INTRODUCTION
Cleft lip with and without cleft palate and cleft palate alone (occurring without a cleft lip) are two common congenital birth defects. A cleft is a separation in the lip or roof of the mouth (palate). Cleft lip and palate occurs twice as often in boys as in girls, while cleft palate alone is slightly more common in girls. Immediately after birth, individuals with cleft lip and palate have facial deformities, feeding problems and recurrent middle ear infections. Treatment requires interventions from multiple disciplines. At the age of speech acquisition, speech therapy is often needed to correct problems resulting from muscular defects of the cleft. As individual continues to grow, defects in tooth development and malocclusion require dental and sometimes surgical treatment.[1]

Cleft lip and palate varies in severity, from small notches in the lip to clefts that extend through the alveolar ridge in the maxilla and involve the floor of the nostril/palate. Cleft classification is based on embryologic development and is defined by the cause and the extent of physical involvement. Category causes include: (1) nonsyndromic cleft lip and palate (2) nonsyndromic cleft palate (3) syndromic cleft lip and palate (4) syndromic cleft palate (Schutte & Murray, 1999). Nonsyndromic is defined when no physical or developmental anomalies except the cleft lip and palate and no known teratogenic exposures that cause cleft lip and palate are involved. Syndromic is when cleft lip and palate appears with other malformations in recognizable patterns. When cleft lip and palate are described by the extent of the tissue involvement, they are unilateral or bilateral, and incomplete or complete. Cleft lip and palate involving only one side of the face are unilateral and when both sides are involved they are bilateral. If the cleft is unilateral, which side is involved is noted. Incomplete cleft lip and cleft palate are defined as involving the lip and the anterior part of the maxilla. (Figure 1) Complete cleft lip and cleft palate includes the lip, anterior part of the maxilla, and the hard and soft palate.[2]
PATHOPHYSIOLOGY

Clefts of the lip are the result of the failure of the lateral nasal and median nasal processes, part of the frontonasal prominence, and the maxillary prominence to merge. Unilateral clefts occur when the maxillary prominence on the affected side fails to unite with the merged medial nasal prominences. A persistent groove results where the epithelial (skin) tissue is stretched and breaks down resulting in cleft lip. Bilateral clefts occur by the same process and result in two grooves. When this tissue breaks down the intermaxillary segment (middle upper lip) hangs free and often projects upwards toward the nose. Closure of the lip is normally completed by the 35th day of embryonic development (Gorlin et al, 1990). Any factor that disrupts the normal embryonic facial development could result in cleft lip and cleft palate.

Delay in the elevation of the palatal shelves from the vertical to horizontal while the head is growing continuously results in a widening gap between the shelves so that they cannot meet, and therefore cannot fuse. When eventually they do become horizontal, this leads to clefting of the palate. Other causes of cleft palate are defective shelf fusion, failure of medial edge epithelial cell death, possible post fusion rupture and failure of mesenchymal consolidation and differentiation. The lines of fusion of the lateral palatal shelves with the primary palate dictate the diversion from the midline of a severe palatal cleft anteriorly to either right or left, or in rare instances to both. If cleft involves the alveolar arch, it usually passes between the lateral incisor and canine teeth.

Cleft lip and palate affects approximately 1/700 live births, with wide variability across geographic origin, racial and ethnic groups, as well as environmental exposures and socioeconomic status. In general, Asian and American populations have the highest reported birth prevalence rates, often as high as 1/500, European-derived populations have intermediate prevalence rates at about 1/1000, and African-derived populations have the lowest prevalence rates at about 1/2500. These observations suggest the relative contribution of individual susceptibility genes may vary across different populations. The frequency of cleft lip and palate also differs by sex and laterality: there is a 2:1 male to female ratio for clefts involving the lip and approximately a 1:2 male to female ratio for clefts of the palate only; and there is a 2:1 ratio of left to right sided clefts among unilateral cleft lip cases.

GENETIC ANALYSIS OF CLEFT LIP AND PALATE

Fogh-Anderson’s (1942) thesis was the first comprehensive study to indicate that there was a genetic cause for cleft lip and palate. Immediately after this study, Warkany, Nelson, and Schraffenberger (1943)
reported that there were also environmental components that could cause cleft lip and palate. As a result of both of these important studies, the inheritance of cleft lip and palate is explained as being multifactorial. Transforming growth factor alpha (TGFA), transforming growth factor beta 3 (TGFB3), and MSX1 are genes that have been identified as having a major role in the development of cleft lip and palate through linkage and association studies. AP2 is another gene that has been identified through the use of linkage that also has a role in the development of cleft lip and palate. Although genetic contributions of cleft lip and cleft palate are thought to be a greater predictor than environmental factors; environmental factors, if identified, could potentially be manipulated.

GENES IN NON-SYNDROMIC CLEFT LIP AND PALATE
Non syndromic clefting arises as a complex multifactorial trait, being a myriad of Mendelian penetrance, sex differences and environmental overlays, with the result that gene identification is difficult.

To date, genetic approaches to non-syndromic cleft lip and palate have included: linkage analysis using large, multiplex families or smaller but inbred families, or analysis of affected relative pairs; association studies using case/control trios or case-control samples; identification of chromosomal anomalies or microdeletions in cases; and direct sequencing of affected individuals. These methods can be applied to candidate genes or genome-wide strategies can be used.

A candidate gene is a gene known to be located in a region of interest in the genome, and whose product(s) has/have biochemical or other properties suggesting that it may be the gene being sought. Candidate gene studies have been at the core of cleft research since Ardinger and colleagues suggested a role for TGFA (transforming growth factor, alpha) variants in risk for non-syndromic cleft lip and palate. The following candidate genes have been identified in the etiology of cleft lip and palate.

Transforming growth factor-alpha (TGFA)
Transforming growth factors (TGFA) gene is located at chromosome 2p13. A significant association between transforming growth factor alpha (TGFA) and cleft lip and palate has been shown to exist. Combined effects of a TGFA mutation and maternal smoking could increase the risk of cleft lip and palate. Furthermore, Shaw et al showed that infants with TGFA genotype whose mothers did not use multivitamins containing folic acid periconceptionally are at a higher risk of being born with cleft lip and palate. In contrast, Lidral et al and Passos-Bueno et al showed no association between TGFA with cleft lip and palate in non-Caucasian population.

Transforming growth factor-beta (TGFB2)
TGFB2 is a member of the highly conserved TGFβ super-gene family and is located at chromosome 1q41. TGFB2 is involved in palatogenesis along with other TGFβ family isoforms. It is expressed in mesenchymal cells adjacent to medial edge epithelium. TGFB2 and TGFB1 regulate mesenchymal cell proliferation and extracellular matrix synthesis of palate, while TGFB3 orchestrates fusion of the palatal seam. Two previous studies in Asian populations found contrasting findings: Tanabe et al reported significant differences in TGFB2 polymorphism between a patient group with non-syndromic cleft lip and palate and control group of Japanese people. In contrast a study in the Philippines conducted by Lidral et al showed no association between this particular gene and cleft lip and palate formation.

TGFB3
TGFB3 is associated with nonsyndromic cleft lip and palate in different populations. TGFB3 is located at chromosome 14q24. This gene is 23kb in size and contains seven exons. Absence of functional gene which encodes transforming growth factor-beta 3 displays cleft palate because of adhesion of opposing palatal shelves. The role of TGFB3 in clefts has emerged from animal studies which indicate that TGFB3 play a crucial role in secondary palate development. In humans a study in Korean populations revealed that the TGFB3 polymorphism was strongly associated with an increased risk of cleft lip and palate patients compared to controls.

MSX1
MSX1 first came to prominence as a candidate for cleft lip and palate following the generation of a gene knockout with cleft palate and oligodontia. A candidate gene-based association study reported significant linkage disequilibrium between both cleft lip and palate and cleft palate with polymorphisms in MSX1. An MSX1 mutation was reported in a Dutch family with tooth agenesis and a mixture of cleft lip and palate and cleft palate, providing another rare example of where a single gene, and in this case single mutation, can give rise to a mixed clefting phenotype.

Evidence of linkage between non-syndromic cleft lip and palate and markers on the long arm of chromosome 4q25 has suggested that a cleft susceptibility locus may reside within this region. Lidral et al found a significant association of MSX1 and TGFB3 with non-syndromic clefting in humans using a linkage-disequilibrium (LD) strategy, suggesting that these genes are involved in pathogenesis of clefting. They further suggested that the combined genetic background of rare variants of TGFB3 and MSX1 could increase the risk of cleft lip and palate, demonstrating the significance of gene-gene interaction in the etiology of non-syndromic cleft lip and palate.

Van den Boogaard et al described a family with a common pattern of tooth agenesis associated with cleft lip and palate. Direct sequencing of MSX1 (two exons and one intron) was performed on 917 cleft and palate lip patients and gene mutation was identified in 16 patients.
with cleft lip and palate. This report demonstrates that the MSX1 mutations appear to contribute about 2% of cases of non-syndromic cleft lip and palate patients.

**Methylenetetrahydrofolate reductase MTHFR**

Methylenetetrahydrofolate reductase (MTHFR) maps on chromosome 1q36 is a key enzyme in folic acid metabolism. The size of this gene is about 19kb and contains 5 exons. The C677T mutation of MTHFR is thermally labile and considered a risk factor of neural tube defects as it lowered the plasma level of folate. Studies in non-syndromic cleft lip and palate patients have shown that, the MTHFR C77T genotype in the mother conferred an increased risk of cleft lip and palate in their offspring. Thus, the importance of periconceptional folate intake were emphasized in these studies and its deficiencies could lead to cleft lip and palate.[12]

**Proto-oncogene BCL3**

BCL3 is related to genes involved in cell lineage determination and cell cycle regulation. Epithelial cell disruption at the edges of the developing maxillary process and growth of underlying mesenchyme leading to mesenchymal continuity and seam formation are critical in palate development.[13] A dominant mutation in BCL3, resulting in increased binding to the transcription factor, could lead to inhibition of the expression of genes important to growth in the developing mesenchyme. Growth failure in these cells could result in cleft lip and palate. Stein et al, demonstrated linkage of nonsyndromic cleft lip and palate to BCL3, a growth factor in 17 multigenerational cleft lip and palate families.[14] Their analyses showed evidence for involvement of chromosome 19 in the etiology of clefting. These results suggest that a major gene does play an etiologic role in the development of cleft lip and palate and that these loci can be detected in linkage studies with sufficient numbers of families. Martinelli et al supported these findings using different methods. He believed that BCL 3 or a nearby gene seems to be implicated in some way in this congenital facial malformation. Thus, it appears that BCL3 plays a role in the etiology of cleft lip and palate. However, it is not known at present whether it acts as a modifier or as additive gene for this malformation.[15]

**Retinoic Acid Receptors (RARA)**

Juriloff and Mah in their study found the chromosomal location of the mouse gene in which mutation occurs that can cause nonsyndromic cleft lip and palate. The region on chromosome 11 associated with CLP in this animal model is homologous to 17q21-q24 in humans. This region, marked by retinoic acid receptor-α (RARA) has shown association with cleft lip and palate in some populations. This study has strengthened the case for cleft lip and palate locus linked to RARA in humans.[16]

**GENES IN SYNDROMIC CLEFT LIP AND PALATE**

Syndromic cleft lip and palate and cleft palate can be broadly divided into those that occur as a part of characterized mendelian disorder, those arising from structural abnormalities of chromosomes, syndromes associated with known teratogens or those whose causation remain obscure and are therefore currently uncharacterized. Some of the candidate genes which are thought to be responsible for several major syndromic clefting disorders are given below.[17]

**T-Box transcription factor-22**

X linked cleft palate is characterized by isolated cleft palate and ankyloglossia. High arched palate, bifid uvula, or ankyloglossia are the presenting signs in the affected males. Female carriers could be asymptomatic or they could exhibit the full features of X linked cleft palate. The syndrome shows a mendelian X-linked semi dominant pattern. Stainer et al located the disease gene locus to chromosome Xq21. Involvement of TBX22 in non syndromic cleft lip and palate has been indicated from a genome wide sibling pair analyses in which the chromosome Xcen-q region, where TBX22 is located.

**Poliovirus receptor- like-1**

Cleft lip/palate ectodermal dysplasia syndrome is characterized by cleft lip with or without cleft palate, hidrotic ectodermal dysplasia, syndactyly and occasional mental retardation. The inheritance of cleft lip/palate ectodermal dysplasia is autosomal recessive.[18] The protein product of PVRL1 was initially identified as poliovirus receptor-related protein. Takahashi et al confirmed the function of PRR as a cell adhesion molecule, and they renamed it nectin-1. It has been speculated that, since Nectin 1 is the principal cell surface receptor for α herpes viruses, the high frequency of heterozygotes form relative resistance to infection by viruses such as HSV1 and HSV2.[19]

**Interferon regulatory factor -6**

Van Der Woude is the most common form of syndromic cleft lip and palate and accounts for 2% of all the cases. This condition is associated with highly characteristic pitting of lower lip mucosa and cleft lip and palate. The locus has been identified as a region of chromosome 1. Through chromosomal analysis and linkage, the critical area for Van Der Woude syndrome was gradually narrowed to 1q32-q41. In 2002, Kondo et al described a pair of monozygotic twins discordant for Van der Woude syndrome whose parents did not have the syndrome. The VDWS in the affected twin was thought to arise from somatic mutation. In most of the cases of VDWS, IRF-6 mutation produced a nonfunctional protein and haploinsufficiency.[20]

A partial or modifying role of IRF6 in nonsyndromic cleft lip and palate has been demonstrated in a study applying the transmission disequilibrium test, in which specific parental alleles at the VDWS locus were
preferentially transmitted to the individuals with non syndromic cleft lip and palate.

**P63**

EEC syndrome is an autosomal dominant disorder of ectrodactyly, ectodermal dysplasia and cleft lip and palate. EEC syndrome was mapped to 3q27 and heterozygous mutations were identified in the p63 gene. One unusual phenomenon with p63 is that mutation to different parts of the gene can influence the cleft phenotype. Missense mutation of the conserved DNA binding domain region gives cleft lip and palate while C-terminal mutations give cleft lip or cleft palate.

Mutation at the N-terminal end outside of the conserved domains gives rise to cleft palate or no clefting at all. In particular Jagged2, a ligand in the notch signalling pathway, is known to act downstream of p63 and homozygous mouse knockouts of Jagged2 exhibit cleft palate.\(^{[21]}\)

---

**ENVIRONMENTAL FACTORS**

Although genetic contributions of cleft lip and palate are thought to be a greater predictor than environmental factors; environmental factors, if identified, could potentially be manipulated.

Environmental factors that could/can increase the risk of cleft lip and palate are divided into four broad categories: womb environment, external environment, nutrition, and drugs. There are several known teratogens, substances that cause birth defects and increase the risk for cleft lip and palate. These include antiepileptic drugs (phenytoin, valproic acid), thalidomide, dioxin (pesticide), retinoic acid, maternal alcohol use, and maternal cigarette smoking. Population studies were used to show that maternal alcohol use is related to higher rates of cleft lip and palate. Migration and differentiation of neural crest cells were interrupted in embryos exposed to alcohol. It has been noted that exposure to four or more drinks per month significantly elevated the risk for cleft lip and palate, especially among those with alterations at MSX1. It was found that embryos exposed to maternal smoking during the first trimester were at increased risk to have cleft lip and palate. The risk for cleft lip and palate as a result of embryonic smoke exposure is also related to the level of exposure. Twenty or more cigarettes per day result in a twofold increase and less than 20 cigarettes per day resulted in a 1.5-fold increase.

Increased risks from exposure to maternal smoking during the peri-conceptual period raises the possibility that genes in certain metabolic pathways may play a role in the development of cleft lip and palate. Specifically, markers in the GSTT1 (glutathione S-transferase theta) or NOS3 (nitric oxide synthase 3) genes appear to influence risk of cleft lip and palate in the presence of maternal smoking. The GSTT1 markers are gene deletion variants, which suggest deficiencies in detoxification pathways may underlie some of this susceptibility. Smoking has also been recently associated with a joint risk with variants in the IRF6 gene and the same study reported interactions between multivitamins and IRF6 variants. These findings provide evidence that gene-environment interactions are important in cleft lip
and palate.\textsuperscript{[22]} Exactly how smoking directly affects fetal growth is not known, however, it is hypothesized that hypoxia alters facial development. Maternal nutrition has also been implicated as one of the environmental factors that may have a role in clefting. Specifically folic acid, vitamin B, was first noted in 1961 to alter embryonic facial growth in rats, resulting in increased rates of clefting. Preliminary data showing that preconceptional vitamins may reduce clefting in humans was first noted in humans in 1982. TGFA type A2, a candidate gene, appears to have an increased risk as a result of folic acid deficiencies.

Nutritional factors, such as folate deficiency, have also been suggested to influence risk of cleft lip and palate, based on both observational studies and interventional trials using folate supplementation to prevent recurrences of cleft lip and palate in families. There are some data to support roles for zinc deficiency in risk of oral clefts in populations in which zinc status is highly compromised, for cholesterol deficiency in facial clefting, as well for as multivitamins in general in cleft prevention.

Besides nutrients and toxins other environmental exposures have been, and should continue to be, assessed for possible roles in clefting. These exposures include hyperthermia, stress, maternal obesity, occupational exposures, ionizing radiation and infection. Pregnancy planning has been shown to have a protective effect and the basis of this observation needs to be more deeply explored.\textsuperscript{[2]}

**COMMON OROFACIAL CLEFT LIP AND PALATE SYNDROMES AND THEIR UNDERLYING GENETIC CAUSES**

**APERT SYNDROME:** Wilkie et al identified one of the two mutations in exon 7 of the FGFR2 gene-S252W or P253R in all 40 unrelated patients with Apert syndrome in their series. Lajemi et al identified S252F mutation in affected fetuses.\textsuperscript{[23]}

**CROUZON SYNDROME:** Kreiborg and Cohen suggested germinal mosaicism as the basis of Crouzon syndrome in two affected siblings with same mother but different fathers. FGFR2 and FGFR3 mutation has also been seen in Crouzon syndrome.\textsuperscript{[24]}

**PIERRE ROBIN SYNDROME:** Deletion 2q32.3-q33.2 has been associated with Pierre Robin syndrome.\textsuperscript{[25]}

**TREACHER COLLIN SYNDROME:** Treacher Collin syndrome is an autosomal dominant disorder with variable expression. There seems to be an increase in affected offsprings from affected females and a decrease in affected offsprings from affected males. Defect in nucleolar trafficking protein has been considered to be responsible for Treacher Collin syndrome. Mutation in Treacher Collins-Franceschetti syndrome 1 gene has also been found to be responsible.\textsuperscript{[26]}

**GENETIC COUNSELING AND PRENATAL DIAGNOSIS**

Improved ultrasound equipment and experience has made prenatal diagnosis of cleft lip and palate possible. The ability to detect cleft lip by ultrasound can occur as early as 13 weeks gestation. However, this is almost always performed with high-resolution ultrasound, and by very experienced health care professionals. Detection can be complicated by fetal positioning and poor resolution when viewed through the abdomen. However, by using vaginal ultrasound, earlier detection has been shown to be successful in some centers. Despite the fact that cleft lip can be diagnosed by ultrasound, parents are often unaware of the presence of cleft lip and palate before delivery. The detection of cleft lip and palate, especially during early pregnancy, results in parental anxiety and an immediate need for information. Parents deserve and want accurate and unbiased information about the health of the fetus to decide about pregnancy outcomes, prepare for immediate care needs, and eventual surgical intervention. Prompt consultation should be planned for parents to meet with specialists in genetics and plastic surgery, and their obstetrical care provider. A genetics consultation provides information about cleft lip and palate and the possible causes of the newborn’s condition. During the consultation, information is obtained to determine if there are any indications toward the cause. Information about chromosome testing, to rule out lethal chromosomal causes of cleft lip and palate, should be offered, especially if additional fetal anomalies are detected. Parents should be offered the option to meet with healthcare providers who are experienced in caring for children with cleft lip and palate to discuss the surgical, dental, and additional interventions their child would require. The ability for parents to discuss and meet individual before the birth of their newborn can provide many benefits, including education about cleft lip and palate, feeding, arranging for initial appointments, and who to call with questions; thereby allowing parents to enjoy their newborn after delivery. Some parents prefer to talk to other parents who have had children with cleft lip and palate to prepare for their child’s birth.

For individuals or families with a confirmed diagnosis of a chromosomal or molecular genetics condition, invasive prenatal testing may be a consideration. The most common types of these tests are amniocentesis and chorionic villus sampling. Amniocentesis is performed at 15-20 weeks of pregnancy and carries a risk of miscarriage of 0.5%. In this test the needle is inserted through the abdominal wall of the pregnant woman into the fetal cavity. The fluid that is removed contains cells from the baby as well as amniotic fluid. Both can be sent for testing to answer a specific genetic question with respect to recurrence risk.

Chorionic Villus sampling is performed at 11 weeks of pregnancy, either through abdomen or through the vagina and cervix. This procedure involves a risk of
miscarriage of 1%. For this reason neither amniocentesis nor chorionic Villus sampling is recommended for individuals or couples for whom the only question is recurrence of isolated cleft lip and/or palate.[6]

**CONCLUSION**

Often pediatric dentists are the first health care practitioners to document dysmorphic features in a child. It is important for them to have an understanding of molecular genetics because the sensitivity and specificity of molecular-based diagnostics have revolutionized how diseases and disorders are defined. These scientific and technological advances translate into improved health, disease prevention, smarter diagnostics, and innovative therapeutic approaches to craniofacial dysmorphogenesis.

**REFERENCES**


