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FACTORS AFFECTING FACIAL DEVELOPMENT AND FORMATION OF CLEFT LIP AND PALATE: A LITERATURE REVIEW

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ABSTRACT

The craniofacial region forms in a complicated developmental process regulated by multiple genes and growth factors. Disruption and dysregulation during facial development can lead to multiple congenital facial anomalies including cleft lip and palate. This literature review collects and analyses the existing information about the interaction of multiple growth factors and genes within the developing facial region and their association with facial pathology. The factors analysed in this review are *DLX4, FOXE1, HOXB3, MSX2, PAX7, PAX9, RYK, SHH, SOX3, WNT3A, WNT9B* and *BARX1*.

Keywords: *cleft lip; cleft palate; genes; growth factors*

INTRODUCTION

Development of the face is a complex process which involves a delicate balance between the action of genes that regulate the formation of the facial structures as well as the interaction of growth and developmental factors necessary for correct differentiation and maturation of the craniofacial region. If this balance is disrupted during the embryonic development, it can lead to multiple craniofacial anomalies, including cleft lip and palate.

This literature review is focused on analysing and collecting the existing information about the interaction of multiple genes and growth factors within the developing facial region. Some of these factors have been associated with craniofacial development abnormalities, but others have not been studied in such great detail regarding the formation of the facial pathologies. The

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literature review was conducted with PubMED (Medline), Google Scholar and ClinicalKey databases using different keywords: genes, gene proteins. By eliminating duplicates and checking article compatibility with the topic, 69 articles were selected and analysed. Data were collected from the last 30 years. The last database search was done on 3 July 2020.

DLX4

The distal-less homeobox (*DLX*) genes are homeodomain-containing transcription factors that are divided into three bi-gene *DLX* clusters. Each cluster contains two closely located gene pairs (*Dlx1*/*Dlx2*, *Dlx3*/*Dlx4*, *Dlx5*/*Dlx6*) that can be convergently transcribed and have been detected in both mice and humans [1]. The subsequent gene products DLX2, DLX3 and DLX4 proteins show high similarity in the structure of their homeodomains and composition of surrounding amino acids [2]. *DLX* genes belong to the homeobox gene super-family. Homeobox genes are expressed at specific time intervals and in specific regions during the embryonic development and control formation of the body axis and morphogenesis of all organ systems [3]. *Dlx* genes play an important role during the development in the process of neurogenesis and limb patterning. The function of *Dlx4* during the development is unclear [3]. In mice, all *Dlx* genes have a differential expression pattern in the branchial region during the process of embryogenesis [4]. Expression of murine *Dlx* genes has been described in the mesenchyme derived from neural crest cells within the first pharyngeal arch or jaw primordia. *Dlx1* and *Dlx2* genes are expressed within the precursor of the upper jaw – the maxillary arch, but *Dlx3*-*Dlx6* are expressed within the precursor of the lower jaw – the mandibular arch [5]. Embryonic expression of *DLX4* has not been well studied in humans, and Dlx4 expression is absent in most adult tissues [6]. A *DLX4* sequence variant (mutation c.546_546delG, predicting p.Gln183Argfs*57) has been reported to be linked to the formation of cleft lip and palate where the pathological *DLX4* variant produced bilateral cleft lip and palate in a mother and her child [1].

FOXE1

Forkhead box protein E1 (FOXE1) is a member of a transcription factor family that contains a DNA-binding forkhead domain and is involved in embryonic pattern formation [6]. Multiple FOXE1 mutations have been linked with the development of the cleft lip/palate [7]. FOXE1 is essential for proper MSX1 and TGFβ3 expression in the developing palate [8]. The proposed function of

FOXE1 is regulation of chondrogenesis [9]. *FOXE1* gene is expressed at the point of fusion between maxillary and nasal processes during palatogenesis [7]. *FOXE1* is expressed in the secondary palate epithelium of both mice [6] and human embryos at week 11 [10]. Newborn mice null for *FoxE1* exhibit cleft palate and thyroid anomalies [6]. *FOXE1* is expressed not only in the oral epithelium but also in the heart and thyroid [11]. Homozygous mutations of *FOXE1* cause the Bamforth–Lazarus syndrome characterized by cleft palate, choanal atresia, bifid epiglottis, thyroid agenesis or dysgenesis, hypothyroidism and spikey hair [12].

HOXB3

Homeobox genes are regulatory genes that encode transcription factors during embryogenesis and normal development in which they regulate cell differentiation and proliferation [13]. There are more than 20 subclasses of homeobox genes; the most notable among these is the homeobox (HOX) gene family that consists of 39 genes [14]. These genes are subdivided into four groups: A, B, C and D [15]. Homeobox B3 (*HOXB3*) plays a role in migration of neural crest stem cells and is important for correct formation of pharyngeal organs and structures derived from the third and fourth pharyngeal arch pouches (thymus, parathyroid glands). *HOXB3* together with *HOXA3* and *HOXD3* overlap in functions to regulate correct migration of the thymus and parathyroid glands during embryogenesis [16]. *HOXB3* has recently attracted attention as its altered expression has been observed in a variety of cancer types [17].

MSX2

Muscle segment homeobox gene 2 (*MSX2*) is a member of the family of divergent homeobox-containing genes. There are three different *Msx* genes in mice and two in humans. Homeobox-containing genes share a well-conserved sequence of 183 bp coding for a helix-loop-helix motif of 64 amino acids. Most homeobox genes are organized in clusters (*HOXA, B, C*, and *D* genes) that control the development of the trunk spatially and temporally. However, other homeobox genes, dispersed around the genome and classified as divergent homeogenes, also include the MSX family which is crucial for the development of the head [18]. *MSX1* and *MSX2* gene mutations cause different cleft lip and palate phenotypes – from cleft palate to bilateral cleft lip and palate [19, 20, 21]. *MSX2* is detectable in the orofacial skeleton: the mandibula and maxilla, Meckel's cartilage and teeth germs [22]. The mutation of the *MSX2* gene causes

Boston-type craniosynostosis in humans [22, 23]. *MSX2* is essential for proliferation of osteoblast progenitors during normal craniofacial development [22].

PAX7

Paired box 7 (PAX7) is a transcription factor that is involved in neural crest development by affecting the expression of neural crest markers Slug, SOX9, and SOX10 [25]. PAX7 expression has been detected in the palatal shelves, Meckel's cartilage, and in nasal structures like the nasal epithelium. Mice with mutant *Pax7* have development malformations of the maxilla and the nose [24, 27]. *PAX7* was previously associated with non-syndromic cleft lip/palate in four human populations in a candidate gene association study [24, 26]. The single SNP at 1p36 associated with cleft lip/palate is located in an intron in the *PAX7* gene (encoding paired box 7) [28]. *PAX7* is functionally involved in craniofacial development [27]. One study investigated seven *PAX7* variants in non-syndromic cleft lip/palate case-parent trios from multiple populations, and two *PAX7* variants showed a strong parent-of-origin effect [26].

PAX9

Paired box 9 (PAX9) belongs to the family of paired-box DNA-binding domain-containing transcription factors, which play key roles in organogenesis [29]. *Pax9* gene deletion is well known to induce cleft palate in mice [30]. The deletion causes defects in palatal shelf elevation and extracellular matrix changes (decrease in hyaluronic acid saturation), defective organ development from pharyngeal pouches and tooth development arrest at the bud stage [31, 32]. *Pax9* expression needs to be balanced and correctly timed for normal palate development – upregulation is seen during palatal vertical growth and elevation, and downregulation happens before the palatal shelves fuse together [33]. *PAX9* downstream affects *SHH* by promoting growth in anterior-posterior palate axis and rugae formation [34]. *PAX9* gene deletion causes a significant loss of BMP pathway signalling (BMP4, MSX1) and causes defects in WNT β-catenin-dependent pathway by the upregulation of genes *DKK1* and *DKK2*, which antagonize WNT signalling, and by the downregulation of *WNT7A*, *WNT3*, *WNT9B* [30,34,35]. PAX9-deficient mice die shortly after birth, exhibiting complete cleft palate [36].

RYK

Receptor-like Tyrosine Kinase (RYK) protein has an extracellular domain similar to WIF1 (WNT inhibitory factor 1), a transmembrane domain, and a kinase-dead tyrosine kinase domain, and it is able to bind to WNT5A protein [37, 38]. RYK is involved in multiple molecular events including heterodimerization with other receptor tyrosine kinases (RTKs) [39], activation of Src kinase [40], binding to frizzled (Fz) receptors [38]. WNT can induce the nuclear translocation of the RYK intracellular domain, which promotes neuronal differentiation [37, 41]. RYK is essential for normal development and formation of craniofacial structures like the secondary palate. Mice deficient in RYK have a specific craniofacial appearance, shortening of limbs and increased postnatal mortality caused by feeding and respiratory complications associated with a complete cleft of the secondary palate [39].

SHH

One of the signalling pathways involved in craniofacial development is the Hedgehog family, in particular, the Sonic hedgehog (*SHH*) and the Indian hedgehog (*IHH*) [42]. *IHH* is required for normal ossification and bone development of the craniofacial region [42, 43]. IHH null mice exhibit reduced expression of osteogenesis factors and decreased ossification process in the palate [44]. SHH is one of the most studied signalling pathways of lip and palate morphogenesis [45]. SHH is essential for craniofacial development, particularly the palate and frontonasal development, and is predominantly found at sites of epithelial-mesenchymal interactions, inducing mesenchymal cell proliferation [46, 47, 48, 49]. SHH is required for mesenchymal cell survival in early stages of development and for cell proliferation in later stages [19, 49]. Before the palatal shelve elevation and fusion, SHH is found in the oral side of the palatal epithelium, afterwards only in spots of thickened epithelium (rugae) [43, 50]. The enhanced SHH signalling might have a restrictive role in WNT signalling by enhancing WNT antagonist signalling [51]. SHH signalling plays an important role in fusion of facial processes and formation of the upper lip. Enhanced SHH signalling caused by mutated Patched1 during head development can lead to cleft lip with craniofacial abnormalities (hypertelorism) [51, 52].

SOX3

The SRY-Box Transcription Factor 3 (*SOX3*) gene found in the X-chromosome belongs to the SOXB1 (SOX1-3) subfamily of transcription factors [53, 54]. *SOX1*, *SOX2* and *SOX3* are expressed in neural progenitor cells where they help to sustain the undifferentiated state of progenitor cells and counteract the activity of proneural differentiation factors, which is important for the development of the neural tube and various placodes [55]. The *SOX2* gene is the closest relative of *SOX3* and is one of the main pluripotency factors involved in the regulation of stem cell activity and differentiation [54, 56]. *SOX3* is known as one of the earliest neural markers in vertebrates and is currently the most studied functional aspect of *SOX3* action [57]. In murine telencephalon, *Sox3* is expressed in neural stem/progenitor cells during embryonic development and is later downregulated during neuronal differentiation [58].

WNT3A

The Wingless-Type MMTV Integration Site Family (*WNT*) genes were discovered in 1982, and similar homologous genes have been reported in other organisms like mice (*Int* gene) and Drosophila (wingless gene) [59]. The WNT gene family encodes 19 different proteins, including WNT1, WNT2, WNT2b (WNT13), WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A (WNT14), WNT9B (WNT14B), WNT10A, WNT10B, WNT11, and WNT16. These proteins are characterized by being secretory glycoproteins that are rich in cysteine [59, 60]. WNT proteins can bind to cell surface receptors and are important in the process of autocrine and paracrine regulation through the WNT signalling pathway [59]. Wingless-Type MMTV Integration Site Family, Member 3A (*WNT3A*) together with other *WNT* genes play an important role in craniofacial morphogenesis, which has been studied in mouse models. *WNT3A* expression has been detected in the upper lip region, also in the primary and secondary palate, and they play a role in regional specification within the developing face of vertebrates [61]. WNT3A together with WNT11 and WNT8A mediate neural crest cell migration and differentiation within the pharyngeal/branchial arches, contributing to the formation of connective tissue and bone in the head and neck region [61]. *WNT* genes, including *WNT3A*, have been suggested as candidate genes for the development of the cleft lip and palate [61, 62].

WNT9B

Wingless-Type MMTV Integration Site Family, Member 9B (*WNT9B*), belongs to the *WNT* gene family. It has also been suggested as a potential candidate gene in the formation of the cleft lip/palate [61, 63]. Recessive knockout mutation of *WNT9B* (*WNT9B-/-*) gene in murine models showed the formation of the cleft lip with or without the cleft palate with incomplete penetrance [64]. *WNT9B* together with *WNT3* are located in the *clf1* region of chromosome 11, which has been associated with the cleft lip and palate [61, 65]. The WNT signalling pathway is essential for the proper development of the craniofacial region. Typically, the loss of function of *WNT* genes is associated with defects in the craniofacial region, including the cleft lip [65].

BARX1

BarH-like homeobox 1 (*BARX1*) is a homeobox gene expressed in ectomesenchymal cells of the developing mandibular and maxillar prominences and plays a role in the formation of pharyngeal osteochondrogenic condensation [66, 67, 68]. *BARX1* is also expressed in the anterior and posterior palatal shelves and is present in a restricted epithelial localization of the anterior domain [68]. *BARX1* mesenchymal expression seen in the posterior palate is complemented by the anterior expression of *MSX1* [69]. WNT3A can significantly increase the expression of *BARX1* by activating the WNT signalling pathway. Reduced *BARX1* expression can potentially cause defects in osteochondrogenic cell condensation and subsequently cause maxillary hypoplasia [66].

CONCLUSION

The amount of information about genes *HOXB3, SOX3, WNT9B* in the development of the craniofacial region is quite limited. More detailed information is available about *SHH* and *WNT3A* about their involvement in craniofacial development and pathogenesis of facial malformations. For genes *DLX4, FOXE1, PAX9, RYK*, and *WNT9B* most information about their functionality comes from morphological and genetical research of mice or other model animals, but the information from human studies is limited to genetical studies or specific case studies. This limits the possibility of accurate prediction of the formation of craniofacial pathologies in humans and causes difficulties in understanding the possible mechanisms of cleft and other facial anomaly formation in unclear or multietiological cases.

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