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Roles of BMP Signaling Pathway in Lip and Palate Development

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Abstract

Cleft lip with or without cleft palate (CLP) and cleft palate only (CP) are severe disruptions affecting orofacial structures. Patients with orofacial clefts require complex interdisciplinary care, which includes nursing, plastic surgery, maxillofacial surgery, otolaryngology, speech therapy, audiology, psychological and genetic counseling, orthodontics and dental treatment, among others. Overall, treatment of clefts of the lip and palate entails a significant economic burden for families and society. Therefore, prevention is the ultimate objective and this will be facilitated by a complete understanding of the etiology of this condition. Here we review the current concepts regarding the genetic and environmental factors contributing to orofacial clefts and emphasize on the roles of BMP signaling pathway components in the normal and aberrant development of the lip and palate.

Epidemiology and Genetics of CLP and CP

Orofacial clefts affecting lip and palate may be classified as: (1) complete cleft palate with cleft lip; (2) cleft of the primary (anterior) palate, in which the cleft is limited to the anterior incisive fossa, and may or may not involve cleft lip; (3) cleft of the secondary (posterior) palate, in which the cleft defect is limited to the posterior incisive fossa and (4) submucosal cleft including bifid uvula [1–7]. CLP and CP can be isolated features (non-syndromic) or part of a syndrome (syndromic). Approximately 70% of all cases of CLP and 50% of cases of CP are considered to be non-syndromic. Non-syndromic cases of CLP and CP occur with a high prevalence in diverse human populations. Non-syndromic CLP affects approximately 1 in 700 live births, with large variability in populations depending on the ethnic group, geographic origin as well as the environmental exposures and social and economic conditions. Asian and Native American populations have the highest prevalence, as high as 1 in 500. Caucasian populations have an intermediate prevalence of 1 in 1,000, and African-derived populations have the lowest prevalence at 1 in 2,500. The occurrence of CLP and CP also changes by gender and laterality of the cleft with males being more affected than females by clefts involving the lip at a 2:1 male to female ratio. On the other hand, the ratio for clefts affecting only the palate is inverse, a 1:2 male to female. Among unilateral cases of CLP, a 2:1 ratio of left- to right-sided clefts has been reported [8].

The etiology of CLP and CP in humans is heterogeneous, involving genetic and environmental factors. Orofacial clefts have an elevated rate of familial recurrence compared with other birth defects. It has been reported that the risk of cleft recurrence in first-degree relatives is 32 for cleft lip (CL) and 56 for CP compared to the reference populations, which suggest a stronger genetic background for CP compared with CL [9]. In studies involving twin pairs, the concordance rate of 40–60% in monozygotic (MZ) twins is

significantly higher than that observed in dizygotic (DZ) twins, which is only 3–5%. The high concordance rate in MZ twins provides substantial evidence supporting a strong genetic component [10]. However, the fact that few pedigrees of patients affected by CLP or CP show mendelian inheritance and most cases appear to be sporadic rules out a purely genetic or purely environmental cause for orofacial clefting. In fact, environmental risk factors have been reported to be an important component of the CLP and CP etiology [11].

A multifactorial model in which multiple and probably small genetic risk factors might interact with environmental agents has been suggested [8, 11]. The identification of individual genetic factors for clefting has been a goal of numerous research groups since the first reported association between CLP and gene variants of the Transforming Growth Factor Alpha (TGFA) by Ardinger et al. [12]. From this study, the list of candidate genes for CLP and CP has increased quickly. Currently, this list includes *IRF6*, *MSX1*, *TGFB3*, *ABCA4*, *FOXE1*, *FGFR1*, *FGFR2*, *FGF8*, *MAFB*, *PDGFC*, *CRISPLD2*, *PVRL1*, *GABRB3*, *MSX2*, *SATB2*, *TBX10*, *TBX22*, *GLI2*, *JAG2*, *MTHFR*, *RARA*, *LHX8*, *SKI* and *SPRY2*, among others [8, 10, 13–20]. Methods to identify those genes include association studies using case-parent trios or case-control samples; identification of chromosomal anomalies or microdeletions in cases, and direct sequencing of DNA samples from affected individuals as well as genome-wide strategies [8]. There have also been several resequencing studies of candidate genes to identify specific variants that may possess statistical associations with orofacial clefting. The strongest existing data is for mutations in *MSX1* [21], *FGFR1* and *FGF8* [19], and bone morphogenetic protein 4 (*BMP4*) [22].

An environmental component to clefting has been also recognized, especially in regions of low socioeconomic status. Among the environmental factors contributing to CLP and CP, maternal exposure to certain drugs such as phenytoin, valproic acid, retinoids and thalidomide has been reported [23]. Increased risk of CLP and CP has been connected to maternal alcohol or cigarette use [24, 25], herbicides such as dioxin [26], altitude [27], hyperthermia [28] and infectious diseases [29]. Specifically, maternal smoking appears to contribute to 4% of the total CLP cases and 12% of bilateral CLP [30]. Several meta-analyses support a significant interaction between maternal smoking and CLP [31, 32]. However, a meta-analysis of five published studies demonstrated that maternal smoking and infant TGFA genotype are associated with CP but not with CLP [33]. Smoking has also been associated with variants of Glutathione S-transferase-01 (*GSTT1*), Nitric Oxide Synthase 3 (*NOS3*) and *IRF6* in the etiology of CLP [8, 34]. Other studies also support interactions between smoking, alcohol consumption, nutritional factors and the *MSX1* and *TGFB3* genes in addition to TGFA [reviewed in 23]. Regarding alcohol exposure, among the anomalies characterizing fetal alcohol syndrome are the alterations of the craniofacial region, including orofacial clefts. The specific association between CLP and CP with alcohol consumption has also been suggested from case-control studies but it is more inconsistent compared to maternal smoking [8]. Some studies indicate that high doses of alcohol in short periods of time increase the risk [35] and others suggest an association of polymorphic variants of the Alcohol Dehydrogenase 1C (*ADH1C*) gene and CLP [36]. More research is needed to understand the complex gene-environment interactions leading to CLP and CP.

Lip and Palate Development

Although CLP is the most frequent type of cleft in humans, most mouse models for clefting display CP only. The upper lip and nose form before the fusion of the secondary palatal shelves [37]. A failure of correct lip fusion may secondarily have an effect on palatal fusion, which explains why CP accompanies clefts of the lip. At embryonic day 10.5 (E10.5) in the mouse and in the fifth week of pregnancy in humans, the surface ectoderm of the frontonasal

prominence thickens developing the nasal placodes. Next, the lateral and medial nasal processes emerge from the prominence around the placode. The nasal pit is established in the center. The maxillary processes of the first branchial arch grow towards the medial and lateral nasal processes at E10.5 in mice and the 35th day in humans and together form the upper lip. The fusion processes between the medial and lateral nasal prominences begin at this stage. Subsequently, the maxillary and medial processes also undergo fusion. At E12.5 in mouse and seventh week in humans, the formation of the upper lip is finished after complete disappearance of the epithelial seams between the processes [37].

The palate in mammals forms from two primordia: the primary and the secondary palate. The primary palate, which shares embryological origin with the lip, represents only a small part of the adult hard palate. The development of the secondary palate begins at E12.5 in mice and the sixth week in humans with the emergence of palatal primordia from the maxillary process [38]. The palatal shelves are composed of cranial neural crest-derived (CNCC) mesenchyme covered by pharyngeal ectoderm-derived epithelium [38, 39]. Using the *Wnt1-Cre;R26R* model, we have shown that more than 90% of palatal mesenchymal cells are derived from CNCC [39, 40]. From E12.5 to E13.5, the palatal shelves grow vertically along the two sides of the tongue. Once the mandible starts growing in length providing physical space for the tongue to descend, the palatal shelves reorient to acquire a horizontal position. Then, during E14.5–15.5 in mice and week 9 in humans, the palatal shelves grow toward each other and establish contact in the midline, where a midline epithelial seam is formed (MES) [41]. The MES must degenerate in order for the mesenchyme to become continuous and several theories have been proposed for this process: programmed cell death [42], migration of the epithelial cells to the oral or nasal side of the palate [43], and/or epithelial-mesenchymal transformation (EMT) [44]. However, epithelial cell mapping using the *K14-Cre;R26R* model shows that midline epithelial cells mainly disappear due to apoptosis and migration, but not EMT [45]. By E16.5 in mice and the 12th week in humans, fusion of the palatal shelves is complete. Next, ossification occurs in the anterior two-thirds of the palate to form the hard palate whereas the posterior third develops into the soft palate without ossification [reviewed in 46]. The nasal and oral epithelia at this stage are differentiated pseudostratified and squamous epithelia, respectively [reviewed in 7].

Lip and palate development rely on a very tightly regulated network that includes the activity of secreted proteins and their signaling pathways, cell-surface receptors, ECM components as well as transcription factors [reviewed in 46]. The transforming growth factor-beta (TGF β) superfamily is an important part of this network as deficiency of its members leads to malformations affecting the craniofacial region and particularly the palate in mouse models. The TGF β superfamily includes activin, TGF β and BMP cytokines [47].

BMP Signaling Pathway

BMPs are secreted signaling molecules that trigger intracellular cascades by binding to cell surface receptor serine/threonine kinases [48]. More than 20 members have been identified in the BMP family [49]. BMP ligands are classified into five subfamilies based on phylogenetic studies and sequence similarity: (1) the *dpp* subfamily is comprised of BMP2 and BMP4 because of their similarity to the *Drosophila dpp* gene (decapentaplegic); (2) the 60A subfamily includes BMP5, -6, -7 and -8; (3) BMP3 and BMP3b (GDF10) together constitute a unique subfamily; (4) GDF5, -6, -7 constitute another subgroup in this family, and (5) Nodal and Lefty are distant members of the BMP subfamily [reviewed in 50, 51]. BMPs mainly signal through BMP receptors (BMPRI type I and type II. Three type I (ALK2, ALK3 or BMPRIA and ALK6 or BMPRII) and three type II receptors (BMPRII, ActRIIA and ActRIIB) are known to mediate BMP signals [52]. Upon ligand binding, the

type II receptor phosphorylates the type I receptor. In turn, type I receptor phosphorylates and activates a group of transcriptional coactivators called Smads, specifically Smad1/5/8. These Smads associate with Smad4 (common Smad) forming a complex that is able to translocate into the nucleus where it regulates the transcription of downstream targets, including *Msx* genes [reviewed in 50]. Vertebrate *Msx* genes are unlinked, homeobox-containing genes that are homologous to the *Drosophila muscle segment homeobox* gene. They are regulatory proteins that function as transcriptional repressors in vitro and in vivo. *Msx* proteins also interact with other homeodomain proteins to regulate transcription. In fact, heterodimers formed between *Msx1* and other homeodomain proteins such as *Dlx2*, *Dlx5*, *Lhx2* and *Pax3* appear to result in mutual functional antagonism [reviewed in 53]. *Msx* genes are considered readouts of the BMP signaling pathway (see below).

Fine-tuning of the BMP pathway is primarily achieved through the action of antagonists, co-receptors and intracellular regulatory proteins. BMP antagonists are secreted peptides that bind to BMP ligands, blocking their receptor epitopes, and consequently prevent ligand-receptor oligomerization. BMP antagonists are structurally similar to BMP ligands as they are homodimeric proteins that consist of a cystine knot motif. BMP antagonists include: the Chordin/Noggin family, Twisted Gastrulation (Tsg) and the DAN/Cerberus family [51, 54]. On the other hand, BMP signaling can be modulated by co-receptors. Repulsive guidance molecules (RGM) a and c, Dragon (RGMb), c-Kit, Endoglin and Betaglycan are co-receptors that enhance BMP signal transduction [55, 56]. The decoy-receptor BMP and activin membrane-bound protein (BAMBI), Ror2 and TrkC receptors regulate BMP signaling negatively [57, 58]. Finally, there is a set of intracellular proteins that act as signal transducers or regulators of BMP signaling. Among them, BMPRIa interacts with the TGF β -activated kinase (TAK) binding protein 1 (TAB1) through the BMP receptor-associated molecule 1 (BRAM1), which downregulates BMP-mediated Smad signaling [59]. Likewise, the X-chromosome-linked inhibitor of apoptosis (XIAP) connects BMPRI with TAB1 and may act as a positive regulator of MAP kinase signaling [60]. In addition to proteins that regulate the MAP kinase pathway, BMPRI also interacts with membrane endocytic factors (caveolin1, Eps15R), phosphatases (Dullard, PP2A), kinases (Rack1) and cytoskeletal proteins (Trb3), among others (for more details on BMP modulation, see Sieber et al. [51]).

BMP Signaling Pathway in Palate Development

Expression Patterns

The BMP signaling pathway regulates a wide array of developmental processes such as cell proliferation, apoptosis, differentiation, and morphogenesis [reviewed in 61]. *Bmp2* and *Bmp4* mRNAs are expressed in a dynamic pattern in the facial primordia of chick and mammal embryos. *Bmp4* expression is highly restricted to the distal epithelia of the medial nasal, lateral nasal, maxillary and mandibular processes [62]. The expression patterns of *Bmp2* and *Bmp4* in the facial ectoderm correlate with the underlying mesenchymal expression domains of *Msx1* and *Msx2* in the facial primordia. Furthermore, ectopic *Bmp2* or *Bmp4* is able to induce *Msx1* and *Msx2* gene expression in the facial mesenchyme [63]. This finding suggests that *Msx1* and *Msx2* are downstream targets of the Bmp pathway. In fact, a BMP-responsive region of *Msx2* has been recently identified in mammalian cells [64]. *Bmp4* expression has also been detected in the distal ectoderm of the facial primordia neighboring the stomodeum prior to and during lip fusion in mouse embryos [65]. Accordingly, *Msx1* and *Msx2* expression is detectable in the adjacent facial mesenchyme.

In the mouse developing palate, *Bmp4* mRNA is found in the palatal epithelium and mesenchyme at E12.5 and it is subsequently restricted to the mesenchyme at E13.5 and E14.5. *Bmp4* expression is absent in the posterior region of E13.5 and E14.5 palatal shelves.

Bmp2 presents a similar pattern of expression. At E12.5 and E13.5 it is expressed in both the epithelium and mesenchyme of the anterior region of developing palatal shelves and is absent in the posterior palate [66]. Reports of a weak, diffuse expression of *Msx1* in the palatal mesenchyme provided the first evidence that *Msx1* may have a direct role in palate development. A more detailed analysis by Zhang et al. [66] has reported that *Msx1* expression in the palatal mesenchyme is confined to the anterior portion of the developing palatal shelves. Recently, it has been shown that phospho-Smad1/5/8, which marks the activation of BMP signaling, appears to be restricted to the nasal side of the palatal mesenchyme although the expression of *Bmp4* and *Bmp2* is homogeneous throughout the palatal mesenchyme [66, 67]. The expression pattern of *Bmpr1a* and other receptors in the palatal mesenchyme needs to be examined to explain the asymmetrical activation of BMP signaling in the palatal mesenchyme. This asymmetry might be due to (1) regional differential expression of *Bmpr*, which determines the establishment of the BMP-responsive domain in the palatal mesenchyme and/or (2) inhibition of BMP signaling activation on the oral side of the palatal mesenchyme.

Animal Models

The first functional analyses of BMP and its downstream targets were performed in chick embryos. For instance, ectopic application of *Bmp2* or *Bmp4* protein induces overgrowth and changes the patterning of the chick facial prominences [63]. Conversely, inhibiting *Bmp* signaling by application of *Noggin* in the chick facial primordia causes reduced mesenchymal proliferation and outgrowth [68, 69]. More recently, *Bmp* signaling has been suggested to be crucial in the evolution of facial shape and size in fish and birds [reviewed in 70]. Analyses of mouse models have provided information regarding the mechanisms that BMP uses to exert its functions in palatogenesis.

The direct study of *Bmp* signaling pathway components in craniofacial development has been hampered by the early embryonic lethality of the *Bmpr*-null mutant mice and functional redundancy among BMP ligands [71–73]. Liu et al. [74] used conditional null alleles of *Bmpr1a* and *Bmp4* to overcome the early embryonic lethality of conventional *Bmpr1a*- and *Bmp4*-null mice. *Nestin-Cre;Bmpr1a* mutants display bilateral CLP and arrested tooth formation with complete penetrance. The cleft palate of *Nestin-Cre;Bmpr1a^{fl/fl}* embryos is associated with reduced mesenchymal cell proliferation in the maxillary process and defective anterior-posterior patterning. In contrast, there is increased apoptosis in the fusing region of the medial nasal process in *Nestin-Cre;Bmpr1a^{fl/fl}* mice. Moreover, conditional inactivation of the *Bmp4* gene using the *Nestin-Cre* transgenic line results in isolated cleft lip. These findings contrast with previous results in which inhibition of BMP signaling in the chick facial primordia with *Noggin* increased epithelial survival [68], suggesting that the epithelial function of BMP during craniofacial development is still not completely understood. Liu et al. [74] also reported that numerous mouse embryos in which *Bmp4* is inactive in facial epithelium have delayed lip fusion that, however, is repaired by E14.5 in most mutant mice. It is hypothesized that the absence of cleft lip in these mutants is due to functional redundancy with other members of the *Bmp* family. Interestingly, specific inactivation of *Bmpr1a* in CNCC-derived mesenchyme from E10.5 (*Osr2-Cre;Bmpr1a^{fl/fl}*) causes reduced cell proliferation in the primary and anterior secondary palate, which result in partial cleft of the anterior palate. In these mutants, *Msx1* and *Fgf10* expression is downregulated in the anterior palate mesenchyme and expression of *Shh* is reduced in the overlying palatal epithelium. These findings indicate that *Bmp* signaling regulates mesenchymal-epithelial interactions during palatal outgrowth. Additionally, the development of the palatal processes of the maxilla is deficient and formation of the palatal processes of the palatine is significantly delayed, which lead to a submucous cleft of the hard palate in *Osr2-Cre;Bmpr1a^{fl/fl}* mice [75]. Similarly, mice

lacking *Alk2* in the neural crest (*Wnt1-Cre;Alk2^{fl/fl}*) display multiple craniofacial defects including CP and a hypotrophic mandible [76].

Conventional knockout mice for downstream elements of the BMP pathway have been also generated. In the case of *Smad4*, the early embryonic lethality in *Smad4*-null mice makes analysis of its effects on craniofacial structures impossible [77, 78]. Inactivation of *Smad4* in the palatal epithelium using *K14-Cre* does not lead to any defect in palate development. However, this result is due to functional redundancy between *Smad4*-dependent and -independent pathways, specifically p38 MAPK [79], and needs a careful interpretation because *Smad4* acts as a common Smad for both TGF β and BMP signaling pathways. Inactivation of *Smad4* in CNCC-derived mesenchyme, in *Wnt1-Cre;Smad4^{fl/fl}* mice, shows that *Smad4* is not required for the migration of CNCC although embryonic development is arrested at E11.5–E12.5. There is underdevelopment of the first branchial arch and failure of fusion in the middle of the frontonasal process and in the middle of the mandibular process of the first branchial arch in *Wnt1-Cre;Smad4^{fl/fl}* embryos. The defects in lateral development of the first branchial arch are more severe than those in anterior-posterior development due to a dramatic increase in the number of apoptotic cells in the CNCC mesenchyme of *Wnt1-Cre;Smad4^{fl/fl}* mice [80]. The analysis of *Smad4* function during palatogenesis in these conditional mutant embryos is impeded due to the early arrested development. To overcome this issue, *Osr2-Cre;Smad4* embryos have been generated. These mice display complete CP due to a significant reduction in mesenchymal cell proliferation, which occurs from E14.5 [pers. unpubl. data].

Satokata and Mass [81] generated *Msx1*-deficient mice and neonatal mortality, CP and anodontia are their main phenotypic features. Mice lacking both *Msx1* and *Msx2* gene function exhibit bilateral CLP [82]. *Msx1* and *Msx2* play critical roles in facial mesenchymal proliferation because *Msx1*-null mutant mice have shortened maxilla and mandible as well as defects in palatal mesenchyme proliferation [66, 81]. Using a transgenic approach, Zhang et al. [66] expressed a *Bmp4* transgene ectopically in the *Msx1* mutant palatal mesenchyme, resulting in rescue of the cleft palate phenotype and neonatal lethality. Associated with this rescue was a restored pattern of *Shh* and *Bmp2* expression, as well as a recuperation of normal cell proliferation in the palatal mesenchyme. Therefore, BMP4 appears to avoid a requirement for *Msx1* and to function upstream of *Shh* and *Bmp2* to regulate palatogenesis [66]. In addition, *Shh* derived from the midline epithelial cells is able to activate *Bmp2* expression in the mesenchyme, which in turn stimulates cell proliferation. Thus, *Msx1* controls a genetic hierarchy involving BMP and *Shh* signals regulating the growth of the anterior palate during development.

The role of *Msx1* in the palate has been further analyzed by generating double (*Msx1^{-/-};Dlx5^{-/-}*) conventional knockout mice [67, 83]. Inactivation of *Dlx5* significantly stimulates palatal mesenchymal proliferation in the background of *Msx1*-null mutation, although proliferation activity is not altered in *Dlx5*-null single mutants [67]. Rescue of cell proliferation is linked with a complete rescue of the *Msx1^{-/-}* cleft palate defect in *Msx1^{-/-};Dlx5^{-/-}* mutant mice. The fact that *Dlx5* expression is not compromised in *Msx1*-null palates suggests an indirect relationship between these two genes in palatogenesis as well as a complex mechanism leading to the rescue of *Msx1*-null phenotype. Interestingly, in *Dlx5*-null embryos, the oral side of the palatal shelves is expanded. This finding is based on the expansion of *Shh* expression into the midline epithelium. This is an expansion, not a shift, as the oral side *Shh* expression persists. The change in *Shh* expression in *Dlx5*-null mice is accompanied by an expansion of *Gli1* in the palatal mesenchyme from the oral region into the nasal portion and a reduction of nasal markers such as Phospho-Smad1/5/8 and *Fgf7* [67]. Previous studies have shown that increased *Shh* signaling has a stimulatory role on cell proliferation in the palatal mesenchyme [66]. Accordingly, *Shh* expansion is

responsible for the rescue of cell proliferation in the *Msx1*^{-/-};*Dlx5*^{-/-} palates [67]. Moreover, *Dlx5* is required for *Fgf7* expression and FGF7 inhibits the expression of *Shh* in the palatal epithelium.

Regarding BMP modulators, previous studies have shown that loss of Noggin function in palatal epithelial cells leads to overactive BMP signaling, particularly in the palatal epithelium. This results in alteration of cell proliferation and excessive cell death leading to complete CP. The integrity of the palatal epithelium is compromised due to the excessive cell death, which in turn leads to abnormal palate-mandible fusion and blocks palatal shelf reorientation. This phenotype is also observed in ectopic expression of a constitutively active form of BMPR-IA but not BMPR-IB in the palatal epithelium [84]. These findings underscore the importance of modulation of BMP signaling during palatogenesis.

BMP and Orofacial Clefts in Humans

Suzuki et al. [22] have identified missense and nonsense mutations in the BMP4 gene in 1 of 30 cases of microform clefts, 2 of 87 cases with subepithelial defects in the orbicularis oris muscle, 5 of 968 cases of overt CLP, and 0 of 529 controls. These results provide confirmation that microforms and subepithelial orbicularis oris muscle defects are part of the spectrum of CLP. Heterozygous variations in BMP7, including a frame shift, missense, or Kozak sequence mutation, in individuals with developmental eye anomalies and a range of systemic abnormalities, including developmental delay, deafness, scoliosis, and CP have also been reported [85]. Historically, *MSX1* has been the subject of human geneticists since at least the early 1990s. In 1997, Lidral et al. [86] proposed a possible role for *MSX1* in non-syndromic CLP but they did not detect disease-linked mutations. In addition, some studies have supported an interaction between environmental factors and *MSX1* [21]. For instance, the risk of CLP and CP related to maternal cigarette smoking and alcohol consumption during pregnancy, increases due to the interaction of such exposure and specific polymorphic variants at the *MSX1* gene [86]. Interestingly, *MSX1* mutation was observed in patients with tooth agenesis and CLP or CP only [21]. Since anodontia and hypodontia have been observed in patients with CP it may be possible that *MSX1* correlates with a syndromic form of cleft including tooth agenesis. Thus, *MSX1* may be involved in syndromic and non-syndromic CLP and CP. Jezewski et al. [87] identified a mutation in the *MSX1* gene in patients with CLP in Filipinas. Suzuki et al. [88] and Vieira et al. [89] detected a missense mutation in the *MSX1* gene in Vietnamese and Chilean CLP patients, respectively. More recently, positive association between *MSX1* gene variants and non-syndromic CLP was found in South Indian patients [90], Malay population [91], Han Chinese in Western China [92], Estonian population [93] and Colombian populations [94], among multiple studies in diverse populations.

Concluding Remarks

Numerous studies in animal models and humans have shown that BMP signals exert diverse and crucial functions during craniofacial development and particularly during palatogenesis. More research is needed to elucidate specifically how BMP signaling regulates different cellular activities, what downstream targets are activated and how it interacts with other signaling pathways during lip and palate development. This information will provide us with a comprehensive understanding of this pathway and with the opportunity of therapeutic approaches for malformations of the lip and palate in humans.

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