

Genetical Basis of Cleft Lip & Palate : A Review

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SUMMARY

Orofacial clefts, non-syndromic cleft lip with or without cleft palate (CL/P) in particular are the mostly occurring craniofacial deformities, affecting one newborn in every 700 to 1000 newborns worldwide. The advancements in the recent past in the fields of genetics and molecular biology have revealed the basis of craniofacial development. Often contradicted and indecisive results have been obtained by various genetic approaches and techniques such as genome-wide and candidate gene association studies as well as linkage analysis. These results reveal the heterogeneity of etiological factors among population and involvement of multiple genes in causative factors of CL/P. In the presences of all the problems there have been several genes which are associated with the occurrence of CL/P.

Keywords: Cleft lip, cleft palate, craniofacial deformity

INTRODUCTION

Orofacial clefts, non-syndromic cleft lip with or without cleft palate (CL/P) in particular are the mostly occurring craniofacial deformities, affecting one newborn in every 700 to 1000 newborns worldwide¹. It has been tendered as a major public health problem all over the world due to its facial growth, function and social integration. Ethically, there is great diversity in the incidence of CL/P. The highest rates of incidences are seen in Asian and North – American populations, intermediate rates is seen in Caucasian population, whereas lowest rates are seen in African American population^{2,3}. Even though environmental influences on facial development have been described, a strong genetic component has been demonstrated in these processes⁴. The nature of genetic contribution to the etiology of CLP is still under studied. Although earlier investigations suggested a multifactorial threshold model⁵. More recently complex segregation analysis of several populations have supported mixed models with major gene influences^{6,7,8}. Researchers worldwide are currently studying the genes mutations associated with CL/P. Recently, advances in genetics and molecular biology have revealed the means to identify a number of genes associated with CL/P. Therefore, the purpose of this review is to present current concepts on the etiology of non-syndromic CL/P, in particular the genetic and environmental factors that have been identified in the scientific literature.

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EMBRYOLOGY

Formation of the primary palate: The first, second and third branchial arches play a critical role in the development of the face, mouth and tongue. The development of the face is well described in terms of its formation and merging of various processes or prominences⁹. At around embryonic day 24 (E24) the fronto-nasal process can be clearly identified, bounded on each side by maxillary processes that are derived from the first branchial arch. By the end of fourth week, bilateral oval-shaped thickenings of the surface ectoderm (nasal placodes) develop on each side of the lower part of fronto-nasal prominence followed by the migration of neural crest cells into that region, and the region from which the maxillary prominences will develop. This migration subsequently causes the mesenchyme to proliferate at the margins of the nasal placodes, producing horseshoe shaped medial and lateral nasal processes. The maxillary processes at this time grow medially and soon approach both the medial and lateral nasal processes. The frontonasal and maxillary processes grow downward and forward but the mechanism of this interaction and its coordination is unclear¹⁰. The medial growth of the maxillary prominences push the medial nasal processes toward the midline, where they merge, eliminating the frontonasal process. This merging mechanism occurs between E40 and E48 (around sixth week of human development). At the same time, the medial nasal prominences merge with each other to form the intermaxillary segment. This segment gives rise to the middle portion or philtrum of the upper lip and the primary palate, an area of the palate bounded by two lines from the incisive foramen along the alveolar bone between the lateral incisor and canine on each side.

Previously it was highlighted the importance of the positioning of the olfactory (nasal) placode¹⁰. Abnormal midline positioning of the placodes could possibly cause facial clefting. In another article it was mentioned that failure in the growth of the median and lateral nasal processes prevents the subsequent merging of these structures¹¹. As a consequence, clefts develop between their derivatives.

Development of the Secondary Palate: The secondary palate is a structure that separates the nasal passage from the oral cavity. The palate proper develops from both primary and secondary components. The secondary palate begins its development in the sixth week from medial projections of the paired maxillary processes of the first branchial arches, termed palatal shelves or lateral palatine processes. Initially these shelves grow medially towards each other and lie in a vertical position on each side of the developing tongue, but as development proceeds the shelves become horizontal and fuse with the primary palate. An intrinsic shelf-elevating force, generated by the hydration of hyaluronan^{12,13,14} primarily causes elevation of the palatal shelves. This osmotic shelf-elevating force is directed by the collagen fibers, mesenchymal cell orientation, and contraction within the palate.

Fusion begins anteriorly in the palate during the ninth week and is completed posteriorly by the twelfth week of embryonic life. During fusion, the apposed epithelia form an epithelial seam that undergoes apoptosis, migration or transformation and results in mesenchymal continuity¹². The basal epithelial layer constitutes the medial edge epithelium (MEE) of each shelf. The shelves grow toward the midline, and the MEE of each shelf approximates and forms the midline epithelial seam. This seam is subsequently disrupted, leading to mesenchymal confluence between the two shelves.

Perturbations caused by genetic, mechanical, or teratogenic factors can occur at any of these steps, and may result in a cleft palate^{11,13,14,15}. Probably the event most subject to error in human palate development is removal of the tongue from between the palatal shelves¹¹. This event appears to involve active movement such as jaw opening and tongue protrusion, as well as differential growth of the lower jaw¹⁰.

Candidate genes or loci for non-syndromic cleft lip and palate: 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^{16,17}. The following candidate genes have been identified in the etiology of CLP

Transforming growth factor-alpha (TGFA):

Transforming growth factors (TGFA) are an extensively studied family of growth factors. The gene is located at chromosome 2p13¹⁸. TGFA have been shown to be present in the regulation of palate development¹⁹ and are present at high levels in the MEE of palatal shelves. Previous genetic studies have demonstrated a significant association between transforming growth factor-alpha (TGFA) and CL/P^{20,21}. In contrast, with two studies which showed no association between TGFA with CL/P in non-Caucasian population^{17,22}. There is also evidence from some studies that reported that the combined effects of a TGFA mutation and maternal smoking could increase the risk of CL/P²³. Furthermore, another study showed that infants with TGFA genotype whose mothers did not use multivitamins containing folic acid preconceptionally are at a higher risk of being born with CL/P²⁴.

Transforming growth factor - B₂ (TGF B₂):

TGFB2 is a member of the highly conserved TGFB supergene family and is located at chromosome 1q41¹. 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^{25,26}. Two previous studies in Asian populations found contrasting findings. In Japanese significant differences in TGF β 2 polymorphism between a patient group with non-syndromic CL/P and control group of Japanese people²⁷. In contrast a study in the Philippines conducted showed no association between this particular gene and CL/P formation¹⁷.

Transforming growth factor - B₃ (TGF B₃):

The role of TGFB3 in clefts has emerged from animal studies which indicate that TGFB3 play a crucial role in secondary palate development¹⁷. In humans, TGFB3 is associated with non-syndromic CL/P in different populations²⁸. TGFB3 is located at chromosome 14q24. There are mixed reports regarding the role of this gene in relation to incidence of CLP. For South American it was suggested that the mutation of MSX1 and TGFB3 in South American populations may contribute to CL/P²⁹. A similar study in Korean populations revealed that the TGFB3 polymorphism was strongly associated with an increased risk CL/P patients compared to controls³⁰.

MSX1: Evidence of linkage between non-syndromic CL/P and markers on the long arm of chromosome 4q25 has suggested that a cleft susceptibility locus may reside within this region³¹. It was found that 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 clefting³². They

further suggested that the combined genetic background of rare variants of TGF β 3 and MSX1 could increase the risk of CL/P, demonstrating the significance of gene-gene interaction in the etiology of non-syndromic CL/P. Subsequently, another study described a family with a common pattern of tooth agenesis associated with CL/P³³. Recently, direct sequencing of MSX1 (two exons and one intron) was performed on 917 CL/P patients and gene mutation was identified in 16 patients with CL/P. This report demonstrates that the MSX1 mutations appear to contribute about 2% of cases of non-syndromic CL/P³⁴.

MTHFR: Methylene tetrahydrofolate reductase (MTHFR) maps on chromosome 1q36 is a key enzyme in folic acid metabolism³⁵. 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 folate³⁶. During investigating Irish cases, found that the homozygosity for the common folate-related polymorphism associated with thermo-labile form of MTHFR is significantly more frequent in CL/P³⁵. Previous studies in non-syndromic CL/P, the MTHFR C77T genotype in the mother conferred an increased risk of CL/P in their offspring^{36,37}. Similarly another study demonstrated a significantly higher mutation frequency of MTHFR in mothers of children with CL/P³⁴. Thus, the important of peri-conceptual folate intake were emphasized in these studies and its deficiencies could lead to CL/P.

Proto-oncogene BCL3: Although the role of B-cell leukemia/lymphoma 3 (BCL3) in the etiology of CL/P is unknown, 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 development¹². A dominant mutation in BCL 3, 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 CLP.

It was demonstrated that the linkage of nonsyndromic CL/P to BCL 3, a growth factor in 17 multigenerational CL/P families (38). Their analyses showed evidence for involvement of chromosome 19 in the etiology of clefting. Another study supported these findings using different methods (39). In addition the data reported for the chromosome region 19q13.2 provided "suggestive" linkage, i.e., statistical evidence expected to occur one time at random in a genome scan. Although suggestive linkage is only

indicative, by definition, so far three different groups have found a suggestive linkage for this locus, a sign that the locus is relevant for different populations³⁸. It was believed that BCL 3 or a nearby gene seems to be implicated in some way in this congenital facial malformation³⁹.

Retinoic Acid Receptors (RARA): Retinoic acid receptor alpha (RARA) showed a significant association with CLP in an Australian population²¹, but no association in a British population⁴⁰. A study on mouse gene they found the chromosomal location of the mouse gene in which mutation occurs that can cause non-syndromic CLP⁴¹. 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-a (RARA) has shown association with CLP in some populations²¹. This study has strengthened the case for CLP locus linked to RARA in humans.

CONCLUSION

In general, the genetic basis of CL/P is still controversial because of genetic complexity of clefting. Results from previous studies support the presence of heterogeneity among populations and the presence of multiple genes involved in the etiology of CL/P. Genetic interaction with environmental factors will become apparent through further studies involving maternal and fetal genotypes along with differing environmental exposures. Furthermore, recent technical advances in gene manipulation promises a stimulating time ahead for CL/P research.

REFERENCES

1. Murray JC. Gene/environment causes of cleft lip and/or palate. *Clin Genet*.2002;61:248–256. [PubMed]
2. Marazita ML, Spence MA, Melnick M. Major gene determination of liability to cleft lip with or without cleft palate: A multiracial view. *J Craniofac Genet Dev Biol*.1986;8:47–51. [PubMed]
3. Melnick M, Marazita ML, Hu DN. Genetic analysis of cleft lip with or without cleft palate in Chinese kindreds. *Am J Med Genet*. 1986;21:183–190. [PubMed]
4. Murray JC. Face Facts: genes, environment and clefts (invited editorial) *Am J Hum Genet*. 1995;57:227–332. [PMC free article] [PubMed]
5. Fraser FC. Research revisited. The genetics of cleft lip and cleft palate. *Am J Hum Genet*. 1970;22:336–352. [PMC free article] [PubMed]
6. Marazita ML, Hu DN, Spence MA, Liu YE, Melnick M. Cleft lip with or without cleft palate in Shanghai, China: evidence for autosomal major locus. *Am J Hum Genet*. 1992;51:648–653. [PMC free article] [PubMed]
7. Chung CS, Bixler D, Watanabe T, Koguchi S, Fogh-Anderson P. Segregation analysis of cleft lip with or without cleft palate: a comparison of Danish and Japanese data. *Am J Hum Genet*. 1986;39:603–611.

8. Hecht JT, Wang YP, Blanton SH, Michels VV, Daiger SP. Cleft lip and palate: no evidence of linkage to transforming growth factor alpha. *Am J Hum Genet.*1991;49:682–686. [[PMC free article](#)] [[PubMed](#)]
9. Moore KL. *The Developing Human, Clinical Oriented Embryology.* Philadelphia: W.B Saunders; 1982. The branchial apparatus; pp. 156–187.
10. Johnston MC, Bronsky PT. Prenatal craniofacial development: New insights on normal and abnormal mechanisms. *Crit Rev Oral Biol Med.* 1995;6:368–422.
11. Young DL, Schneider RA, Hu D, Helms JA. Genetic and teratogenic approaches to craniofacial development. *Crit Rev Oral Biol Med.* 2000;11:304.
12. Ferguson MW. Palate development. *Development (Suppl)* 1988;103:41–60. [[PubMed](#)]
13. Singh GD, Moxham BJ, Langley MS, Waddington RJ, Embery G. Changes in the composition of glycosaminoglycans during normal palatogenesis in the rat. *Arch Oral Biol.* 1994;39:401–407. [[PubMed](#)]
14. Singh GD, Moxham BJ, Langley MS, Embery G. Glycosaminoglycan biosynthesis during 5-fluoro-2-deoxyuridine-induced palatal clefts in the rat. *Arch Oral Biol.*1997;42:355–363. [[PubMed](#)]
15. Singh GD, Johnston J, Ma W, Lozanoff S. Cleft palate formation in fetal Br mice with midface retrusion: tenascin, fibronectin, laminin, and type IV collagen immunolocalization. *Cleft Palate Craniofac J.* 1998;35:219–241. [[PubMed](#)]
16. Wyszynski DF, Beaty TH, Maestri NE. Genetics of nonsyndromic oral clefts re-visited. *Cleft Palate Craniofac J.* 1996;33:406–417. [[PubMed](#)]
17. Lidral AC, Murray JC, Kenneth HB, Masart AM, Scheerer H, et al. Studies of the candidates genes TGFB2, MSX1, TGFA, and TGFB3 in the etiology of cleft lip and palate in the Philippines. *Cleft Palate Craniofac J.* 1997;34:1–6. [[PubMed](#)]
18. Ardinger HH, Buetow KH, Bell GI, Bardach J, VanDemark DR, Murray JC. Association of genetic variation of the transforming growth factor alpha gene with cleft lip and palate. *Am J Hum Genet.* 1989;45:348–353. [[PMC free article](#)] [[PubMed](#)]
19. Fitzpatrick DR, Denhez F, Kondaiah P, Athurst RJ. Differential expression of TGFβ isoforms in murine palatogenesis. *Development.* 1990;109:585–595. [[PubMed](#)]
20. Holder SE, Vintiner GM, Farren B, Malcolm S, Winter RM. Confirmation of an association between RFLPs at the transforming growth factor-alpha locus and nonsyndromic cleft lip and palate. *J Med Genet.* 1992;29:390–392. [[PMC free article](#)][[PubMed](#)]
21. Chenevix-Trench G, Jones J, Green A, Duffy DL, Martin N. Cleft lip with or without cleft palate: association with transforming growth factor-alpha and retinoic acid receptor loci. *Am J Hum Genet.* 1992;51:1377–1385. [[PMC free article](#)] [[PubMed](#)]
22. Passos-Bueno MR, Gaspar DA, Kamiya T, et al. Transforming growth factor-alpha and non-syndromic cleft lip with or without palate in Brazilian patients: results of large case-control study. *Cleft Palate Craniofac J.* 2004;41:387–391. [[PubMed](#)]
23. Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, et al. Orofacial clefts, parental cigarette smoking, and transforming growth factor alpha gene variants. *Am J Hum Genet.*1996;58:551.
24. Shaw GM, Wasserman CR, Murray JC, Lammer EJ. Infant TGF-α genotype, orofacial clefts, and maternal periconceptional multivitamin use. *Cleft Palate Craniofac.* 1998;35:366–367. [[PubMed](#)]
25. Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R et al. TGFB2 knockout mice have multiple developmental defects that are nonoverlapping with other knockout phenotypes. *Development.* 1997;124:2659–2670.
26. Alvarez J, Sohn P, Zeng X, Doetschman T, Robbins DJ, et al. TGFB2 mediates the effects of Hedgehog on hypertrophic differentiation and PTHrP expression. *Development.*2002;129:1913–1924.
27. Tanabe A, Taketani S, Endo-Ichikawa Y, et al. Analysis of the candidate genes responsible for non-syndromic cleft lip and palate in Japanese people. *Clin Science.*2000;99:105–111. [[PubMed](#)]
28. Wong FK, Hagg U. An update on the aetiology of orofacial clefts. *Hong Kong Med J.* 2004;10:331–336.
29. Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis. *J Dent Res.* 2003;82:162–165. [[PubMed](#)]
30. Kim MH, Kim HJ, Choi JY, Nahm DS. Transforming growth factor beta3 gene Sfn1 polymorphism in Korea nonsyndromic cleft lip and palate patients. *J Biochem Mol Biol.* 2003;36:533–537. [[PubMed](#)]
31. Carinci F, Pezetti F, Scapoli L, Martinelli M, Avantiaggiato A, et al. Recent developments in Orofacial Cleft Genetics. *J Craniofac Surg.* 2003;14:130–143. [[PubMed](#)]
32. Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ, et al. Association of MSX1 and TGFB3 with nonsyndromic clefting in human. *Am J Hum Genet.*1998;63:557–568. [[PMC free article](#)] [[PubMed](#)]
33. van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet.*2000;24:342–343. [[PubMed](#)]
34. Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, et al. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. *J Med Genet.*2003;40:399–407. [[PMC free article](#)] [[PubMed](#)]
35. Mills JL, Kirke PN, Molloy AM, Burke H, Conley MR, et al. Methylene tetrahydrofolate reductase thermolabile variant and oral clefts. *Am J Med Genet.* 1999;86:71–74. [[PubMed](#)]
36. Prescott NJ, Winter RM, Malcolm S. Maternal MTHFR genotype contributes to the risk of nonsyndromic cleft lip and palate. *J Med Genet.* 2002;39:368–369. [[PMC free article](#)] [[PubMed](#)]
37. van Rooij I, Vermeij-Keers C, Kluijtmans L, Ocke M, Zielhuis G, et al. Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphism affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol.* 2003;157:583–591. [[PubMed](#)]
38. Stein J, Mulliken JB, Stal S, Gasser DL, Malcolm S, Winter R, et al. Nonsyndromic cleft lip with or without cleft palate: evidence of linkage to BCL3 in 17 multigenerational families. *Am J Hum Genet.* 1995;57:257–272. [[PMC free article](#)][[PubMed](#)]
39. Martinelli M, Scapoli L, Pezzetti F, Carrinci F, Carrinci P, Baciliero U, Padula E, Tognon M. Suggestive linkage between markers on chromosomes 19q13.2 and nonsyndromic orofacial cleft malformation. *Genomics.* 1998;51:177–181. [[PubMed](#)]
40. Vintiner GM, Lo KK, Holder SE, Winter RM, Malcolm S. Exclusion of candidate genes from a role in cleft lip with or without cleft palate: linkage and association studies. *J Med Genet.* 1993;30:773–778. [[PMC free article](#)] [[PubMed](#)]
41. Juriloff DM, Mah DG. The major locus for multifactorial nonsyndromic cleft lip maps to mouse chromosome 11. *Mamm Genome.* 1995;6:63–69. [[PubMed](#)]