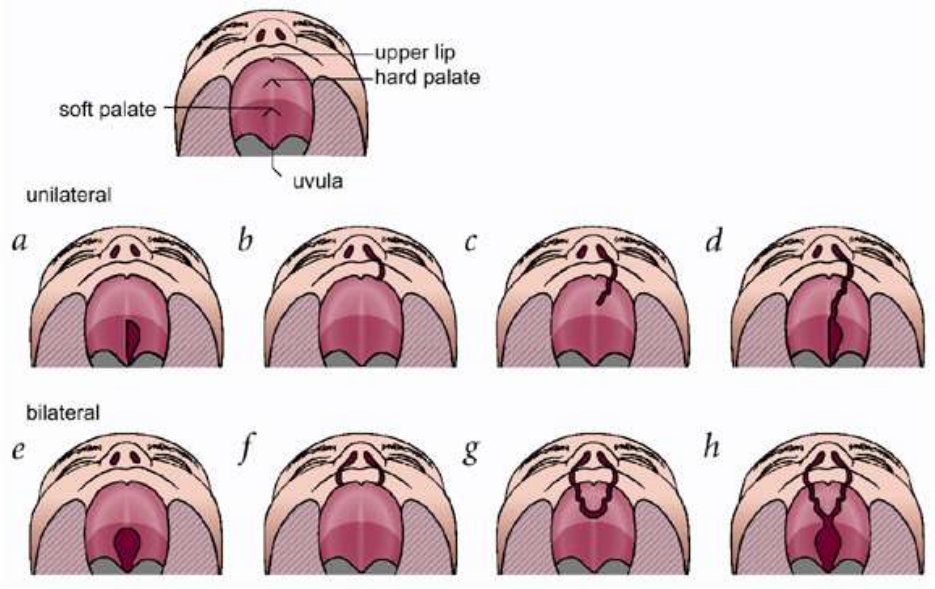


Figure 2: Cleft lip, cleft palate classification

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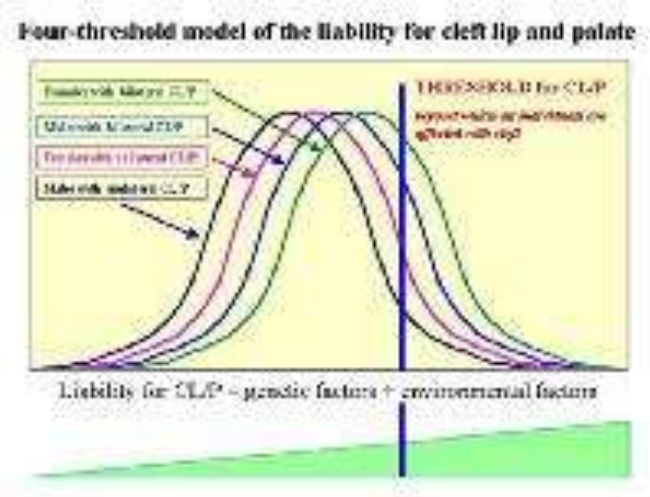
Epidemiology

Clefts of the palate, alveolus and lip (CLAP) are syndromic or non-syndromic. Syndromic types are by definition associated with other malformations, and include the Pierre-Robin Sequence, Treacher-Collins Malformation, Trisomies 13 and 18, Apert's Syndrome, Stickler's Syndrome and Waardenburg's Syndrome. At last count, more than 300 syndromes were associated with CLAP (Sulik *et al.*, 1988; Melnick, 1986). Syndromic etiologies include single gene transmission such as Trisomies; teratogenic causes such as fetal alcohol syndrome; or environmental causes such as amniotic band syndrome or maternal diabetes mellitus (Ferguson, 1988; Sulik *et al.*, 1988; Melnick, 1986; Poswillo, 1968). Table.2

Table 2: Pediatric Cleft Lip and Palate



Non-syndromic CLAP is a diagnosis of exclusion, and is considered to be of multifactorial inheritance with known predicted rates of recurrence (Amaratunga, 1989).

An isolated cleft palate (CP) is genetically distinct from an isolated cleft lip (CL) or a combined CLAP. Cleft lip with or without cleft palate has an incidence of 1:1000 births in the United States and approximately 1:600 births in the United Kingdom, but is different among ethnic groups (Sulik *et al.*, 1988; Melnick, 1986). American Indians have the highest incidence, at 3.6:1000 births, followed by Asians, whites and blacks (0.3:1000). In contradistinction, isolated cleft palate is the same among all racial groups, with an incidence of approximately 1:2000 births. The male:female ratio for CL or CLAP is 2:1, whereas for isolated CP it is 1:2 (Sulik *et al.*, 1988).

Normal Anatomy

The upper lip is longer than the lower lip, and is shaped liked a flattened 'M'. The lower lip is shaped like a flattened 'W'. Cupid's bow defines the central portion of the upper lip and the apices of the bow join the philtrum. The nadir bisects the apices. The lip extends laterally to approximately the lateral limbus of the eye. Surrounding the lips is the orbicularis oris, the sphincter of the mouth (Moore *et al.*, 1988; Sulik *et al.*, 1988). The maxilla has several distinct anatomical areas. The nasal spine is the anterior projection of the maxilla and alveolus. The alveolar process of the maxilla surrounds the palate and houses the teeth. The incisive canal is located posterior to the incisors, and transmits the lesser palatine artery, one of the distal branches of the internal maxillary artery. Posteriorly and laterally along the palate is the greater palatine foramina, which transmit the greater palatatine artery, a branch of the internal maxillary artery (Melnick, 1986). The palate itself is formed from the maxilla, the horizontal process of the palatine bone and the pterygoid plates. The soft palate attaches to the posterior portion of the hard palate and interdigitates with the lateral pharyngeal wall via several muscular attachments. From the nasopharyngeal to the oral cavity surface, the muscles of the soft palate consist of the palatopharyngeus, the

salpingopharyngeus, the levator and tensor palatini, the muscular uvula, the palatoglossus and the superior constrictor muscle (Poswillo, 1968; Amaratunga, 1989).

The superior constrictor muscle is the primary sphincter of the pharyngeal phase of swallowing and is responsible for preventing regurgitation into the nasopharynx (Velopharyngeal Insufficiency, VPI). The tensor veli palatini connects from the eustachian tube to the scaphoid fossa of the sphenoid bone and then to the lateral soft palate. It tenses the palate, but is not believed to play a major role in palatal elevation. Tubal dilitation from the tensor palatini is probably minimal (Ferguson, 1988; Sulik *et al.*, 1988; Poswillo, 1968). The levator veli palatini originates from the bony cartilaginous junction of the eustachian tube and wraps around the hamulus before connecting to the soft palate. The levator is responsible for palatal elevation and perhaps tubal dilitation (Ferguson, 1988; Sulik *et al.*, 1988). The salpingopharyngeus is a consistently small muscle with probable minimal effects upon palatal and tubal function (Ferguson, 1988; Sulik *et al.*, 1988).

PATHOGENESIS OF CL AND CP

In studying different types of orofacial malformation, animal specimens have been proved to be especially helpful because they permit observation of embryological and fetal stages that lead to malformations found at birth.

The majority of congenital craniofacial malformations occur during the 5-12 weeks of development (Moore *et al.*, 1988). The embryonic period (from 3-9 weeks) is the most sensitive period during which teratogens can be particular damaging. This is especially true for midline morphologic disorders such as cleft lip and palate. They are considered to be a polygenic multifactorial problem in which genetic susceptibility is influenced by multiple and probably cumulative environmental factors, interacting altogether to shift the complex process of morphogenesis of the primary and secondary palates towards a threshold of abnormality at which clefting may occur (multifactorial/threshold model). Both the genetic and the environmental factors have not been established yet (Melnick, 1986; Poswillo, 1968).

Cell death is a normal phenomenon seen in the developing embryo (PCD). It is also a common feature seen in embryos after exposure to a variety of teratogenes that induce craniofacial malformations. There are three distinct types of PCD (Sulik, 1988). Type 1 is characterized by cellular condensation, fragmentation, phagocytosis and finally lysosomal degradation. Type 2 is characterized primarily by the appearance of large lysosomes which initiate cellular degradation. Type 3 occurs without the involvement of lysosomes and without apparent phagocytosis (Poswillo, 1968; Amaratunga, 1989).

The sites of cell death vary depending upon the teratogen (or genetic insult) and the exposure time (i.e. developmental stage of the embryo). There seems to be a selective sensitivity of cells; tissues with high proliferative activity are more likely to show cell death than tissues that proliferate more slowly. Other factors may also been involved i.e. state of cellular differentiation, differential drug distribution or other specific cellular characteristics. Both the disappearance and expansion of areas of PCD may have a role in teratogenesis (Sulik *et al.*, 1988).

Pathogenesis is probably caused by one of the following mechanisms:

1) Anatomic obstruction i.e. the tongue obstruction hypothesis-only when associated with mandibular underdevelopment (Melnick, 1986). In the cases where the chin is compressed against the sternum, the tongue may interpose in the space between the ascending shelves. The resultant palatal deficiency is U-shaped not V-shaped and it is considered to be a deformation of tissues with a normal growth potential rather than a malformation of tissues that may have been affected by disturbances of ectomesenchyme or other phenomena at cellular level (Poswillo, 1968).

2) Interference with cell differentiation or migration, either through hormonal defect, biochemical defect, or extrinsic biochemical interference. Numerous studies have substantiated the association between teratogens and clefting. Such teratogens may be individually operative in a subgroup of individuals that is genetically and biologically susceptible. Conversely, several different teratogens may act together on a single mechanism controlled by only a few genes. At present our knowledge of the teratogens that are associated with clefting is very limited. Only a few substances such as retinoic acid

(used in the treatment of acne and psoriasis), have been confirmed as teratogens with direct effect on facial morphogenesis.

Several other factors that may influence genetic behavior and early morphogenesis have received attention in investigation of the etiology of CL and CP (Amaratunga, 1989).

Seasonal variation in the incidence of the CLP has been reported by several authors while others have reported the opposite. This phenomenon has not been satisfactorily explained. One possible reason is viral infection, which may have a seasonal trend. However, a correlation between clefts and viral infections has not clearly been established.

Also some authors report that CLP is higher in the earlier born children while others conclude the opposite. When birth rank is raised, maternal age also could be raised. Mutations of genes can occur with advanced parental age.

Monozygous twins discordant for clefting are interesting. Examinations of the developing fetus by ultrasound have shown that there are altered rates of fetal growth, both of the whole body and of its parts, so that at any one time twins may exhibit different stages of development. Therefore the variable expression of clefting could result from the same factor acting on both twins at the same time, but at relatively different stages of their early growth.

With regards to lip clefting, it seems that the critical stage of lip formation is when the medial and lateral nasal processes contact each other and fuse.

- Anatomical variations (differences in the size, shape or position of the facial processes), based possibly on ethnic or other factors, may predispose to the problem of lip formation. Where the size of the facial processes is reduced and they are not in tight apposition there is an increased possibility of cleft lip. Experimental support of this is found in the work of Trasler (1968) and Brown *et al.,* (1985) reviewed by Poswillo (1988), where the spontaneous development of cleft lip and palate in A strain mice is attributable to the pointed facial processes that prevent wide areas of contact. On the other hand in the C57 black strain of mouse the larger facial processes facilitate wider contact of the processes and therefore clefting does not develop.

While anatomical variation is one potential predisposing factor in the development of cleft lip and palate, there are also other factors. It is well established that at the time of consolidation of the facial processes there is a concurrent program of spontaneous cell death (PCD) involved in the removal of the epithelial debris from the developing nasal placode. When this PCD is more extensive than necessary and repair of mesenchyme is disturbed, a weakness develops in the forming lip and alveolus. The continued action of growth traction forces may further disrupt the association of the facial processes with the lip margins being pulled apart. Resultant clefts of the lip may vary from a simple groove in the muscle to a complete cleft into the nasal floor (Poswillo, 1988).

With regards to the submucous cleft palate and bifid uvula, both can be considered as microforms of isolated palatal clefting and are probably the result of disturbances in the local mesenchyme at the time of ossification of the palatal bridge and merging of the margins of the soft palate. These phenomena occur late in morphogenesis, between 7-10 weeks of human development (Poswillo, 1974).

There is a frequent association between clefts of the lip and cleft palate. Animal studies suggest that following the failure of lip closure there is an overgrowth of the prolabial tissues which then divert the tongue into the nasal cavity. The mesenchymal obstruction ~~of~~ by the tongue can delay the movement of one or both palatal shelves, so that opportunities for palatal fusion are lost (Poswillo, 1988).

The sequence of lip and palate formation extends over 15 days in man. Therefore in many syndromes cleft lip and palate may accompany anomalies of other parts of the body. Many developing systems can be disturbed simultaneously by teratogenic influences which operate over a long period of morphodifferentiation. But despite the fact that there are over 150 recognized disorders in which CL, CP or both may represent one feature, it is widely believed that the majority of affected individuals are otherwise structurally normal (Jones, 1988). Recent studies (Shprintzen *et al.*, 1985) have emphasized the fact that a significant portion of children with clefts have the cleft as one feature of a broader pattern of malformation. It is important to recognize that structural defects are not, for the most part, randomly

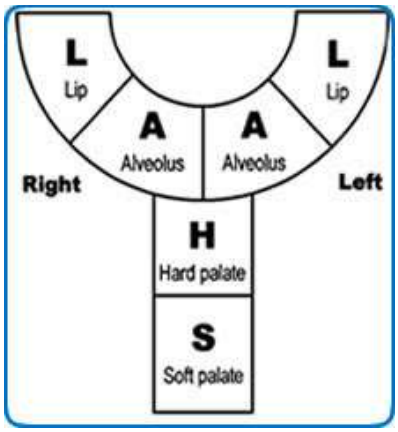
associated. The presence of other major and minor malformations in association with a cleft implies that a single etiologic factor - genetic, chromosomal or teratogenic - produced the pattern as a whole.

Although CL is frequently associated with CP, CL with or without CP and CP alone are distinctly different in etiology. Subsequent studies have consistently confirmed that these two conditions indeed differ in etiology and also in incidence, sex predisposition and their relationship to associated birth defects. CL results from the non-fusion of the upper lip and the anterior part of the maxilla during weeks 5-7 and occurs at an incidence of approximately 1/1000 births (Thompson and Thompson, 1986). CP alone results from failure of the mesenchymal masses of the palatine processes to fuse during weeks 7-12 and has an average incidence of 0.7/1000 births. The incidence of CL with or without CP varies from 2.1/1000 in Japan to 0.4/1000 Nigeria (rev by Moore, 1988), with the geographical variation being less important than ethnic differences. In contrast the incidence of the CP alone shows little variation in different racial groups. This may mean that CP alone will not fit the purely multifactorial model which includes both polygenic origin and undefined environmental factors that would increase the variation in incidence both geographically and to some extent racially. Generally CL with or without CP are more frequent in males, whereas CP alone is more frequent in females. Therefore due to both genetic and environmental evidence it seems that CL with or without CP and CP alone are separate entities (Poswillo, 1988).

Classification

There is no universally accepted classification of clefts, although the most commonly used is the Veau classification, which was described in 1931 (Moore *et al.*, 1988; Sulik *et al.*, 1988; Melnick, 1986; Poswillo, 1988). Veau Class I is an isolated soft palate cleft; Class II is a hard/soft cleft palate; Class III is unilateral cleft lip and palate; Class IV is a bilateral cleft of the lip and palate. Most surgeons describe the defect rather than using the Veau system. For example, a Veau Class III would be described as a unilateral complete cleft of the lip, alveolus, primary and secondary palates (Moore *et al.*, 1988; Sulik *et al.*, 1988; Poswillo, 1988; Jones, 1988). Table 3.

Table 3: Cleft-classification



The Unilateral Cleft Lip. The CLAP can be divided into defects of the lip, alveolus and palate. The cleft lip is a failure of mesodermal proliferation resulting in complete or incomplete defects. The complete unilateral cleft lip includes the orbicularis oris muscle, where the medial portion of the muscle attaches to the columella and the lateral portion to the nasal ala cartilage. The medial vermillion border is usually thin (called *the white roll)*. An incomplete cleft lip ranges from a mucosal covering to a slight defect in the bulk of the orbicularis muscle which is barely detectable.

The nasal defect of a unilateral cleft lip is fairly constant. The ipsilateral lower lateral cartilage is usually flattened and rotated laterally and inferiorly, resulting in horizontal, short appearance. The columella is short and often bends and dislocates the septum. The overall appearance is a flattened dome with a wide, horizontal ipsilateral nostril.

The Bilateral Cleft Lip. The bilateral cleft lip is similar to the unilateral defect with the exception of a complete absence of orbicularis muscle on the medial aspect *(the premaxilla or prolabium)*. The prolabium is usually extruded to a varying degree. The nasal deformity is essentially a duplication of the unilateral defect, with a bilaterally flattened dome, short columella and bilateral horizontal nostrils.

Clefts of the Primary Palate and Alveolus. The primary palate is that portion anterior to the incisive foramen. A cleft of the primary palate results in a gap from the incisive foramen through the alveolus. Clefts of this type are always associated with clefts of the lip.

Clefts of the Secondary Palate. Clefts of the secondary palate are a failure of medial growth of the palatal shelves. Fusion begins at the incisive foramen and progresses posteriorly. The vomer is midline, with various attachments to the remnant palate. The defect of the soft palate is failure of midline fusion. The palatal musculature attaches to the posterior hard palate.

There are a wide clinical range of clefts of the secondary palate, from the submucous cleft to a complete cleft of the hard and soft palate. The submucous cleft palate is as a midline diasthasis of the velar musculature, a bifid uvula and a notch in the posterior hard palate (Poswillo, 1968; Amaratunga, 1989; Jones, 1988; Thompson and Thompson, 1986).

Genetic Factors

Polygenic inheritance refers to conditions determined exclusively by a large number of genes, each with a small effect, acting addictively (i.e. hair color) (Bjornsson *et al*., 1989).

Multifactorial inheritance refers to conditions determined by a combination of factors each with a minor but additive effect (i.e. blood pressure) (Thompson and Thompson, 1986) and has been developed to describe the observed non-Mendelian recurrences of common birth defects. It includes both polygenic origin and undefined environmental factors that will increase the variation in incidence both geographically and to some extent racially. The multifactorial inheritance is more difficult to analyze than other types of inheritance but it is thought to account for much of the normal variation in families, as well as for many common disorders, including congenital malformations. Table 4.

Table 4: Candidate genes or loci implicated in the etiology of nonsyndromic cleft lip and palate and the available evidence

Chromosome	Gene*	Evidence			
		Mutation identified	Linkage	Linkage disequilibrium	Association
1p36	*MTHFR*			☑	☑
1q32	*IRF6*	☑			
2p13	*TGFA*			☑	☑
2q32	*SATB2*	☑			
4p26	*MSX1*	☑		☑	☑
4q21	*ACOD4*	☑			
6p23	-	☑	☑	☑	
11q23	*PVRL1*	☑			☑
14q24	*TGFB3*			☑	☑
19q13	*CLPTM1*	☑	☑	☑	
Xq12	*TBX22*	☑		☑	

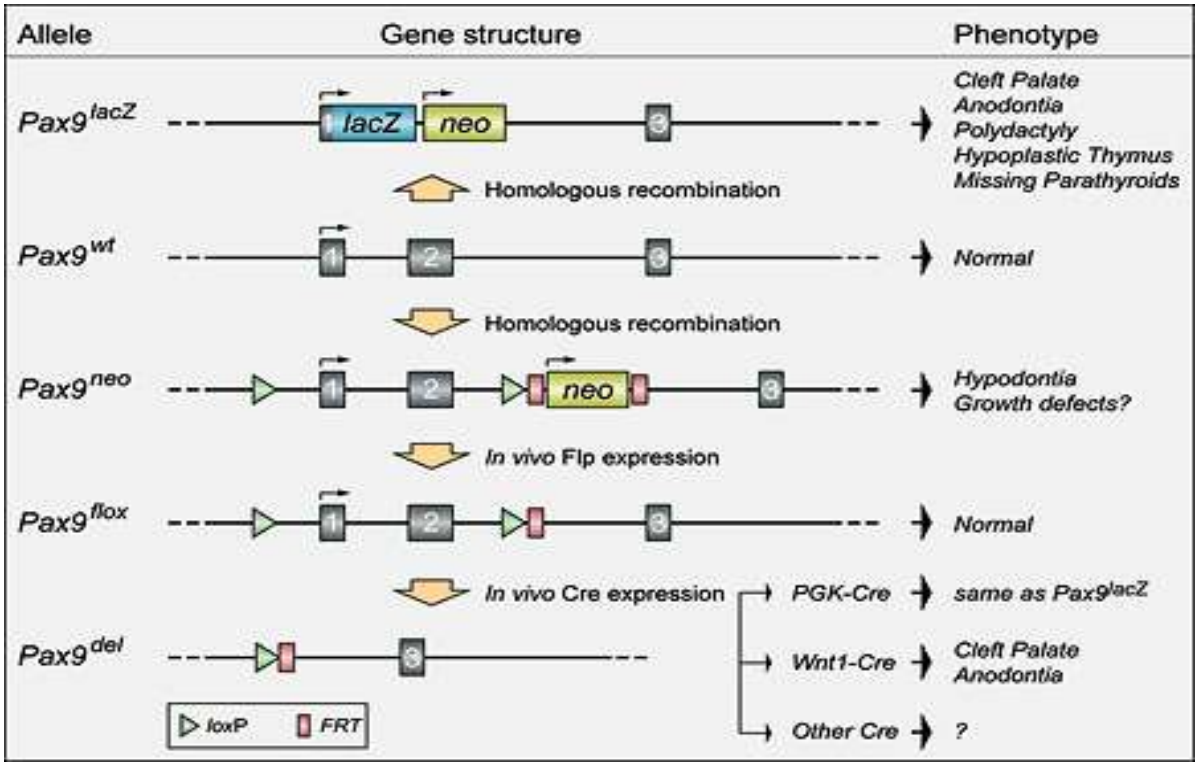
**MTHFR* - 5,10-methylenetetrahydrofolate reductase, *IRF6* - Interferon regulatory factor-6., *TGFA* - Transforming growth factor-alpha, *SATB2* - Special AT-rich sequence-binding protein-2, *MSX1* - *Drosophila* msh homeobox homolog-1, *ACOD4* - Acyl-coenzyme A desaturase-4, *PVRL1* - Poliovirus receptor like-1m, *TGFB3* - Transforming growth factor beta-3, *CLPTM1* - Cleft lip and palate–associated transmembrane protein-1, *TBX22* - T-box transcription factor-22

The normal rate of development can be thought as a continuous distribution that if it is disturbed a serious malformation may result, dividing the continuous distribution into normal and abnormal class**es** separated by a threshold. This has been described as the multifactorial/threshold model and several human congenital malformations show family patterns that fit this model. CL with or without CP and CP alone are included in this category.

CL with or without CP shows both geographical and racial variations which means that it could be explained by the multifactorial/threshold model. In contrast CP alone shows little variation in different racial groups. This may mean that CP alone will not fit the purely multifactorial model. Table 5.

To date, there have been three pedigrees reported in which CP is clearly inherited as a single-gene X-linked disorder (Moore *et al.*, 1987; Moore *et al.*, 1990; Melnick *et al.*, 1980).

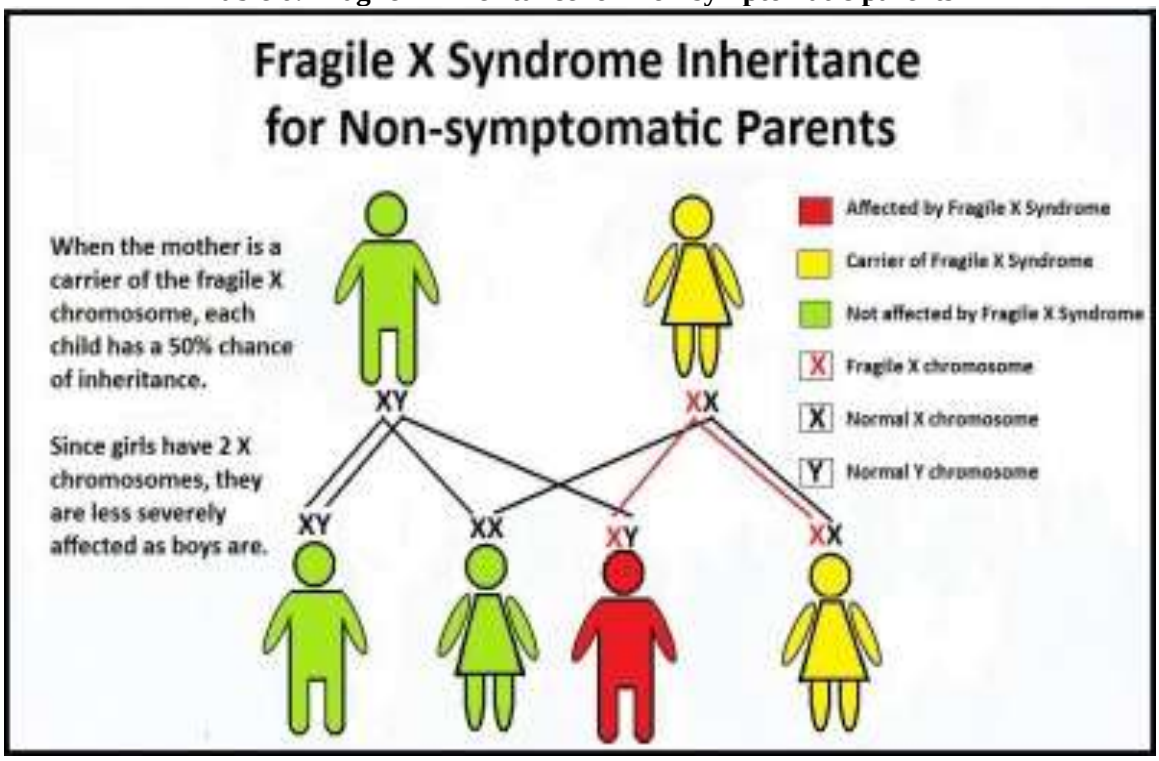
Table 5: Orofacial clefting such as cleft lip and cleft palate often occurs sporadically and do not follow an obvious Mendelian inheritance



One of these pedigrees is described in a large Icelandic family (293 individuals) that shows Mendelian inheritance of X-linked secondary cleft palate and ankyloglossia (Melnick *et al.*, 1980). Family analysis showed that the frequency of CP among all those relatives was much higher among the male than among the female CP probands. There was no incidence of male to male transmission in this large family. The X-linked mode of inheritance of CP is indicated by the family distribution. **In addition** the large size of this family together with the availability of many well localized X-chromosome probes has made it possible to localize the defect subchromosomally (using RFLP-restriction fragment length polymorphism studies for linkage) to the q13-q21.1 region of the X chromosome (Farrall and Holder, 1992). Finer mapping and the use of cell lines from patients with deletions of the X chromosome have further localized the defect to Xq21.31-q21.33 (Lammar *et al.*, 1985). **Table.6**

In the case of CL with or without CP, Melnick *et al.*, (1980) reviewed worldwide CL/P recurrence risk data and found that both a multifactorial-threshold model and a monogenic with random environment component model fitted poorly.

Table 6: Fragile X inheritance for Non-symptomatic parents



Farrall and Holder (1992) refer to the work of several investigators. According to their report: Marazita 1984 in his analysis of a subset of Danish CL/P families, found no support for a MF/T model but suggested the possibility of a major gene. Also Marazita *et al.*, (1986) have reported an analysis of ten English multigenerational CL/P families (collected by Carter, 1982). They were able to reject an MF/T model and demonstrated that major locus acting on a multifactorial background (mixed-model) gave a reasonable fit. Chung (1986) analyzed a series of Danish and Japanese CL/P families and concluded that the best fitting model predicted recessive major gene acting on a multifactorial background (mixed-model). Chung *et al.*, (1989) analyzed Hawaiian families from several racial groups and found that the data were consistent with a major-gene/multifactorial model (mixed model). Ardinger (1988) have provided additional evidence for an association between the locus for transforming growth factor alpha (TGFA) and CL/P locus. TGFA is believed to be the embryonic form of epidermal growth factor, which is believed to regulate the proliferation and differentiation of palatal epithelial cells both in vitro and in vivo. Hecht et al, 1991, analyzed Midwestern U.S. Caucasian families and showed consistency with a major-locus model. He found that the dominant or co-dominant models with decreased penetrance fitted the best. Both the MF/T model and the mixed model with a dominant major gene effect were found to provide an explanation of familiar clustering pattern. Marazita *et al.*, (1992) analyzed almost 2000 Shanghai families and found that the best fitting model was that of an autosomal recessive major locus.

Farrall and Holder (1992) in their own analysis have shown that the extensive published recurrence risk data, which have been interpreted to be consistent with an MF/T pattern of inheritance, are equally compatible with an oligogenic model with perhaps as few as four genes.

In conclusion, the extensive recurrence risk data, which have been widely interpreted as providing evidence of a polygenic multifactorial trait, are now thought to be consistent with a model with a major-gene effect contributing to about 1/3 of CL/P and acting on a multifactorial background. For CL/P, the observed decline in risk with decreasing relatedness to the proband is incompatible with any generalized single-major-locus (gSML) model of inheritance and is suggestive of multilocus inheritance.

Teratogenes

Palatal shelf elevation and fusion depends on fetal neuromuscular activity, growth of the cranial base and mandible, production of extracellular matrix and contractile elements in the palatal shelves, shelf adhesion, PCD of the midline epithelial seam and fusion of the ectomesenchyme between one shelf and the other. All these phenomena must act in perfect harmony over a short period of time in order to produce normal palatogenesis. Factors that interfere with any of these events could lead to a cleft (Poswillo, 1968; Poswillo, 1974; Jones, 1988).

Vitamin A

By introducing into the maternal diet of A strain mice human teratogenic agents such as of excess vitamin A, the malformation threshold in the developing embryos may be shifted to the extent that 100% of the offspring are born with the expected deformity (Poswillo, 1988). Renewed interest in retinoic teratogenicity has followed the introduction of 13-cis-retinoic acid as an effective treatment for severe cystic acne. Inadvertent use of 13-cis-retinoic acid during the first trimester of human pregnancy has been reported to result in a spectrum of malformations termed retinoic acid embryopathy (RAE) (Sulik *et al.,* 1988) and includes microtia or anotia, micrognathia and in some cases CP. Induction of CP following administration of excess vitamin A to pregnant laboratory animals is well documented (Schendel *et al.,* 1989). Most of the early animal studies involved exposure to forms of vitamin A that are stored in the maternal liver and these therefore have a relatively long half-life; these studies also involved multiple administrations of the drug or only examined the developmental end-point ; thereby excluding study of the developmental changes that lead to CP.

The study of Kochhar and Johnson (1965), reviewed by Sulik (1989), describes palatal clefts for which the shelves were very small or entirely absent; these resulted from insufficient maxillary prominence mesenchyme. These investigators also found that size reduction of the palatal shelves occurred only posteriorly in the most cases.

The use of all-trans-retinoic acid, which is of short half-life, has shown the incidence of cleft palate peaks at more than one developmental stage in both hamsters and mice (Kochhar, 1973; Sulik, 1989).

The changing incidence and severity of secondary palatal malformations that may be induced within a narrow window of time (over a 16 hour period) appear to be related to a corresponding change in the pattern of PCD in the first visceral arch. It has been shown that 13-cis-retinoic acid increases the amount of cell death in regions of PCD in C57B1/6J mice, a strain which is particularly prone to spontaneous craniofacial malformations (Sulik *et al.,* 1988; Fraser, 1976). The distribution of excessive cell death in regions of PCD provides a base for understanding the composition of syndromes in which malformation appears to be unrelated by tissue type or location (Fraser, 1976).

Vitamin A cell necrosis has been described as being consistent with type-2 cell death (Sulik, 1988). On the other hand it has been noted that lysosomal membranes of all cells do not lyse. Only those membranes that are at a particular stage of differentiation or which have been perturbed in some other way lyse. Membrane destabilization by the retinoids may interfere with many cellular functions. For example, blebbing of neural crest cells membrane was noticed following retinoic exposure. This may interfere with the migratory ability of these cells. Recovery follows removal of the retinoic acid in vitro. In vivo, recovery from a brief interference with cell migration might also be expected but sufficient recovery probably does not follow the excessive cell death of progenitor cells.

Treatment of female C57B1/6J mice with 13-cis-retinoic acid at an early stage of pregnancy (8d14h to 9d0h) has a more severe effect on the secondary palate (Fraser, 1976). 12 hours after the 8d14h treatment time, embryos have 13 to 20 somites. Extensive expansion of cell death at this time would be expected to have a major effect on almost the entire secondary palatal shelf complex, thereby resulting in severe hypoplasia and clefting. Minor effects would be expected to involve only the posterior portion of the maxillary prominences, thereby resulting in deficiency in the posterior aspect of the secondary palate.

12 hours after the 9h6h treatment time (late treatment), embryos have approximately 30 to 34 somites. Expansion of cell death in embryos at this stage of development results primarily in foreshortening of the secondary palate, which occurs at the expense of its posterior portion. Major effects on the entire palatal

shelves would not be expected at this treatment time. Later treatment times are mostly associated with induction of limb malformations (Lowry, 1970)

Smoking

The relationship between maternal smoking and CLP is not strong, but it is significant. Several studies have consistently yielded a relative risk of about 1.3-1.5. When maternal smoking was considered together with a positive genetic background, the combined effect was more significant. Furthermore, van Rooij *et al.*, (2001) found that a maternal glutathione s-transferase (*GSTT1*) genotype, when combined with smoking, could significantly increase the risk of CLP (odds ratio=4.9). Beaty *et al.*, (2002) reported that maternal smoking and infant *MSX1* genotypes acted together to increase the risk for CLP by 7.16 times (Moore *et al.*, 1987).

Use of Folic Acid and Multivitamins

Shaw *et al* reported that if vitamin supplements namely folic acid and cobalamins were not taken during early pregnancy the risk for CLP could be tripled (Shaw *et al.*, 2002). Folic acid deficiency with a pre-existing *TGFA* TaqI C2 genotype was also found to increase the risk of CLP In addition, defective maternal vitamin-dependent homocysteine metabolism is a risk factor for CLP in the offspring. In a case-control study, mothers of patients with CLP had significantly higher homocysteine level, lower level of whole-blood vitamin B_6, and higher rate of hyperhomocysteinemia (Jugessur *et al.*, 2003; Wong *et al.*, 1999). The role of folic acid supplementation in the prevention of CLP has been investigated in several studies. It seems that low-dose folic acid supplementation by fortification of cereal grain products cannot protect against CLP. Only a very high dose of supplementary folic acid (10 mg/day) could reduce the risk of CLP significantly (65% reduction was observed) (Ray *et al.*, 2003; Tolarova and Harris, 1995).

Phenytoin

Under the influence of teratogenic doses of phenytoin, the lateral nasal process fails to expand to the size necessary for tight tissue contact with the medial nasal process (Poswillo, 1988). Abnormal differentiation of the cellular processes of the ectomesenchymal cells is probably associated with this condition, where the failure of union is at the point of connection which establishes the lip and primary palate (Moore *et al.*, 1990; Lammar *et al.*, 1985).

Ethanol

Ethanol (alcohol) is an important human teratogen. It is estimated to affect severely 1.1/1000 live births and have lesser effects in 3-4/1000 children born. Its abuse during pregnancy results in fetal alcohol syndrome (FAS) which involves a wide variety of malformations in many organs. Abnormalities that are not diagnostic of FAS, but are associated with maternal ethanol abuse are termed fetal alcohol effects (FAE) (Sulik, 1988). Treatment of C57BL/6J female pregnant mice with ethanol when the embryos have approximately 7-10 somites results in a pattern of malformation that is consistent with the DiGeorge sequence (midline clefts in the nose and cleft palate are features of this sequence). The DiGeorge sequence has been described in the offspring of alcoholic mothers (Melnick *et al.*, 1980; Lammar *et al.*, 1985).

Among the cellular effects of ethanol are increased peroxidase activity, interference with cytoskeletal components, diminished DNA synthesis and suppressed rates of cell division, and direct effect on membranes resulting in excessive fluidity (Sulik, 1988). Recent investigations illustrate excessive cell death within 12 hours following maternal treatment. The rates of cell death are similar to the normal rates of cell death seen in PCD, but the areas of cell death are expanded. The reason for this excessive cell death is not yet clear. One possible explanation is that exposure to ethanol results in lipid peroxidase formation that leads to rupture of lysosomal membranes and release of hydrolytic enzymes (type 2 cell death) (Thompson and Thompson, 1986; Lammar *et al.*, 1985; Powsillo and Roy, 1965).

Hyperthermia

Hyperthermia has teratogenic effects and the facial malformations induced include, among others, cleft lip and/or cleft palate. CNS is particularly sensitive to hyperthermia. Facial abnormalities have been associated with human maternal hyperthermia at 4-7 weeks (Sulik, 1988). The type and extent of damage depend on the duration of temperature elevation and the extent of elevation. In addition low sustained

temperature elevations appear to be as damaging as repeated spikes of higher elevation. Elevations of 1.5-2.5 degrees of Celsius above normal body temperatures represent the threshold for Teratogenesis in human. Such elevations can result with excessive exercise, the use of hot bath and saunas and febrile episodes (Moore *et al.,* 1987).

Again in the case of heat-induced Teratogenesis, cell death is considered to play a major role, with the mitotic cells being the most susceptible. The pathogenesis of heat-induced malformations in areas other than the CNS has not been studied yet. It has been suggested though that hyperthermia could result in intra and extracellular leakage of lysosomal enzymes which could lead in type-2 cell death (Farrall and Holder, 1992; Powsillo and Roy, 1965; Sulik *et al.,* 1989).

Ionizing Radiation

Ionizing radiation acts as a direct insult to the embryo. The malformations induced are similar to those noted following exposure to ethanol, retinoic acid or hyperthermia. The cellular mechanisms of radiation induced Teratogenesis are not completed understood. They vary from sublethal injuries affecting differentiation and cellular interactions, to effects on rates of proliferation and cell death (Sulik, 1988). Cellular response to the radiation is dependent on cell cycle. Also in some instances cell death is linked to chromosomal damages. Some studies have shown that irradiation results in altered permeability of intracellular structures and enzyme release, i.e. rupture of lysosomal membranes, and suggest that this results from lipid peroxide formation (Sulik *et al.,* 1989; Sulik *et al.,* 1988).

Hypoxia

Of particular interest is the hypoxia-induced cleft lip. Hypoxia in the human embryo may result from cigarette smoking, reduced atmospheric oxygen levels and also placental insufficiency. Previous studies had shown size reduction and abnormal apposition of the facial prominences as possible pathogenetic mechanisms. The presence of cellular debris resulting from cell death in the deepest aspects of the invaginating nasal placodes, as well as overall growth retardation of the facial prominences, lead to inability of the facial prominences to contact and fuse (Sulik, 1988). It has also been suggested that direct effects of oxygen deficiency on cells can lead to glycolysis which is followed by acidification of intercapillary spaces and subsequent necrosis resulting from intra and extracellular leakage of lysosomal enzymes. It is interesting to note that chemicals that interfere with oxidative enzymes such as phenytoin induce cleft lip in the mice (Schendel *et al.,* 1989; Lowry, 1970).

Antimetabolites

Methotrxate and aminopterin are two uncommon antimetabolites that can induce cranial dysplasia and cleft palate in humans. Their action is inhibitory of DNA synthesis through competitive folic acid antagonism. The pathogenesis of methotexate involves fluid imbalance, resulting perhaps from interference with osmoregulatory cells in extraembryonic capillary beds, which is partially responsible for the malformation (Schendel *et al.,* 1989; Fraser, 1976; Lowry, 1970).

Metabolic Disorders

An interesting finding associated with lip clefting was that of mitochondrial myopathy of cleft muscles (Rushton, 1979). Facial muscle specimens from the cleft site were characterized by disorganized fibers, going in many different directions. The number of fibers appeared to be decreased and there was more connective tissue between the muscle fibers. The fiber diameters were also found to be much smaller. NADH staining and electron microscopy revealed large accumulation of mitochondria at the central portion of the fibers, giving a star shaped appearance to each fiber. The mitochondria are more variable in shape than normal, and the cristae are more densely packed than expected.

These abnormalities in mitochondrial size, location, cristae and number suggest a form of metabolic defect that could underlie cleft lip deformities. The suggested explanation is that a defect in energy production could result in insufficient cellular migration and proliferation and thus be the pathophysiologic basis for cleft lip. As mentioned, in addition to the mitochondria, the cleft lip muscles were found to be abnormal. However, no signs of group denervation or re-enervation were found and the motor end plate structures appeared normal. These findings argue against denervation or abnormal innervation as a cause of the abnormalities. Since the innervation was normal, the muscle atrophy was

attributed to an inability of these muscles to function properly, which was furthermore attributed to the mitochondrial energy production abnormality or to the lack of normal fiber orientation. If the causative factor is the fiber orientation, one would expect this to improve following adequate surgical reconstruction of the muscle at the time of lip repair. On the other hand changes secondary to cellular energy problems would not be expected to improve following surgery (Lowry, 1970; Rushton, 1979).

In general common targets for some teratogens (i.e. cells in regions of PCD that represent a developmental weak point) provide reason to expect interactive effects. Repeated exposure of teratogens in subthreshold doses of more than one agent could result in potentiation. Potentiation indeed occurs after repeated exposures to vitamin A and hyperthermia (Schendel *et al.*, 1989; Rushton, 1979).

Alcohol us

Heavy maternal drinking, apart from causing fetal alcohol syndrome, also increases the risk of CLP. Munger *et al.*, (1996) showed that maternal drinking increased the risk for CLP by 1.5-4.7 times in a dose-dependent manner. The results were supported by Shaw and Lammer (1999) who showed that mothers who consumed more than five drinks per occasion had 3.4 times increased risk of delivering an infant with CLP. Low-level alcohol consumption, however, did not seem to increase the risk of orofacial clefts. The link between alcohol consumption and genotypes on the risk of CLP has yet to be demonstrated (Natsume *et al.*, 2000).

Steroids

Corticosteroids form the first-line drugs for the management of a variety of conditions in women of childbearing age. The clefting role of corticosteroid in animal models is well known (Diewert and Pratt, 1981; Melnick *et al.*, 1981). CLP is induced in the progeny of pregnant mice that are given glucocorticoids. The incidence, however, varies among inbred strains and also with the dose given and the stage of gestation when the drug is given. Diewert and Pratt found that cortisone not only affected the content of extracellular matrix (ECM) and the number of palatal shelf cells in A/J mice but that shelf elevation was delayed and only half of the cortisone-treated palates achieved complete horizontal positioning of the shelves in all regions of the palate (Diewert and Pratt, 1981; Melnick *et al.*, 1981). Melnick *et al.*, (1981) studied the teratological effects on lip morphogenesis after the administration of triamcinolone hexacetonide on the eighth day of gestation. The frequency of CLP in treated A/J mice was found to be more than three times greater than the spontaneous frequency in untreated controls. Affected A/J embryos showed a severe reduction in the size of the lateral nasal processes. Gasser *et al*. examined strains of mice for susceptibility to cortisone-induced CLP and confirmed the role of genes linked to H-2 on chromosome 17 (Gasser *et al.*, 1981). Later, the same group refined the chromosome region carrying the cleft palate susceptibility-1 (*Cps-1*) gene (Gasser *et al.*, 1981). Juriloff and Mah mapped a major CL(P)-causing gene to mouse chromosome 11 in a region having linkage homology with human 17q21 through 24 (Juriloff *et al.*, 1995).

Studies have investigated the association between maternal corticosteroid use during the periconceptional period (1 month before conception to 3 months after conception) and the delivery of infants with selected congenital anomalies. Carmichael and Shaw found an increased risk of NSCLP (Carmichael and Shaw, 1999). A Spanish case-control study by Rodriguez-Pinilla and Martinez-Frias found an association between maternal systemic use of glucocorticoids and the birth of an infant with CLP, based on five exposed patients, one of which had multiple malformations and may have been a trisomy 13 (Rodriquez-Pinilla and Martýnez-Frýas, 1998). Park-Wyllie *et al* have also demonstrated that although prednisone does not represent a major teratogenic risk in human beings at therapeutic doses, it does increase the risk of oral cleft by 3.4-fold, which is consistent with the findings of existing animal studies (Park-Wyllie *et al.*, 2000). A retrospective study by Pradat *et al*. found a positive association between systemic corticoids use and the occurrence of cleft lip with or without cleft palate (Pradat *et al.*, 2003). Similarly, as has been reported in a previous study, Carmichael *et al*. in a recent population-based case-control investigation observed a moderately increased risk of CLP in the offspring of women who used corticosteroids during early pregnancy (Carmichael *et al.*, 2007).

Anticonvulsants

Anticonvulsants (phenytoin/hydantoin, oxazolidinones, and valproic acid) are associated with a clearly demonstrated increased risk for congenital defects (Gorlin *et al.,* 1990). All three therapeutic classes are liable to produce CLP, although inconsistently, as part of severe and significantly overlapping embryopathies. It is also worth noting that a significant increase in benzodiazepine use was detected in mothers of infants with cleft palate alone, and a nonsignificant increase was found in mothers of CLP infants. Safra and Oakley reported the association of CLP with first trimester exposure to diazepam (Saxen and Saxen, 1975; Safra and Oakley, 1975). In a further study, Czeizel (1988) addressed the question of benzodiazepine teratogenicity as a whole. Although diazepam at high doses is a weak teratogen in susceptible mice, its interference with fetal face development is probably modest or nonexistent. It is well known that women with epilepsy have an increased risk of having offspring with orofacial clefts. This risk has been attributed mostly to the teratogenic effects of antiepileptic drugs, but other risk factors have also been suggested, including epilepsy per se or some underlying genetic defects associated with epilepsy (Durner *et al.,* 1992).

Stress

Previous studies revealed increased risk of clefts among infants born to women with higher stress. t has been hypothesized that maternal stressors may cause birth defects through increased production of corticosteroids. Animal studies in rodents and rabbits have demonstrated that high doses of corticosteroids consistently cause cleft palate Stressful life events have been shown to be associated with increased levels of maternal corticotrophin-releasing hormone and corticosteroid during pregnancy. Increased risk of oral clefts was observed in the infants born to women who took corticosteroid medications during the first trimester of pregnancy (van Rooij *et al.,* 2001; Jugessur *et al.,* 2003; Munger *et al.,* 1996; Shaw and Lammer 1999; Juriloff *et al.,* 1995)

Conclusion

There is strong evidence that nonsyndromic orofacial clefts have genetic predisposition, but in the absence of a classic mendelian inheritance pattern. The orofacial clefts are caused by the interactions of multiple interacting genes, each gene having its own variable tendency to be expressed. In addition, the expression of orofacial clefts requires the presence of appropriate environmental triggers. Markers in more than 16 chromosomal regions have shown evidence of linkage to orofacial clefts or its related phenotypes in many genome-wide studies, suggesting that these genetic loci may contain major genes influencing the expression of orofacial clefts. These include, but are not exclusive to or limited to, genes that regulate transcription factors, growth factors, cell signaling and detoxification metabolisms. However, it remains to be determined whether polymorphisms in these genes account for the reported linkages in these regions. Studies are under way in many laboratories around the world to identify the disease-causing variations in these genes that account for the linkages discussed above. Although no major gene has been confirmed, it is noteworthy that the field of cleft palate genetics has evolved in a relatively short time into a research field. If successful, the genetic approach is likely to provide a new plane of understanding on the pathophysiology of the orofacial clefts. It is, however, clear that there is no single major genetic risk factor for the development of orofacial clefts; and the development of the orofacial clefts in an individual will depend on the interaction of several genes of moderate effect with environmental factors. Identifying specific genetic polymorphisms that influence orofacial cleft phenotypes will shed light on the molecular pathways involved in these complex disorders and provide a better understanding of the pathophysiology of orofacial clefts. Figure 3.

At present our knowledge of the teratogens that are associated with clefts is not extensive. Some of these substances (such as retinoic acid) have been confirmed to have direct effects on facial morphogenesis but many more await identification.

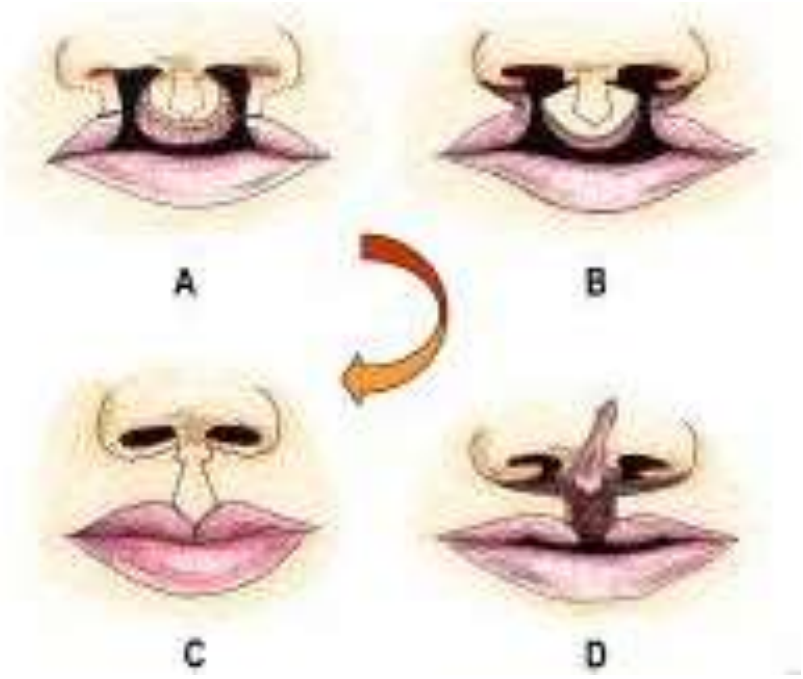


Figure 3: Bilateral Cleft Lip Repair

Metabolic disorders inherited or not, may play a role in the pathogenesis of clefting. Our knowledge of cell biology increases rapidly and may eventually lead to the understanding and possibly prevention of clefts of the lip and palate. This can particularly apply in cases with monogenic etiology and in chromosomal disorders.

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