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Cleft lip and palate - The culprit gene- Identified yet? : A review

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Abstract: *Various genetic approaches, including genome-wide and candidate gene association studies as well as linkage analysis, have been undertaken to identify aetiologic factors, but results have often been inconclusive or contradictory. These results may support the presence of aetiologic 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. Even though the environmental factors have been understood and can be avoided to stop the formation of this facial anomaly, the genetic pathways still show the whirlphool effect and need to be understood and refined in their expression so that the genes and their actions could be identified and the probably existing single gene or family could be identified and reparative measures to stop its expression could be learned.*
Key words: cleft lip +/ palate, genetics, nonsyndromic, etiology.

I. Introduction

Malformations of the facial regions are usually seen as congenital anomalies, which include cleft lip and palate as one of the most common and psychologically distressing defect with a prevalence rate of about 1 in 600 – 700 new births taking place in Asian countries. As the occurrence of this unfortunate oral cleft which includes both cleft lip with or without cleft palate is seen in both syndromic and nonsyndromic conditions, the genes responsible for causing syndromic cleft lip and palate have been understood so far, however the identification of genes responsible for nonsyndromic CLP (cleft lip +/ palate) remains a challenging task. The etiology of this congenital birth defect is found to be polygenic as a plethora of genes have been identified as responsible, making the exact etiological factor still unknown, which would be the first step towards prevention of this birth defect. Extensive medical and behavioral interventions are needed to treat these common structural birth defects, which impose substantial economic and personal health that can persist from infancy to childhood and throughout life. Mounting evidence suggests that multiple genes and environmental factors influence the risk of orofacial clefts, either individually or through their interactions in complex biological pathways. Technological advances and collaborative efforts have led to major advances in gene-mapping for clefts, with the first wave of genome-wide association (GWA) studies identifying several key candidate genes and loci. By contrast, efforts to identify gene–environment (G3E) interactions have not been as successful, most likely because of a combination of insufficient sample size, study heterogeneity, differential assessment of environmental exposures, and a lack of robust methodology to detect these higher order interactions.[1]

Although cleft lip+/palate is usually not a life-threatening condition, many functions such as feeding, digestion, speech, middle-ear ventilation, hearing, respiration, facial and dental development can be disturbed because of the structures involved. These problems can also cause emotional, psychosocial and educational difficulties. A multidisciplinary care from birth until adulthood is needed for these children.[2] Orofacial clefts pose a burden to the individual, the family, and society, with substantial expenditure, and rehabilitation is possible with good quality care. The probable genes having a causal role in the etiology could be associated with various other functions such as, growth factors (eg, TGF-α, TGF-β), transcription factors (MSX1, IRF6, TBX22), nutrient metabolism (MTHFR, RARA) or immune responses (PVRL1, IRF6).

The various identified genes and their signalling pathways which are involved directly or indirectly in causing this birth defect are worth discussing. These are:

Homeobox gene (Msx1 –msh): MSX genes are homeobox-containing genes homologous to the Drosophila msh gene. MSX proteins function as transcriptional repressors in cellular differentiation and interact with other protein factors to modulate differentiation and proliferation.[3] Embryonic expression patterns of MSX genes are consistent with the role of Msx proteins in epithelial-mesenchymal tissue interactions during craniofacial development.[4] The role of Msx proteins in active morphogenesis is suggested by the lack of Msx1 expression in cells undergoing terminal differentiation and by restricted cellular expression of Msx1 transcript during periods of rapid cellular proliferation. Point mutations in MSX1 appear to contribute to approximately 2% of all CL/P cases.[5] Msx1-deficient mice develop craniofacial abnormalities of the nasal, frontal, and parietal bones, as well as CP. The occurrence of CP in Msx1 knockout mice aided the identification of a MSX1 mutation co-segregating with tooth agenesis, CL/P and CP. It has been proposed that CP in Msx1 knockout mice is due to insufficient palatal mesenchyme.[6] Also, rare human mutations have been observed in MSX1 that are associated with tooth agenesis, with and without CL/P.[7] Association and linkage studies further support a role for MSX1 in different populations.

Transforming Growth Factor-Beta (TGF-): It is one of the strongest candidate gene for cleft lip and palate in humans. TGF-β (located on 14q24) has a broad spectrum of biological activities and is known to induce palatal fusion and in recent years a large number of studies have been conducted to elucidate the relationship of TGF-β and cleft lip and palate. Transforming growth factor betas (TGF-β) mediate many cell-cell interactions that occur during embryonic development.

Transforming growth factor alpha- TGF- -: The physiological role of TGF-α is probably the control of epidermal development during development and differentiation of the cells.[8] TGF-α also affects bone formation and remodelling by inhibition of the synthesis of collagen and release of calcium. TGF-α also promotes the generation of osteoblast-like cells in long-term bone marrow cultures.[9] A study combining 13 linkage scan studies, revealed positive results, corroborating the hypothesis that TGF-α is a modifier rather than being necessary or sufficient to cause clefting.[10]

BMP Signaling Pathway: The BMPs are a collection of secreted cell signalling molecules of the TGF-b superfamily of growth factors. They regulate important developmental processes, including cell proliferation, differentiation and apoptosis. Members of this signaling pathway are expressed throughout the orofacial primordia in a strictly regulated spatio-temporal pattern, and outgrowth and patterning of the facial primordia are BMP-dosage sensitive . Conditional inactivation of the type 1 Bmp receptor gene (Bmpr1a) in the orofacial primordia causes bilateral CL/P with tooth agenesis; whereas, conditional deletion of its ligand Bmp4 in the same tissue results in isolated cleft lip only.[1]

FGF Signaling Pathway: The FGF signaling pathway plays a central role in craniofacial development, essentially through induction and migration of cranial neural crest cells and regulation of epithelial–mesenchymal interactions during fusion of the facial prominences. The majority of the FGF ligands and the receptors FGFR1 and FGFR2 are broadly expressed in the developing facial primordia. Several members of this family of signaling molecules have been implicated in various birth defects that also afflict craniofacial structures.[1]

Methylene tetrahydrofolate reductase (MTHFR): folic acid appears to play a very crucial role in palate development during maternal nutrition. Palate development delay was observed in folic acid-deficient mice.[11] Low dietary intake of B-complex vitamins and exposure to deficient or excessive amounts of vitamin A, have been linked to increased risks of clefts. Methylenetetrahydrofolate reductase deficiency leads to homocystinuria. Significantly higher serum homocysteine levels were detected in mothers of babies with orofacial clefts compared with mothers of unaffected babies.[12,13]It has also been hypothesized that genetic variants in the enzymes controlling folate metabolism might play a role in the susceptibility of oral clefts. It is reported that at least 400 μg/day of folic acid supplementation during early pregnancy was significantly associated with reduced risk of cleft lip even after adjustment for multivitamins, smoking, and other potential confounding factors.

RARA-retinoic acid receptor, alpha: Retinoic acid has a well-established role during development, and members of the retinoic acid receptor family mediate its activity. Transgenic and knockout mice studies have shown that these genes are important for facial development.[14] Zhang et al. first reported a significant difference in the frequency of alleles at the RARA locus between nonsyndromic CL/P patients and unrelated controls.

IRF6-Interferon regulatory factor 6: Variation at the IRF6 locus is responsible for 12% of the genetic contribution to CL/P at the population level and triples the recurrence risk for a child with a cleft in some families.[15] A positive association between IRF6 variants and OC has been confirmed in multiple populations and independently replicated. Meta analysis of 13 genome scans confirmed that IRF6 is one of the main candidates' genes that have common polymorphic variants, which can increase the risk of CL/P.[16]

TBX22: It is a recently described member of the T-box containing transcription factor gene family that is conserved throughout metazoan evolution. These genes play essential roles in early development and in particular mesoderm specification. The first T-box gene, Brachyury or T, was originally identified in mice.[17] Subsequently, a family of T-box proteins have been described including 18 in humans, all characterized by a similar DNA binding domain. In addition to TBX22, several other T-box genes have been implicated in human syndromes, emphasizing their importance in development.

Jagged-2 precursor (JAG2-Protein) : The Notch family of receptors is important signalling molecules regulating cell fate during development. Jagged 1 and Jagged 2 proteins play a role in craniofacial and limb development. Jag2 is expressed throughout the oral epithelium and is required for Notch1 activation during oral periderm differentiation. The mutant homozygotes exhibited CP and fusion of the tongue with the palatal shelves. Jag2 mutant mice have CP mainly due to failure of the palatal shelves to elevate and fuse. It is also shown that Irf6/Jag2 doubly heterozygous mice displayed fully penetrant intraoral epithelial adhesions, resulting in CP.[18]

BCL3 : It is that protein which is involved in cell proliferation, differentiation and apoptosis. Previous evidence has implicated the role of the BCL3 gene in the etiology of nonsyndromic clefting. Several studies have observed an association between BCL3 alleles and oral cleft, and the association has been suggested to be due to either an allele of low penetrance or BCL3 acting as a modifier locus.[19,20]

FOXE1-forkhead box E1 (thyroid transcription factor 2): The involvement of FOXE1 during primary palatogenesis is supported by the previously uncharacterized epithelial expression in the medial nasal and maxillary processes that will undergo fusion. Mutations in FOXE1 are associated with congenital hypothyroidism, thyroid agenesis and CP in humans and mice.[21] Isolated cleft palate was also associated, indicating that FOXE1 may play a role in two phenotypes thought to be genetically distinct.

Endothelin-1 (EDN1): It is synthesized by vascular endothelial cells and is found in plasma. EDN1 is released from an inactive transitional form in a step catalyzed by endothelin-converting enzyme (ECE). The mouse deficient in ECA or in endothelin-A receptor genes also has shown almost identical abnormalities to those of EDN1-deficient mice.[22] EDN1 knockout mice have shown craniofacial abnormalities, including cleft palate.[23]

Conclusion: To date, genetic approaches to nonsyndromic CLP have included: linkage analysis; association studies; identification of chromosomal anomalies or microdeletions in cases; and direct sequencing of DNA samples from affected individuals . These methods can be applied to candidate genes or genome-wide strategies can be used. Each approach has its own advantages and disadvantages, some of which will depend on the underlying genetic architecture of the disease, as well as the realities of economics and technology. 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 the identification of the probable culprit gene in the causation of CL/P research.

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