GENETICS OF CLEFT LIP AND PALATE: A REVIEW

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Orofacial clefts, particularly non-syndromic cleft lip with or without cleft palate (CL/P) are the most common craniofacial deformities, affecting one in every 700 to 1000 newborns worldwide. Numerous efforts have been made to understand the etiology of CL/P so as to predict its occurrence and to prevent it from occurring in the future. In the recent years, advances in genetics and molecular biology have begun to reveal the basis of craniofacial development. Various genetic approaches, including genome-wide and candidate gene association studies as well as linkage analysis, have been undertaken to identify aetiological factors, but results have often been inconclusive or contradictory. These results may support the presence of aetiological heterogeneity among populations and the presence of multiple genes involved in the aetiology of CL/P. Despite these difficulties, several different genes have been implicated in harbouring genes that contribute to the aetiology of CL/P. In conclusion, the genetic basis of CL/P is still controversial because of genetic complexity of clefting.

Key words: Orofacial clefts, genetics, candidate genes

Introduction

Orofacial clefts, particularly non-syndromic cleft lip with or without cleft palate (CL/P) are the most common craniofacial deformities, affecting one in every 700 to 1000 newborns worldwide (1). Due to their disturbing appearance in many cases, these deformities have attracted much attention in terms of treatment and research. The large impact of the defects on facial growth, function and social integration renders them a major public health problem worldwide.

There are significant ethnic differences in the incidence of CL/P, with the highest rates in Asian populations and Native-Americans, intermediate rates in Caucasians, and lowest rates in African American (2,3). Even though environmental influences on facial development have been described, a strong genetic component has been demonstrated in these processes (4). The nature of genetic contribution to the etiology of CLP is still being studied. Although earlier investigations suggested a multifactorial threshold model (5), more recently complex segregation analysis of several populations have supported mixed models with major gene influences (6,7,8). Many researchers worldwide are currently studying the location and nature of genes mutations associated with CL/P.

In recent years, advances in genetics and molecular biology have begun to disclose the basis of craniofacial development, and a number of genes associated with CL/P have been identified. 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.

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 prominences (9). At around embryonic day 24 (E24) the frontonasal 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 unclear (10). 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 inter-maxillary 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.

Johnston and Bronsky (1995) highlighted the importance of the positioning of the olfactory (nasal) placode (10). Abnormal midline positioning of the placodes could possibly cause facial clefting. Young et al. (2000) in their review mentioned that failure in the growth of the median and lateral nasal processes prevents the subsequent merging of these structures (11). As a consequence, clefts develop between their derivatives. In the mildest cases, the clefts may be limited to the vermillion border of the upper lip. In progressively more severe cases, the cleft develops through the tissue of the lip (unilateral or bilateral cleft lip), and can also involve the lateral aspect of the nose (typically referred to as oblique facial clefts).

**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 formation of the primary palate has already been described. 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 continuity (12). Before merging of the palatal shelves, the outer epidermal layer is lost, leaving the basal epithelial layer. 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 (11,13-15). Probably the event most subject to error in human palate development is removal of the tongue from between the palatal shelves (11). This event appears to involve active movement such as jaw opening and tongue protrusion, as well as differential growth of the lower jaw (10).

**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 2p13 (18). TGFA have been shown to be present in the regulation of palate development (19) 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, Lidral et al. (17) and Passos-Bueno et al. (22) showed no association between TGFA with CL/P in non-Caucasian population. 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/P (23). Furthermore, Shaw et al. (24) showed that infants with TGFA genotype whose mothers did not used multivitamins containing folic acid periconceptionally are at a higher risk of being born with CL/P.

**TGFB2**

TGFB2 is a member of the highly conserved TGFB super-gene family and is located at chromosome 1q41 (1). TGFB2 is involved in palatogenesis along with other TGFB 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 (25, 26). Two previous studies in Asian populations found contrasting findings. Tanabe et al. (27) reported significant differences in TGFâ2 polymorphism between a patient group with non-syndromic CL/P and control group of Japanese people. In contrast a study in the Philippines conducted by Lidral et al. (17) showed no association between this particular gene and CL/P formation.

**TGFB3**

The role of TGFB3 in clefts has emerged from animal studies which indicate that TGFB3 play a crucial role in secondary palate development (17). In humans, TGFB3 is associated with non-syndromic CL/P in different populations (28). TGFB3 is located at chromosome14q24. This gene is 23kb in size and contains seven exons. There are mixed reports regarding the role of this gene in relation to incidence of CLP. Vieira et al. (29) suggested that the mutation of MSX1 and TGFB3 in South American populations may contribute to CL/P. A similar study in Korean populations revealed that the TGFB3 polymorphism was strongly associated with an increased risk CL/P patients compared to controls (30).

**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 region (31). Lidral et al. (32) 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 increased the risk of CL/P, demonstrating the significance of gene-gene interaction in the etiology of non-syndromic CL/P. Subsequently, van den Boogaard et al. (33) described a family with a common pattern of tooth agenesis associated with CL/P. 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/P. The authors further suggested that these issued will need future studies to confirm the these particular mutations are causal (34).

**MTHFR**

Methylenetetrahydrofolate reductase (MTHFR) maps on chromosome 1q36 is a key enzyme in folic acid metabolism (35). 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 (36). Mills et al. (35) 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/P. 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 Carinci et al. (34), demonstrated a significantly higher mutation frequency of MTHFR in mothers of children with CL/P. Thus, the important of peri-conceptional 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
development (12). 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.

Stein et al. (38) demonstrated linkage of non-syndromic CL/P to BCL 3, a growth factor in 17 multigenerational CL/P families. Their analyses showed evidence for involvement of chromosome 19 in the etiology of clefting. These results suggest that a major gene does play an aetiologic role in the development of CLP and that these loci can be detected in linkage studies with sufficient numbers of families. Martinelli et al. (39) supported these findings using different methods. In addition the data reported by Stein et al. (38) 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 populations. Martinelli et al. (39) believed that BCL 3 or a nearby gene seems to be implicated in some way in this congenital facial malformation. However, the difficulties in demonstrating significant linkage indicate the 19q13.2 gene is not a major clefting gene. Thus, it appears that BCL3 plays a role in the aetiology of CLP. However, it is not known at present whether it acts as a modifier or as additive gene for this malformation.

Retinoic Acid Receptors (RARA)

Retinoic acid receptor alpha (RARA) showed a significant association with CLP in an Australian population (21), but no association in a British population (40). Juriloff and Mah (41) in their study found the chromosomal location of the mouse gene in which mutation occurs that can cause non-syndromic CLP. 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 populations (21). 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.

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