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BMP4 Gene and Pathophysiology of Cleft Lip and Palate.

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Short Communication

ABSTRACT

The rapid proliferative expansion and complex morphogenetic events that coordinate the development of face underpin the sensitivity of the structure to genetic and environmental insult and provide an explanation for the high incidence of midfacial malformation. Most notable of these malformations is cleft lip with or without palate. Despite the global impact of the disorder and some recent progress in identifying the causative genes for the non - syndromic forms of CLP, our knowledge on the key genetic factors and the environmental mutants therein contributing to CLP remains still remarkably patchy. The current understanding of the molecular and cellular processes that orchestrate morphogenesis of the midface, with emphasis on events leading to the fusion of the lip and primary palate is detailed in this review. The role of the candidate gene BMP4 in nsCLP, identified from relevant animal model systems and the likely events perturbed by the key gene pin pointed in human studies are discussed in this light. In this present review the normal craniofacial patterning and morphogenesis of lip and palate are discussed with a focus on the role of BMP signaling therein.

INTRODUCTION

Development of head and face comprises one of the most complex events during embryonic development, coordinated by a network of transcription factors and signaling molecules together with proteins conferring cell polarity and cell – cell interactions. Disturbance of this tightly controlled cascade can result in facial cleft where the facial primordial ultimately fail to meet and fuse or form appropriate structures. Collectively craniofacial anomalies are among the most common features of all birth defects. The most frequent of these are the orofacial clefts of lip and/or palate are immediately recognizable disruptions of normal facial structure [1]. although not a major cause of mortality in developed countries, CLP does cause considerable morbidity to affected children and imposes a substantial financial risk for families with a concomitant societal burden. Individuals with CLP may experience problems with feeding, speaking, hearing and social integration that can be corrected to varying degrees by surgery, dental treatment, speech therapy and psychosocial intervention [2]. the complex etiology of clefts affords opportunities to identify gene – gene interactions/ gene – environment interactions that can shed light on human embryology and its disturbances [3]. Recent successes in genome wide linkage and association studies have identified novel loci significantly associated with CLP [4,5,6,7,8]. Researchers are currently striving to identify the etiologic variants at these novel loci to understand the developmental disturbances leading to CLP, and this knowledge should eventually result in improved prevention, treatment and prognosis for individuals with these conditions.

Normal Craniofacial Development and Palatogenesis

The formation of mammalian palate is a multistep process that includes palatal shelf growth, elevation of shelves, fusion between paired shelves and the disappearance of midline epithelial seam [9]. Development of the human face begins in the fourth week of gestation when migrating neural crest cells from the dorsal region of the anterior neural tube (cranial neural crest cell) combine with mesodermal cells to establish the facial primordial [1]. Mesenchyme from the first branchial arch forms the maxillary, lateral nasal, and medial nasal processes. The upper lip, primary palate and secondary palate form sequentially from connective tissue derived from neuroectoderm. By the 4th week of gestation in humans, the facial primordial consists of five facial swellings called facial prominences. The frontonasal mass and medial nasal processes will develop into the mid face and contribute to the forehead,

ridge of the nose and primary palate, which includes the premaxillary segment of the upper jaw. The sides of the nose and cheeks develop from the lateral nasal and maxillary prominences respectively while the mandibular processes from the lower jaw. The five prominences encircle the stomodeum or the primitive mouth. The development of the upper face requires complex morphogenetic movements and occurs in 2 stages; an early phase for lip development and a later phase for secondary palatogenesis. The maxillary processes grow medially, pushing the lateral nasal process superiorly and allowing the maxillary process to fuse with both the lateral and medial nasal process. Together with the maxillary prominences, the lateral and medial nasal processes fuse to form a continuous upper lip. After fusion of the upper lip, proliferation of mesenchyme causes the intermaxillary segment to project into the oral cavity, forming the primary palate. The secondary palate is formed from the downward projections of the maxillary processes that descend into the oral cavity and lie lateral to the tongue (lateral palatine processes). In this first stage, the bilateral palatal shelves subsequently grow down vertically along the two sides of the tongue [10].The second stage of palatogenesis, relies on the morphogenetic movements/growths of the lower jaw, to lower the tongue relative to the palatal shelves. As the mandible starts to grow in length, the tongue descends, allowing the two lateral palatine shelves to elevate, grow medially, and meet together. Movement from vertical to horizontal is likely to be the consequence of an intrinsic force resulting from increased turgidity through recruitment of water in response to elevated levels of glycosoaminoglycans such as hyalouron [9].The occurs concomitantly with rapid remodeling of the extracellular matrix (ECM). Following elevation, the bilateral palatal shelves grow toward each other and adhere by the glycoprotein coat and desmosomal junction of medial edge epithelia (MEE) to form the midline edge seam (MES) [3]. The palatal shelves initially contact in the mid portion and the zipper is closed towards both the primary palate and uvula [1].
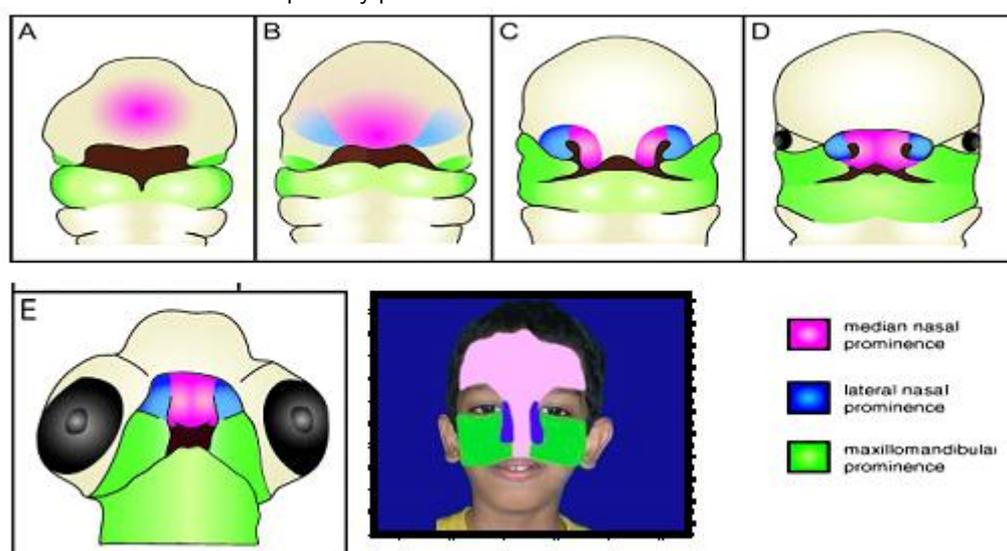
Figure 1: Development of the craniofacial primordial from different facial processes [1,2].

Mechanism of Palatal Tissue Fusion

Midline edge seam, which is composed of periderm and basal medial edge epithelia cells, essentially degenerates by apoptosis; dying cells activate basal lamina degradation and the fusion is complete [11,12]. The exact fate of the epithelia in MES is controversial and three major pathways have been put forth namely: (1) Apoptosis [13,14], (2) Migration to the oral or nasal side of the palate [15] (3) Epithelial – mesenchymal transformation [16]. The process of palatal fusion is complete by 12th week in man when the MES disappears entirely [17, 18]. Epithelial cells on the nasal side of the palate differentiate in pseudo stratified ciliated columnar epithelium. The cells in the oral side differentiate into stratified squamous non-keratinizing epithelium. Once fusion is complete ossification occurs in anterior two thirds of the palate and form hard palate [3]. Posterior third develops into soft palate without ossification. Peptide growth factors are known to play crucial roles as inductive signals that mediate epithelial – mesenchymal interactions during histologically the stages of the process of primary palatal fusion are explained in seven stages. In the first stage the pre fusion contact epithelia is demarcated by elevated BMP4. This in turn initiates a cascade of cellular changes in the epithelium, including apoptosis, which is stage 2. The Stage 3 is characterized by apical surface bulging and stage 4 by induction of filopodia. In stage 5, apposing epithelial sheets subsequently join forming full junctional complexes that includes adherens junctions, tight junctions, and desmosomes. These eventually undergo epithelial – mesenchymal interactions in stage 6, with concomitant breakdown of basal lamina. Finally, in stage 7, the establishment of mesenchymal confluence to form primary palate is seen [19].
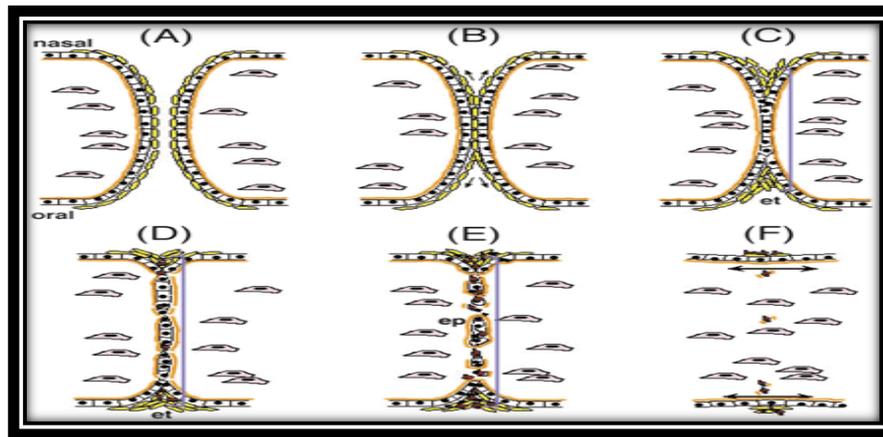
Figure 2: Diagrammatic representation of seven stages of fusion of palate [3,4,5].

Development of Cleft Lip And Palate

Orofacial union is an important mechanism for craniofacial development. The facial primordia, palate, tongue - all undergo midline union in early development. Perturbation of this process leads to various orofacial clefts such as cleft lips, cleft palate, oblique facial cleft, lateral facial cleft, mandibular cleft, and cleft tongue. Hence cleft lip and palate is the result of improper fusion of the processes that form the face, caused by abnormal morphogenesis, misguided epithelial movement, disrupted epithelial-mesenchymal (EMT) transformation, or disrupted apoptosis [16]. Failure of any of the above mechanisms result in insufficient growth, decreased nutrients or a diminished degradation of the epithelial seam covering the growth processes, each predisposing to cleft lip and palate.

Non Syndromic Cleft Lip and Palate: Novel Genes Unravelled.

Craniofacial morphogenesis is a complex process involving the interaction of cell growth, growth factors and receptors, and apoptosis to guide the convergence and fusion of the facial and palatal processes. Many genes are involved and regulate the development of the craniofacial region. In the past few years the list of NSCLP candidate genes has rapidly increased and their study has been mainly focused in the search of coding mutations [20]. A few to list are [1]

Sl No	Function	Genes
1	Polarizing Signals	Shh, Bmp2, Bmp4 and Bmp7, Wnt5a, Smad2-4
2	Growth Factors And Receptors	Fgf, Egfr, Tgfα, Tgfβ1-3, Fgf1, Fgf2, Fgf8, Fgfr1, Fgfr2
3	Transcription Factors	Ap2α, Dlxl-6, Gli2-3, Hoxa2, Irf6, Lhx8, Pax9, Pitx1, Pitx2, Prxl2, Msxl, Thxl, Tbx22
4	Cell Adhesion Molecules	Pvrll, Connexin43, E-Cadherin
5	Extracellular Matrix	Col2Al, col1Al, and Col1A2, Mmp2, Mmp3, Mmp9, Mmp13, Timp1-3, Fibronectin

Brief Overview of BMP4 Gene

The presence of BMP was initially implicated by the pioneering work of Urist who first discovered bone autoinduction by bone matrix. Thereafter a number of BMPs were isolated and cloned. The candidate gene [21]. Bone morphogenic protein 4 is a member of the transforming growth factor - beta super family is extensively studied and has gained the attention of many researchers in the yester years [6]. Bmps are now recognized as multifunctional growth factors that mediate a variety of biological functions essential for gastrulation, organogenesis, and embryonic and post natal growth. BMPs are also important candidate genes in craniofacial patterning located on chromosome 14q22 – 23 [22] in humans. More than 20 BMP genes have been identified. Currently, Bmp 2 and 4 are grouped as dpp sub-family due similarity to the dpp gene in Drosophila. Bmp 5, 6, 7 and 8 are grouped as the 60A subfamily; Bmp3 and 3b (GDF10) together constitutes a separate subfamily. BMPs mainly function through BMP receptor (BMPR) type I and type II (BMPRI and BMPRII) [21]. Bmp signaling plays crucial roles in normal craniofacial development, and *Bmp4* deficiency results in craniofacial anomalies, such as cleft lip and palate, in mouse and humans. Lidral and Moreno [23] performed a genome wide scan Meta-analysis that showed evidence of linkage between NSCLP and chromosomal region of 14q21 – 24 [24]. How this conserved signal regulates the development of the phylogenetically new mammalian head remains interesting and

incompletely known issue. The use of mouse models and modern molecular techniques have greatly facilitated the process of craniofacial research and extended our understanding of craniofacial development in the past decade.

The BMP signaling pathway has been shown to be involved in a number of developmental processes and critical in the formation of variety of craniofacial elements including cranial neural crest, facial primordial, tooth, lip and palate [21]. there is strong evidence that Bmp signaling regulates craniofacial morphological change during evolution, Both gain-of-function studies and comparative expression data revealed *Bmp4* to be a crucial regulator of beak shape in Darwin's finches, a classic model of evolutionary diversification. Other experiments in Cichlid fish also support the notion that *Bmp4* is a major regulator of craniofacial cartilage shape and morphological adaptive radiation.The transcripts of both BMP2 and BMP 4 have also been detected in developing mouse shelves [25, 26, 27]. Bmp-regulated genes control self-renewal, osteoblast differentiation and negative feedback regulation, suggesting that Bmp signaling regulates facial skeletal morphogenesis by controlling the balance between self-renewing progenitors and differentiating lineage-restricted cells.

BMP signaling is an important mediator in the process of orofacial union is indicated by Alk deficient mice, in which orofacial clefts is one of the major anomalies [21]. BMP4 which is highly expressed in the putative orofacial epithelia prior to facial union is implicated a good candidate for this role [28]. A recently generated mouse model with inactivation of BMP4 from facial primordial provides convincing evidence of this role. In those mice, lip development is delayed and Fgf8, a critical gene for early facial development, fails to be upregulated at a crucial developmental stage and results in cleft lips. Those authors demonstrated that the BMP4/ BMPrla pathway is critical for the lip development this model also implies that BMP 4 is a possible candidate gene for isolated cleft lips [29].

BMP Signalling In Palatogenesis

Gong and Guo have reported that the Bmp4 expression localizes at the site of fusion of the mice facial prominences [28]. The conditional inactivation of Bmp4 in a transgenic mice line results in an isolated cleft lip The findings of these studies imply that the function of Bmp4 in the ectoderm of the facial processes is to regulate lip fusion. BMPs have been implicated in mammalian palate development [33]. Several BMP genes including BMP2, BMP 4 and BMP 7 are expressed in developing mouse palatal shelves [34], Wall Nancy A et al [35] and Levi et al [36]. On the other hand found inactivation of promiscuous TGF – beta antagonist Follistatin causes a cleft palate phenotype raising a possibility that an elevated level of BMP signaling also impairs palate development [37]. The importance of BMP 4 signaling in palate development was initially demonstrated in Msx 1 mutant mice which exhibit cleft palate phenotype. In the Msx1 mutant palate, BMP4 expression is abrogated and ectopic expression of its human ortholog rescues the cleft palate phenotype in MSX 1 mutant [25]. Msx 1 was required for the expression of BMP4 and BMP2 in the palatal mesenchyme and Shh in the medial edge epithelium. BMP4 appears to bypass a requirement for Msx 1 and to function upstream of Shh and BMP2 to regulate palate development [35]. Inactivation of BMP4 – BMPr1a genetic pathway by Nestin Cre transgenic line mutant mice demonstrated orofacial clefting, thus, revealing the role of BMP signaling in orofacial union [38].

Recent Human Studies Done on BMP Gene and Cleft Lip And Palate

Suazo et al [39], detected 3 novel variants in BMP 4.1 (c.-5514G >A, c. 5365 C> T and c.–5049C> T) and said it could be considered as cleft risk factors due to their absence in controls. Additionally rs28555330G allele (BMP 4.2) carriers showed an increased risk of NSCLP restricted to males. The nonsynonymous polymorphism rs17563 T>C (p.V152A) in the *BMP4* gene has been associated to the risk of nsCLP and the C allele had a protective effect against the occurrence of nonsyndromic CL±P]. Lately, Chen et al [40], provided further evidence of association between *BMP4* gene and nonsyndromic CL±P. Nominal significant evidence of linkage and association was observed for three SNPs (rs10130587, rs2738265, and rs2761887). Lin et al performed an association study in Chinese population using non synonymous single nucleotide polymorphism of BMP4, rs17563 (p Val152Ala) and described that C allele carriers showed an increased risk for NSCLP [41]. The BMP4 coding sequence was analyzed by Suzuki et al [42] in a sample of patients with sub epithelial, microform and overt cleft lip detected missense and nonsense mutations in 0.7% of these patients. All these findings support a role for genetic variation of BMP4 in the pathogenesis of NSCLP. According to L Jianyan et al [43], there was significant relationship between genetic variations at BMP 4T538C and several common environmental exposures aka parents' tobacco and alcohol consumption, mother's multivitamin supplement and passive smoking during the 3 months before conception to the 1st trimester of pregnancy and non-syndromic cleft lip and palate. All these studies provided evidence that BMP4 polymorphisms could be used as genetic susceptibility markers for non syndromic cleft lip with or without cleft palate.

CONCLUSION

The completion of genome sequence has contributed to the recent successes in identifying novel CL/P genes1. The direct study of non-syndrome CL/P has been previously hampered due to the general lack of well-

defined multiplex families with sufficient power to enable a genome wide linkage study to provide localization. The use of model organisms and in particular the mouse has for some time been a rich source of information for craniofacial development. Transgenic and knockout technology has often and sometimes quite unexpectedly, provided a long list of genes that confer a CLP phenotype. The number and diversity of targeted genes that result in a cleft probably reflects why CL/P is one of the most common features seen in human birth defects. The use of mutant inbred strains to tease out causative genes and provide models is an exceptionally powerful tool. Nevertheless, the problem is to directly relate them to the complex human situation where genetic heterogeneity and varying environmental/socio – economic status is found. Our general failure is to pin point the precise molecular events that lead to human CL/P most likely stems from our lack of knowledge about gene networks and regulation of gene expression during palatal development. A decade back we were faced with the question "Can this complex trait be too complex?" Clearly we are now in a better position to address this [44] we have now a long list of candidate genes that contribute to the incidence of nsCL/P. This can be combined with latest generation of expression profiling techniques. Therefore there is now every reason to be optimistic about our understanding of human CLP. For many, the benefits of precise diagnosis, accurate risk assessment and genetic counseling can be achieved. When integrated with tissue specific expression profiling and targeted developmental studies, the potential for treatments and preventive therapies may also become a reality. The complex etiology of clefts affords opportunities to identify gene – gene interactions/ gene – environment interactions that can shed light on human embryology and its disturbances [3]. Recent successes in genome wide linkage and association studies have identified novel loci significantly associated with CLP. Researchers are currently striving to identify the etiologic variants at these novel loci to understand the developmental disturbances leading to CLP, and this knowledge should eventually result in improved prevention, treatment and prognosis for individuals with these conditions. Fortunately, the state of art research tools are being used to unveil more secrets of palate development and clefting [45].

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