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Cellular and developmental basis of orofacial clefts

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Abstract

During craniofacial development, defective growth and fusion of the upper lip and/or palate can cause orofacial clefts (OFCs), which are among the most common structural birth defects in humans. The developmental basis of OFCs includes morphogenesis of the upper lip, primary palate, secondary palate, and other orofacial structures, each of which consisting of diverse cell types originating from all three germ layers: the ectoderm, mesoderm, and endoderm. Cranial neural crest cells and orofacial epithelial cells are two major cell types that interact with various cell lineages and play key roles in orofacial development. The cellular basis of OFCs involves defective execution in any one or several of the following processes: neural crest induction, epithelial-mesenchymal transition, migration, proliferation, differentiation, apoptosis, primary cilia formation and its signaling transduction, epithelial seam formation and disappearance, periderm formation and peeling, convergence and extrusion of palatal epithelial seam cells, cell adhesion, cytoskeleton dynamics, and extracellular matrix function. The latest cellular and developmental findings may provide a basis for better understanding of the underlying genetic, epigenetic, environmental, and molecular mechanisms of OFCs.

Keywords

Cleft lip/palate; Neural crest cells; Epithelial-mesenchymal transition; Primary cilia; Epithelial seam; Periderm; Convergence and extrusion; Cell adhesion; Cytoskeleton dynamics; Extracellular matrix

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA SHARING AND DATA ACCESSIBILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

1. Introduction

Orofacial clefts (OFCs) are among the most common structural birth defects and result from defective tissue fusion of the upper lip, palate (the roof of the mouth), or both, during craniofacial development (Mossey, Little, Munger, Dixon, & Shaw, 2009; Reynolds et al., 2019; Schutte & Murray, 1999). The incidence of OFCs varies with geographic factors, ethnic background, and socioeconomic status, averaging 1 in 700 newborns or a range of 0.5 – 2.6 per 1,000 live births (Carmichael, Nelson, Shaw, Wasserman, & Croen, 2003; Croen, Shaw, Wasserman, & Tolarova, 1998; Panamonta, Pradubwong, Panamonta, & Chowchuen, 2015; Vanderas, 1987). This translates to approximately 220,000 babies per year born with OFCs (Mossey et al., 2011).

The most common types of OFC are cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) (Figure 1). Children born with OFCs suffer severe feeding problems, speech difficulties, frequent middle ear infections, and dental defects (Mossey et al., 2009). Long-term multidisciplinary treatments for these afflictions are heavy medical, psychological, social, and economic burdens on OFC patients and their families (Cassell, Meyer, & Daniels, 2008; Christensen, Juel, Herskind, & Murray, 2004; Marcusson, Akerlind, & Paulin, 2001; Sischo, Wilson-Genderson, & Broder, 2017; Wehby & Cassell, 2010). Although vertebrate faces are highly diversified, the basic mechanism driving orofacial development is relatively conserved at the cellular and molecular levels (Chai & Maxson, 2006). This review summarizes current understanding of the main cellular and developmental mechanisms underlying OFCs in vertebrate models, which may provide a foundation for developing novel approaches to improve the quality of care and efficacy of treatments for OFC patients.

2. Embryogenetic basis of orofacial clefts

Midfacial morphogenesis primarily involves development of the upper lip and primary palate. It has been precisely described using scanning electron microscopy on embryonic faces of several vertebrates, including chicks, mice, and humans, to observe the structures at various developmental stages (Timothy C Cox, 2004; Gaare & Langman, 1977a, 1977b; Klaus Hinrichsen, 1985; Jiang, Bush, & Lidral, 2006; Millicovsky, Ambrose, & Johnston, 1982; Millicovsky & Johnston, 1981; O'rahilly, 1972; Senders, Peterson, Hendrickx, & Cukierski, 2003; D. Trasler & Ohannessian, 1983; D. G. Trasler, 1968; Yee & Abbott, 1978). Midfacial morphogenesis occurs from the fourth to the seventh week of human embryogenesis (around Carnegie Stages 10 to 18) (O'rahilly, 1972) or embryonic day (E) 8.5 to E12.5 in mice. Craniofacial development begins with the ventral migration of cranial neural crest cells (CNCCs) from the dorsal edge of the cephalic neural tube to the frontonasal process (FNP) and BA1 (Cordero et al., 2011; Le Lievre & Le Douarin, 1975). The first pharyngeal arch then gives rise to the paired mandibular prominences which develop into the lower jaw (Bush & Jiang, 2012; Chai et al., 1997). Meanwhile, the upper jaw is assembled by three paired orofacial primordia: the lateral nasal prominence (LNP), medial nasal prominence (MNP), and maxillary prominence (MxP) (Gerard F Couly, Coltey, & Le Douarin, 1992; Gérard F Couly, Coltey, & Le Douarin, 1993; Noden, 1978; Drew M Noden, 1983; Noden, 1988), as demonstrated in the mouse model (Figure 2) (L. Song et al.,

2009). Disrupting the developmental processes of the upper jaw may lead to OFCs; thus, this review will predominantly focus on the upper lip and the palate.

2.1. Morphogenesis of the upper lip and primary palate and clefts

By E9.5 of mouse embryogenesis, or stage 12 of human embryogenesis, the facial prominences consist of a symmetrical FNP, which is localized to the ventrolateral sides of the forebrain, and a pair of MxPs at the lateral side of the stomodeum (K. Hinrichsen, 1985). From stage 14 to 15, the FNP grows and bulges rapidly around the nasal placodes, resulting in the formation of the MNPs and LNPs (Abramyan, Thivichon-Prince, & Richman, 2015; K. Hinrichsen, 1985). Through the proliferation of the primordial cells, the MxPs also swell during this period (Bailey, Minkoff, & Koch, 1988; Minkoff & Kuntz, 1978). By stage 15 of human embryogenesis (E10.5 in mouse embryogenesis), the three paired prominences have enlarged sufficiently, and the fusion between the MNPs and the LNPs is initiated (Senders et al., 2003). By stage 16 (E11.0 in mouse embryogenesis), rapid mesenchymal growth pushes the distal ends of the MNPs, LNPs, and MxPs into direct contact, forming the junction point known as the lambdoid or lambdoidal junction (λ-junction) (Depew & Compagnucci, 2008; Tamarin & Boyde, 1977) (Figure 2B).

The union of the individual prominences into facial structures occurs mainly through two mechanisms. The first mechanism, fusion, is employed at the λ-junction between the tips of the MNPs, LNPs, and MxPs (Jiang et al., 2006). During fusion, an epithelial seam forms in this region. Subsequently, the epithelial seam degrades to allow for a continuous mesenchyme across the prominences (Abramyan & Richman, 2015). The second mechanism, merging, takes place at the nasolacrimal groove between the MxPs and the LNPs, and between the two MNPs during midfacial formation (T. C. Cox, 2004). During merging, a deep groove between the involved prominences fills with mesenchymal cells, creating a smooth external surface over the face. By E12.5 of mouse embryogenesis and stage 19 of human embryogenesis, the formation of the upper lip is complete with a confluent mesenchyme between the MNPs, LNPs, and the MxPs (Jiang et al., 2006).

The intermaxillary segment, which is derived from the distal part of MNPs, continuously grows into the oral cavity and generates the anterior part of the palate (K. Hinrichsen, 1985). This anterior section is termed the “primary palate.” Later, the primary palate fuses with the MxP-derived secondary palate. Failed union of the MNPs, LNPs, and MxPs causes cleft lip. Because lip formation precedes the fusion of the two secondary palatal shelves to each other and to the primary palate, failure to properly construct the upper lip can affect the proper contact of the palatal structures. Therefore, cleft lip and cleft palate have a high co-morbidity. Although CL/P is the most common OFC, the cellular basis of upper lip development is not fully elucidated as there are limited usable models for upper lip fusion.

2.2. Secondary palatogenesis and cleft palate

From mid to late gestation, the MxPs develop into paired palatal shelves which eventually grow toward the midline of the oral cavity and form a continuous secondary palate posterior to the primary palate, in a process that is similar across all mammalian species. In humans, the development of the secondary palate spans from the end of the sixth week post-

conception through ninth week during which an intact palate is formed (Burdi & Faist, 1967). In mice, the development of the secondary palate is initiated at approximately E11.5. Around E12.5, the palatal shelves grow vertically downward on either side of the tongue before orthogonally shifting their direction and elevating on both sides of the tongue (E13.5) (Figure 3A). At E14.5, palatal shelves have achieved a horizontal position above the tongue, and the palatal shelves continue growing toward the midline at which the two shelves cohere (Figure 3B). The transient midline epithelial seam (MES) is formed around E15 (Figure 3C) and is eliminated once the two palatal shelves have fused (Bush & Jiang, 2012). At E16.5, palatogenesis is considered complete (Figure 3D).

Palatogenesis is a complex operation involving many cellular processes, including mesenchymal and epithelial cell proliferation, epithelial-to-mesenchymal transition (EMT), cell migration, apoptosis, signaling transduction through primary cilia, and interaction with the extracellular matrix (L. Yu et al., 2005; Z. Zhang et al., 2002). Any disruptions in these processes can cause cleft palate (CP) (Figure 1F).

3. Neural crest cells and orofacial clefts

Neural crest cells are a group of multipotent cells induced at the dorsal edge of the neural tube (Ji, Hao, Reynolds, McMahon, & Zhou, 2019). The cranial neural crest cells (CNCCs) generated from the cephalic neural tube migrate long distances into the frontonasal prominences and branchial arches to form a diverse cell lineage, including orofacial bones, cartilages, connective tissues, melanocytes, and facial nerve cells (Cordero et al., 2011; Knight & Schilling, 2006; Lumsden, Sprawson, & Graham, 1991). During development, signals from the local microenvironment regulate the proliferation and differentiation of CNCCs in branchial or pharyngeal arch 1 (BA1 or PA1) (Graham, 2001). Defective CNCC development may cause syndromic orofacial clefts. For example, cell-intrinsic defects causing CNCC disorders (neurocristopathies) can lead to Treacher Collins Syndrome (OMIM 154500), often presenting with cleft palate (Jones et al., 2008). Signaling aberrations from the pharyngeal environment may cause DiGeorge Syndrome (OMIM 188400) (Piotrowski et al., 2003) and Van der Woude Syndrome (OMIM 119300) (Kondo et al., 2002), which are also associated with OFCs. Therefore, it is crucial to understand the mechanisms that guide CNCCs to their correct target regions and the signals that induce them to differentiate into craniofacial structures with the proper shape and function. The development of CNCCs includes induction, delamination, migration, and differentiation (Ji et al., 2019).

3.1. Neural crest induction

Neural crest cells are induced at the interface between the neural plate and non-neural ectoderm, a region known as neural plate border (NPB) (Ji et al., 2019). The morphogenetic Wnt molecules expressed in the NPB, dorsal neural tube, and the paraxial mesoderm, have been shown to induce NCC formation (Ji et al., 2019). Studies in frog and fish models show that bone morphogenetic protein (BMP) signaling also generates its concentration gradient, which is required for NCC formation, within the dorsal neural plate (Mayor, Morgan, & Sargent, 1995; Morgan & Sargent, 1997). Moreover, fibroblast growth factor (FGF)

signaling from the paraxial mesoderm can also induce the formation of NCCs in *Xenopus* (Hong, Park, & Saint-Jeannet, 2008; Monsoro-Burq, Fletcher, & Harland, 2003). Because the frog and fish models corroborate the evidence that Wnt, Bmp, and Fgf signaling are essential to NCC inductive regulation, the same processes were examined in a murine model to determine consistency throughout amniotes. In mouse embryos, the Wnt, Bmp, and Fgf signaling pathways regulate the survival and fate determination of NCCs (Crane & Trainor, 2006); however, the specific roles of these signals in mammalian NCC induction is less understood than in its amphibian and fish counterparts.

3.2. EMT and CNCC migration

After induction, NCCs undergo an epithelial to mesenchymal transition (EMT) and delaminate from the neural tube. Unlike trunk NCCs that undergo EMT individually, CNCCs emigrate collectively from the dorsal neural tube in mice (Nichols, 1981). During delamination, NCCs adjust their intercellular adhesion and obtain migration ability (Ji et al., 2019). Several signaling molecules have been shown to regulate NCC motility, such as Rho, cadherins, Wnt, Bmp, Fgf, and Yap (Chalpe, Prasad, Henke, & Paulson, 2010; Clay & Halloran, 2011; De Calisto, Araya, Marchant, Riaz, & Mayor, 2005; Kumar, Nitzan, & Kalcheim, 2019; Martinez-Morales et al., 2011). Rather than randomly migrate, CNCCs follow segregated migration streams into the FNP, BA1, and BA2 (Lumsden et al., 1991). The proper positioning of CNCCs is believed to be determined by *Hox* gene activity and influenced by the specific rhombomeres from which the CNCCs had arisen. For instance, CNCCs that originate from rhombomeres 2 and 3 of the hindbrain and lack *Hox* gene expression will migrate to BA1 (Lumsden et al., 1991).

3.3. CNCC proliferation

After arrival in the orofacial primordia, CNCCs continue oriented cell divisions and organized relocations of cell groups to extend and shape the face (Kaucka et al., 2016). Disrupted regulation of CNCC proliferation causes syndromes with severe craniofacial malformation, such as the aforementioned neurocristopathy Treacher Collins syndrome (TCS, OMIM 154500) (Sakai & Trainor, 2009). TCS presents phenotypically when *TCOF1*, which encodes a nuclear protein that regulates rDNA transcription, is mutated (Sakai & Trainor, 2009). Studies in murine models show that a *Tcof1* mutation causes a significant decrease in CNCC population due to decreased CNCC proliferation as well as increased apoptosis (Dixon et al., 2006).

Many signaling pathways have been shown to be involved in the regulation of CNCC proliferation, including Wnt and Shh, and disruption of such pathways causes craniofacial defects including OFCs, which are expanded upon in a companion review (Reynolds et al., in this issue) and others (Jeong, Mao, Tenzen, Kottmann, & McMahon, 2004; Ji et al., 2019). The transduction of Shh signaling depends heavily on structures within the primary cilia. Mutations in the components of the primary cilia can disrupt Shh signaling and result in craniofacial malformation. The structure and function of primary cilia will be discussed in section 6 of this review.

3.4. Cell lineage interactions during CNCC differentiation

CNCCs give rise to major craniofacial structures. Two notable theories have been proposed to describe the differentiation of CNCCs. The first theory suggests that CNCCs are pre-patterned. This would assume that CNCCs each contain a molecular “blueprint” for facial patterning, dictating the structure into which they will develop (D. M. Noden, 1983). The second theory suggests that CNCCs are plastic, responsive to intercellular communication. As this premise does not suppose predetermination, CNCCs must instead receive facial patterning information from the surrounding environment, the extracellular matrix and neighboring cells, and interaction between the CNCCs with those surrounding cells influences CNCC fate (del Barrio & Nieto, 2002).

In the orofacial primordia, CNCC-derived mesenchyme is covered by the epithelium and contains a cranial paraxial mesoderm (CPM) core with muscle precursor cells (Chai & Maxson, 2006; Graham, 2003). The coordinated development of the CNCC-derived facial skeleton and its associated CPM-derived musculature contributes to the formation of functional orofacial structures. Therefore, interactions between epithelial cells, CNCCs, and CPM cells are believed to be critical for craniofacial patterning and CNCC differentiation (Cordero et al., 2011). Disruption of these interactions can cause OFCs and other craniofacial malformations (Twigg & Wilkie, 2015). For example, in zebrafish, the facial epithelia and mesoderm secrete the peptide Endothelin 1 (Edn1), which regulates skeletal formation by CNCCs in BA1 (Kimmel, Miller, & Moens, 2001; Miller, Schilling, Lee, Parker, & Kimmel, 2000). A loss-of-function mutation in *Edn1* impairs proper bone development, causing mandibular abnormalities in mice (Kurihara et al., 1994). Studies in mouse and chick embryos demonstrate that CNCCs also generate signals to regulate the differentiation of the CPM into myoblast precursors (Grenier, Teillet, Grifone, Kelly, & Duprez, 2009; Rinon et al., 2007).

3.5. CNCC defects as the cause of orofacial clefts

Although CNCCs generate many cell and tissue types, they only exist transiently during embryonic development. Therefore, it is crucial that embryos produce and maintain enough CNCCs to migrate and differentiate into the craniofacial complex (Trainor, 2010). Disruption of the formation, migration, proliferation, or survival of CNCCs results in fewer CNCCs in their final destinations, which can cause various craniofacial anomalies including syndromic and non-syndromic OFCs (Dixon et al., 2006; Jones et al., 2008; Q. Wang et al., 2019). However, if only the differentiation of CNCCs is disrupted, a dysmorphic cranial shape may occur (Morriss-Kay & Wilkie, 2005).

Because development of the upper lip and the palate is dependent primarily on the growth of the orofacial primordia, attaining and maintaining the proper cell count is of utmost importance to ensure properly formed facial structures (W. Yu, Serrano, Miguel, Ruest, & Svoboda, 2009). Defective post-migratory CNCC proliferation or abnormal apoptotic frequency within the orofacial primordia may affect their morphology, potentially resulting in OFCs. For instance, Wnt signaling is essential for CNCC division within the maxillary prominences (Brugmann et al., 2007; Brugmann, Tapadia, & Helms, 2006). Embryos with a *Wnt9b* loss-of-function mutation exhibit cleft lip and palate, likely due to defective

mesenchymal proliferation leading to insufficient growth of the orofacial prominences (Y. R. Jin, Han, Taketo, & Yoon, 2012). Consider also that palatogenesis relies on the growth of the palatal shelves, which is primarily driven by oriented cell divisions and organized relocation of CNCCs (Jones & Trainor, 2005; Kaucka et al., 2016). In this case, excessive apoptosis of CNCCs may prevent the palatal shelves from achieving contact at the midline, thus leading to a cleft palate (Alappat et al., 2005; Dixon et al., 2006; Dudas et al., 2006; Jeong et al., 2004; Suwa et al., 2001; Wilson et al., 2016).

In summary, defective CNCC development causes disruptions in the outgrowth of the facial prominences, resulting in OFCs. In fact, all craniofacial defects are caused either by cell-intrinsic defects, which can affect CNCCs, or signaling disorders within the orofacial primordia, leading to disruptions in CNCC development (Dworkin, Boglev, Owens, & Goldie, 2016). Therefore, understanding the mechanisms that regulate the development of CNCCs is essential for designing potential treatments and therapies of OFCs and related disorders. Currently, much of the gathered knowledge regarding craniofacial development comes from genetically engineered mouse models. Recently developed single-cell RNA sequencing technology allows us to reveal the cell lineage, track cell fate determination, as well as understand the molecular state of developmental processes from a wholly unique perspective at a level of precision that was before impossible. The exploitation of new techniques combined with genetically engineered mouse models will hopefully provide a detailed description of CNCC development at a single-cell resolution and suggest a possible treatment for craniofacial malformations.

4. Midfacial epithelial cells and cleft lip

Following the rapid expansion of neural crest-derived mesenchymal cells, the midfacial primordia extend tri-dimensionally, according to genetic predetermination, and communicate via superficial epithelial cells (Miyake, Cameron, & Hall, 1996). These epithelial cells fuse between the primordia in different directions depending on the structures involved: “upward” between a MNP and a LNP, “downward” between a MNP and a MxP, and “outward” between a LNP and a MxP. These directions are relative to the site at which all three primordia establish contact, identified as the lambdoidal junction (Jiang et al., 2006) (Figure 2B). Disruption of the fusion between a MxP and a MNP (Ashique, Fu, & Richman, 2002; D. Sun, Baur, & Hay, 2000) or between the MNP and LNP (Timothy C Cox, 2004; Gong & Guo, 2003; D. G. Trasler, 1968) can cause the characteristic bilateral or unilateral cleft lip (Figure 1). In rare occasions, median cleft lip and bifid nose can also occur in humans with undetermined causes (Eppley, van Aalst, Robey, Havlik, & Sadove, 2005).

4.1. Epithelial seam formation during the upper lip fusion

Before lip fusion, the epithelium that covers midfacial prominences (LNPs, MNPs, and MxPs) is formed by bilayer basal cells; the outer layer of these basal cells subsequently differentiates into flattened (those outside from the fusion zone) or rounded (those within the fusion zone) peridermal cells during lip fusion (Li, Jones, Hooper, & Williams, 2019; Millicovsky et al., 1982; Millicovsky & Johnston, 1981). After lip fusion, the epithelial seam between the prominences breaks down and is replaced by mesenchymal tissue (Gaare &

Langman, 1977a; Jiang et al., 2006; D. Sun et al., 2000; D. G. Trasler, 1968; K. Y. Wang, Juriloff, & Diewert, 1995). However, compared to the degeneration of the medial epithelial seam during palatal fusion, the mechanisms that drive the breakdown of the lip epithelial seams remain poorly understood. Nevertheless, as will be elaborated in a later section, it is possible that both programmed cell death (apoptosis) and epithelial-mesenchymal transition (EMT) are involved in this process.

4.2. Epithelial apoptosis during lip fusion

In 1977, Gaare and Langman observed cellular degeneration within the epithelial seam between the MNP and LNP during normal upper lip fusion in mice (Gaare & Langman, 1977b). They referred to these regions of fusing epithelial cells as “cell-death zones,” explicitly conveying a prominent trait of the area. Further experiments on cell proliferation and programmed cell death demonstrated that the constituents of the “cell-death zones” are indeed non-proliferative and are actively undergoing apoptosis (Figure 4) (Ferretti et al., 2011; Jiang et al., 2006; L. Song et al., 2009). These data indicate that apoptosis may play an essential role in lip formation, and disruption in its regulation, both positive and negative, can result in OFCs.

Deficient apoptosis in the upper lip region may prevent the desired continuous mesenchyme across the MNPs and LNPs from developing, thus causing a cleft lip. Immunostaining for activated Caspase-3 and in situ hybridization for *p63* in a wild type mouse model displayed apoptotic orofacial epithelial cells both prior to and during fusion (Ferretti et al., 2011; Jiang et al., 2006). This evidence suggests programmed cell death is an essential process required for degeneration of the epithelial seam. Furthermore, it is possible that apoptosis may also be responsible for removing peridermal cells, described in the previous section, from the surfaces of the midfacial prominences.

Nevertheless, excessive apoptosis is equally problematic. In 2011, Ferretti et al. proposed a Pbx regulatory network that controls the apoptosis of epithelial cells at the λ junction (Ferretti et al., 2011). *Pbx* genes, which encode TALE homeodomain-containing transcription factors, have been established as Hox cofactors during development (Bürglin, 1998; Tümpel, Wiedemann, & Krumlauf, 2009). Previous studies in murine models demonstrated that disrupting the interaction between Hox proteins and Pbx1 can induce apoptosis in many cancer cells, suggesting a strong link between the genes on which cell survival depends (Morgan, El-Tanani, Hunter, Harrington, & Pandha, 2017; Morgan, Plowright, Harrington, Michael, & Pandha, 2010; Shears, Plowright, Harrington, Pandha, & Morgan, 2008). During normal lip fusion, *Pbx1*, *Pbx2*, and *Pbx3* are highly expressed at the λ junction. Inactivating *Pbx* genes in the surface cephalic ectoderm using Foxg1-Cre recombination is sufficient to cause cleft lip as it ruptures any communication dependent on Pbx, thus increasing the rate of apoptosis.

4.3. EMT during lip fusion

After lip fusion, all epithelial cells are removed from the seam along the midfacial primordia, allowing the mesenchyme to connect at the λ junction. Although the majority of epithelial seam cells are removed through apoptosis, some of them appear to remain healthy

(Gaare & Langman, 1977a). Sun et al. examined apoptosis during lip fusion in chick embryos using a TUNEL assay and found very few TUNEL-positive epithelial seam cells in the chick model. This indicates that among the cells in the fusing epithelium, a small minority maintain function while the rest die (D. Sun et al., 2000). To observe this phenomenon, they labeled the surface epithelial cells with a lipophilic dye during lip fusion. After the breakdown of the epithelial seam, the labeled cells were found in the mesenchymal tissue between the MNPs and MxPs (D. Sun et al., 2000). Based on this evidence, Sun et al. proposed EMT as a model to remove epithelial seam during lip fusion in chicks. However, there are still many questions about the final fate of epithelial seam cells. For example, after these cells transdifferentiate into mesenchyme, do they contribute to facial structures or die after EMT?

Recently, the temporal sequence of EMT and apoptosis during upper lip fusion in mice has been revealed (Losa et al., 2018). Before orofacial prominences fuse, apoptosis is already detectable by E10.5 in mice. A subpopulation of epithelial seam cells has been found to express epithelial and mesenchymal markers, indicating that they are transitional cells that undergo EMT (Losa et al., 2018). By E11.5, epithelial cell descendants can be found in the λ junction mesenchyme. The EMT is regulated by a Pbx-Snail1/Smad-E-cadherin pathway, which potentially interacts with Pbx-regulated apoptosis (Losa et al., 2018). Thus, a Pbx-directed gene regulatory network may mediate the dynamic cellular behaviors, therefore controlling the breakdown of the epithelial seam at the λ junction for lip fusion.

In summary, all epithelial cells must be removed from the λ junction during normal lip fusion, and various studies corroborate that this cell removal is executed through epithelial cell apoptosis and EMT. As discussed, these processes rely on two Pbx-dependent signaling pathways in normal midfacial development, resulting in a temporal sequence of apoptosis and EMT. However, further investigation is required to determine how and whether these two pathways communicate and if they interact with other signaling pathways.

5. Palatal epithelial cells and cleft palate

The formation of the secondary palate only happens in amniote lineages. The secondary palate forms from the fusion of paired palatal shelves, which are developed from the outgrowth of the MxPs. During the fusion of palatal shelves, a medial epithelial seam (MES) forms and eventually disappears, allowing mesenchymal cells to form bridges and connect the paired palatal shelves. Many cellular behaviors have been shown to contribute to the fusion of secondary palate, including apoptosis, cell migration, cell extrusion, and EMT. Failure of these cellular processes results in cleft palate.

5.1. Medial epithelial seam (MES) formation during palatogenesis

When the palatal epithelium recognizes that the two palatal shelves have made contact at the midline, it adheres to the epithelium from the opposite shelf (Dudas, Li, Kim, Yang, & Kaartinen, 2007). MES then forms, eventually disappearing once palatal fusion is complete. Several studies report that midline epithelial dysfunction (MED) can prevent palatal fusion (Cecconi, Alvarez-Bolado, Meyer, Roth, & Gruss, 1998; Dudas, Nagy, Laping, Moustakas, & Kaartinen, 2004; Kaartinen, Cui, Heisterkamp, Groffen, & Shuler, 1997; Kaartinen et al.,

1995; Proetzel et al., 1995; Saito et al., 2005). Therefore, proper fate direction of MES cells is essential for palatogenesis. Like the upper lip primordia, the medial edge epithelia of the palatal shelves also contain two layers of cells, a basal layer of cuboidal cells and an outer layer of flat cells, the latter of which is called the periderm (Holtgrave & Stoltenburg-Didinger, 2002). The basal layer, which forms the MES, might not disappear through cell death but may instead transition into mesenchyme through EMT (Fitchett & Hay, 1989; J. Z. Jin & Ding, 2006). The fate of peridermal cells is more complicated. Using light- and electron-microscopy, peridermal cells have been observed gradually desquamating from the epithelial surface before the closure of the secondary palate (Schupbach & Schroeder, 1983; Waterman, Ross, & Meller, 1973). Some of the peridermal cells in the posterior portion of the palate, however, remain trapped within the MES during palatal fusion (Nawshad, LaGamba, & Hay, 2004; D. Sun et al., 2000). These trapped cells must eventually be removed through apoptosis (D. Sun et al., 2000) for if peridermal cells remain in the MES, the palatal shelves may fail to fuse and result in cleft palate (Cuervo & Covarrubias, 2004).

5.2. Periderm formation and peeling during palatogenesis

During early orofacial development, the immature ectoderm contains only a single layer of cuboidal, undifferentiated, proliferative epithelial cells covering the primitive oral cavity (R. J. Richardson et al., 2014). These cells undergo stratification and differentiation as the embryo develops to generate the mature epidermis (R. J. Richardson et al., 2014). The first stratification gives rise to the periderm, which covers the embryonic epithelia until shortly before birth (M'Boneko & Merker, 1988). Using a *Krt17* promoter-driven GFP reporter, Richardson et al. established that peridermal cells form in the secondary palatal shelves at E11.5 (R. J. Richardson et al., 2014). Several studies suggest that the periderm provides a protective barrier for oral cavity formation and prevents pathological oral epithelial adhesion. Therefore, failed peridermal formation can hinder the elevation of palatal shelves, causing cleft palate (Casey et al., 2006; Jiang et al., 1998; R. J. Richardson, Dixon, Jiang, & Dixon, 2009; R. J. Richardson et al., 2014). Consider *Jag2* and *Irf6* knockout mice, which both present disrupted peridermal differentiation. These mutants exhibit abnormal adhesion, such as palate-tongue, maxillary-mandibular, and/or intra-oral epithelial adhesions, which block palatal elevation and lead to the cleft palate phenotype (Casey et al., 2006; Ingraham et al., 2006; Jiang et al., 1998; R. J. Richardson et al., 2006).

Investigations on the differentiation of ectodermal cells into periderm suggest that Jag2-Notch1 signaling is an essential regulatory process (Lan, Xu, & Jiang, 2015). In *Jag2* knockout mice, the oral epithelium is able to undergo stratification; however, the suprabasal cells cannot differentiate into flattened peridermal cells as they should (Casey et al., 2006). Irf6 is also required for peridermal differentiation (R. J. Richardson et al., 2014). Mutations in the human *IRF6* gene present phenotypically as Van der Woude and popliteal pterygium syndromes (OMIM 119500), both of which exhibit OFCs (Kondo et al., 2002). Further studies have demonstrated that both Notch signaling and *Irf6* expression are regulated by the transcription factor p63 (*Tp63*) (Lan et al., 2015). Similar to lip fusion, *Tp63* is expressed in the basal layer of the orofacial epithelium and directly activates *Irf6* expression during early epithelial development (Casey et al., 2006; R. J. Richardson et al., 2014). *Jag2* has also been shown to be up-regulated by p63, suggesting that p63 triggers the Notch signaling cascade

(Casey et al., 2006; R. J. Richardson et al., 2014; Sasaki et al., 2002). Both Irf6 and Notch negatively regulate *Tp63*, reducing its expression during peridermal differentiation (Casey et al., 2006; Moretti et al., 2010; R. J. Richardson et al., 2014; Tadeu & Horsley, 2013). Consequently, *Tp23* is activated due to the low level of p63 in the suprabasal cells, driving the cell cycle exit and differentiation into flattened peridermal cells (Westfall, Mays, Sniezek, & Pietenpol, 2003; Yoshida et al., 2012).

Immediately before palatal fusion, the palatal peridermal cells are sloughed from the surface of the edge epithelia (Yoshida et al., 2012). Once the peridermal layer has been stripped, the two palatal shelves can initiate adhesion and fusion. Electron microscopy reveals that peridermal cells peel out from the palatal shelves away from the surfaces to be fused *in vivo* (Fitchett & Hay, 1989; Schupbach & Schroeder, 1983). The peridermal cells form multiple filopodia and lamellipodia just before two palatal shelves meet (Dudas et al., 2006; Takigawa & Shiota, 2004); however, the function of these protrusions is still not fully understood. Besides aiding peridermal peeling, it is possible that these protrusions are also involved in palatal adhesion or oral and nasal peridermal migration.

5.3. Cell adhesion during MES formation and fusion

Cell adhesion is critical to the initial steps of MES formation and fusion (Lough, Byrd, Spitzer, & Williams, 2017). As discussed, impaired adhesion can cause cleft palate. Unsurprisingly, mutations in the genes encoding adhesion molecules have been identified in humans with cleft palate (Lough et al., 2017), suggesting that cell adhesion is essential to proper palatal fusion. Many adhesion proteins, such as cadherins, nectins, claudin, occluding, vinculin, afadin, and desmoglein are expressed in the MES (Kim et al., 2015; Mogass, Bringas, & Shuler, 2000; R. Richardson et al., 2017; Tudela et al., 2002; Yoshida et al., 2012); however, the precise function of these proteins during palatal fusion remains unclear. One obstacle is the redundancy among protein family members, which complicates efforts to study individual adhesion proteins during palatal fusion (Lough et al., 2017). Within the larger family of cell-adhesion molecules (CAMs), are the cadherins (Gul, Hulpiau, Saeys, & van Roy, 2017), with E-cadherin (*Cdh1*) as the most widely expressed among these (Lough et al., 2017). *CDH1* mutations, including missense or in-frame deletion, have been identified in patients with OFCs from all over the world, such as in Northern Venezuelans, the Chinese Han population, and Caucasians (Frebourg et al., 2006; Y. Song & Zhang, 2011; Sozen et al., 2001). Immunofluorescent studies reveal adherens junctions in the MES (Kitase & Shuler, 2013; Tudela et al., 2002). However, knocking out *Cdh1* in epithelial cells with K14-Cre does not cause cleft palate (Andl et al., 2014). This may be because the K14-Cre used in the study expresses only in the basal layer and not in the periderm, in which proper adhesion dynamics are important (Vasioukhin, Degenstein, Wise, & Fuchs, 1999). It is also possible that P-cadherin, which is also expressed in the MES, is functionally redundant with E-cadherin, resulting in the persistent palatal fusion in the absence of *Cdh1* (Tinkle, Pasolli, Stokes, & Fuchs, 2008).

5.4. Cell migration during MES degeneration

In 1992, Carette and Ferguson proposed cell migration as a model for MES degeneration for the first time (Carette & Ferguson, 1992). In this model, during palatal fusion, individual

MES cells migrate to the oral and nasal sides of the palatal shelves. For more than twenty years, studies have tried to test this model by tracking the fate of MES cells by labeling MES cells genetically, using different promotor-driven Cre recombinases, or chemically, using fluorescent dyes (Aoyama et al., 2019; Griffith & Hay, 1992; J. Z. Jin & Ding, 2006; Kang & Svoboda, 2003; San Miguel et al., 2011). However, different studies produced contradictory data, suggesting that the cell migration model still needs experimental evaluation.

Studies have reported lateral migration of MES cells to the epithelial triangles on both the oral and nasal sides of the palatal shelves (Aoyama et al., 2019; Cuervo & Covarrubias, 2004). In these studies, MES cell migration leads to mesenchymal exposure. The specific direction of MES cell migration may be associated with the region of mesenchymal exposure, meaning the MES cells on the oral side of the exposed mesenchyme will migrate away from the area of exposure on the oral side of the palatal shelf, and the cells on the nasal side will also migrate farther on the nasal side. Contrary to these findings, Jin and Ding only observed lateral migration to the nasal side of the palatal shelves using K14-Cre to label MES cells (J. Z. Jin & Ding, 2006). These studies reveal an anterior-posterior migration of the MES cells from the middle palate to the posterior, which may be required for posterior palatal fusion (Aoyama et al., 2019; J. Z. Jin & Ding, 2006).

A recent study took a novel approach to observing MES cell movement during palatal fusion, using two-photon microscopy and ephrin-B2/GFP (Logan & Benson, 2020). Because *ephrin-B2* is consistently expressed in the epithelium during palatal fusion (San Miguel et al., 2011), ephrin-B2/GFP can be used to track MES cells in real-time. The authors observed a nasal to oral migration of MES cells. Moreover, instead of migrating individually, the cells maintain their positions relative to each other, suggesting collective migration.

The conflicting observations previously discussed may be caused by variations in culture conditions or experimental foci. However, it also suggests that a revised, consistent experimental system is needed to better understand MES migration during palatal fusion.

5.5. EMT during MES degeneration

Among the various theoretical models for MES degeneration in the field of OFC research, EMT is one of the most popular and well-studied (W. Yu, Ruest, & Svoboda, 2009). Evidence suggests that some epithelial cells become mesenchymal cells during palatal fusion. Transmission electron micrographs show that the transforming epithelial cells at the tip of the degrading MES break through the basement membrane by extending filopodia and pseudopodia (Griffith & Hay, 1992). Fluorescent dyes and genetic cell-lineage tracing studies provided more lines of evidence of EMT during palatal fusion. For example, mouse MES cells labeled with DiI (1, 1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindo-carbocyanine perchlorate) before palatal fusion are later found in the mesenchyme after the two palatal shelves fuse, indicating that MES cells undergo EMT (Shuler, Halpern, Guo, & Sank, 1992). K14-Cre and Shh-Cre labeled MES cells are also found in the mesenchyme distanced from the midline in E14.5 and E15.5 palates (J. Z. Jin & Ding, 2006; Vaziri Sani et al., 2005).

Transforming growth factor-beta (Tgfβ) has been shown to be essential for MES degeneration (Kaartinen et al., 1995). During palatal fusion, Tgfβ isoform 3 (Tgfβ3) is highly expressed in the medial edge epithelium (Nakajima et al., 2010). Tgfβ signals can induce both EMT and apoptosis in the same cell types (L. T. Yang & Kaartinen, 2007); however, Tgfβ only induces EMT at the G1 and S phases and not the G2 or M phases (Y. Yang, Pan, Lei, Wang, & Song, 2006). During palatal fusion, MES cells cease DNA synthesis before they are removed from the midline (Cui et al., 2003; Gehris & Greene, 1992). Moreover, *Tgfβ3*-mutated mice show increased MES cell proliferation (Cui et al., 2003). This evidence and many other studies suggest that Tgfβ signaling is associated with EMT during palatal fusion (Brunet, Sharpe, & Ferguson, 1995; Cui, Warburton, Zhao, Crowe, & Shuler, 1998; J. Z. Jin & Ding, 2006; Kaartinen et al., 1995; Martinez-Alvarez et al., 2000; Nawshad & Hay, 2003; D. Sun, Vanderburg, Odierna, & Hay, 1998). The downstream targets of Tgfβ signaling and their roles in EMT during palatal fusion have been recently reviewed by Nakajima et al. (Nakajima, C, Gulka, & Hanai, 2018). Tgfβ3 binding to its receptors results in phosphorylation of Smad2, which is also expressed during the fusion process, and activates the Smad-dependent signaling pathway (Iwata, Parada, & Chai, 2011). The Tgfβ signal also activates Smad-independent pathways, including the Erk, Jnk, and p38 MAPK pathways (Kang & Svoboda, 2002; Nawshad & Hay, 2003; Sorrentino et al., 2008; Xu et al., 2008). Moreover, the Tgfβ signaling pathway interacts with other previously mentioned vital signaling pathways, such as BMP, FGF, Ephrin, and Wnt, during palatal fusion (Nakajima et al., 2018).

5.6. Epithelial apoptosis and cleft palate

Apoptosis as a method of MES regression was first reported by Glucksmann as early as 1951 (Glucksmann, 1951) and has been experimentally supported by many others (Cecconi et al., 1998; Cuervo & Covarrubias, 2004; Cuervo, Valencia, Chandraratna, & Covarrubias, 2002; DeAngelis & Nalbandian, 1968; Holtgrave & Stoltenburg-Didinger, 2002; Martinez-Alvarez et al., 2000; Mori, Nakamura, Okamoto, Osawa, & Shiota, 1994; Taniguchi, Sato, & Uchiyama, 1995). Apoptosis during palatal fusion is characterized by cell cycle arrest, degradation of intracellular organelles, activation of caspase, and fragmentation of genome DNA (Ads, Piddington, Goldman, & Herold, 1983; Dudas et al., 2006; Greene & Pratt, 1976; Knott, Hartridge, Brown, Mansell, & Sandy, 2003). In fact, programmed cell death as a method of dissolving the MES has the most reliable experimental support compared to other methods such as EMT or MES cell migration.

Considering these results, it comes as no surprise that intrinsic apoptosis is critical for palatal fusion. Apoptosis is regulated by the highly conserved caspase family of proteases, which cleave many cellular substrates (Julien & Wells, 2017). Two apoptotic pathways that regulate caspase activity have been identified: the extrinsic and intrinsic pathways (Czabotar, Lessene, Strasser, & Adams, 2014; Green & Kroemer, 2004). Intrinsic apoptosis is induced by developmental cues and controlled by the BCL-2 family of proteins, which in turn activate the multi-BH domain-containing proteins BAX/BAK (Czabotar et al., 2014; Green & Kroemer, 2004). BOK is another highly conserved BH domain-containing protein in apoptotic signaling, downstream of BCL-2 (Yakovlev et al., 2004). A recent study showed that *Bok*−/−*Bax*−/−*Bak*−/− triple knockout mice lack developmental apoptosis, and 45% of

these triple knockout embryos present with complete cleft palate defects (Ke et al., 2018). This study clearly demonstrated that intrinsic apoptosis is required for palatal fusion. Moreover, these mutants exhibited additional midline fusion defects in the nasal septum, philtrum, and neural tube; however, the characteristic lateral cleft lip was not reported or observed in these mutants, suggesting that additional apoptotic molecules may be involved in the lateral lip fusion process (Ke et al., 2018).

Disrupting the timing of apoptosis can also block palatal fusion. The contact of palatal shelves at the midline triggers the programmed cell death in the anterior region of the MES in wild type mouse embryos (Cuervo et al., 2002). In the absence of contact, however, exogenous retinoic acid (RA) can increase apoptosis. Moreover, inhibiting RA signaling using retinol dehydrogenase or a retinoic acid receptor antagonist reduces apoptosis in the MES, which can be rescued with exogenous RA, suggesting that RA is an endogenous mediator of programmed cell death during palatal fusion (Cuervo et al., 2002). These data suggest that the timing of MES apoptosis must be tightly regulated by the contact of the palatal shelves.

5.7. Convergence and extrusion during palatal fusion

Using live imaging, genetic fate mapping, and tissue-specific gene ablation, Kim et al. recently proposed a new model for the formation and degeneration of a single-layered MES (Kim et al., 2015). Actomyosin contractility plays an essential role in this model, including convergence and cell intercalation during the formation of the MES and cell displacement and extrusion during MES degeneration. The initiation of palatal fusion begins once the palatal shelves establish contact with each other through cell protrusions, which transiently create epithelial bridges. These epithelial bridges form along the fusion front and eventually give rise to a multi-layered MES structure that completes the contact of two palatal shelves. Finally, the multi-layered epithelium converges toward the midline and resolves into a shared, single-layered MES. Cell intercalation and displacement of MES cells from a deeper position to the oronasal axis drive the multi-layered MES structure to converge toward the midline. As the two palatal shelves move to the midline, multicellular actin cables form along the apical edge of the palatal shelf. The force that drives the convergence of the MES and, eventually, MES breakdown is generated by actomyosin contractility. Two major kinases, rho-kinase (ROCK) and myosin light chain kinase (MLCK) phosphorylate the regulatory light chains (RLCs) to activate non-muscle myosin IIA (NMIIA) and regulate actomyosin contractility (Kim et al., 2015).

Live imaging reveals multiple cell extrusion events during the resolution of the multi-layered epithelium into a single-layered MES and MES breakage (Kim et al., 2015). In these events, multicellular rosettes form around a supracellular actin ring and squeeze the cells in the center of these rosettes out of the MES; however, the extruding cells do not undergo apoptosis. Previously, a study in a chicken model suggested that apoptosis may not be the primary mechanism for MES degeneration as an excess of dead cells would weaken the fusion site, and apoptosis only occurs among periderm cells (D. Sun et al., 2000). The cell extrusion model shows that at least during the early stages of MES degeneration, apoptosis is not critical to remove cells from the MES.

In conclusion, decades of studies of the fate of the MES show that the formation and removal of MES are essential for the normal formation of the secondary palate. Disrupting the complex cellular behavior during this developmental process leads to cleft palate. Currently, the major research goals are to reveal how the fate of epithelial cells is determined at the molecular level, and to decipher the signaling pathways involved in palate development.

6. Primary cilia and orofacial clefts

Ciliopathies are diseases that are caused by the disruption of structure or function of cilia. Ciliary dysfunction causes a wide range of symptoms, including orofacial clefts. This section briefly discusses the structure of primary cilium and the role it plays during normal midfacial formation.

6.1. Primary cilium

The primary cilium, also known as the sensory/immotile cilium, is an evolutionarily conserved and microtubule-based organelle that extends from the surface of a cell and interacts with the extracellular environment (Badano, Mitsuma, Beales, & Katsanis, 2006; Mirvis, Stearns, & James Nelson, 2018; Zaghloul & Brugmann, 2011). It senses chemicals and mechanical cues outside of the cell and plays critical roles in various signaling pathways that are essential for orofacial development, such as the Hh, Wnt, Tgfβ, and Pdgf pathways (Eggenschwiler & Anderson, 2007; Wheway, Nazlamova, & Hancock, 2018). Ciliary dysfunction leads to ciliopathies with a wide range of defects and syndromes. CNCCs with primary cilia are essential for the formation of the orofacial structures (Brugmann, Allen, et al., 2010; Tobin et al., 2008), and several ciliopathies exhibit OFCs and related craniofacial anomalies (Brugmann, Cordero, & Helms, 2010; Cortes, Metzis, & Wicking, 2015). In mice, knocking out the intraflagellar transport protein Kif3a in CNCCs causes truncation of the primary cilium and excessive Hedgehog (Hh) activity, resulting in enhanced CNCC proliferation and cleft of the secondary palate (Brugmann, Allen, et al., 2010). A study in zebrafish suggested that the protein BBS, which forms the Bardet-Biedl Syndrome (BBS) complex near the basal body of the primary cilium, modulates CNCC migration (Tobin et al., 2008). Therefore, it is essential to gain a better understanding of primary ciliary biology during craniofacial development.

The primary cilium contains three major components (Figure 5). The basal body is located at the base of cilia and anchors the cilium to the cell (Marshall, 2008). The axoneme is a microtubule-based structure that forms the core of the cilium (Mirvis et al., 2018). The ciliary membrane covers the axoneme, which contains membrane-bound receptors (Eggenschwiler & Anderson, 2007). The proteins that localize to each component are essential for ciliary assembly and function; therefore, they are the most closely associated with ciliopathies.

6.2. Basal body

The basal body is a cylinder consisting of nine microtubule triplets (Vertii, Hung, Hehnly, & Doxsey, 2016). It provides a template from which the nine doublet microtubules of the

axoneme can form. Therefore, the basal body is required as the nucleation point for ciliogenesis (Marshall, 2008). During ciliogenesis, the basal body attaches to the cell cortex and interacts with the plasma membrane (Keeling, Tsiokas, & Maskey, 2016; Lemullois, Boisvieux-Ulrich, Laine, Chailley, & Sandoz, 1988; Reiter, Blacque, & Leroux, 2012). Thereby, they bring the ciliogenesis site to the cell surface, attach the cilium at the cortex, and define the orientation of the axoneme (Marshall, 2008). Moreover, the transport of proteins into the cilium is also regulated by the basal body (Deane, Cole, Seeley, Diener, & Rosenbaum, 2001; Stephan, Vaughan, Shaw, Gull, & McKean, 2007). Given various roles the basal body plays in the cell, it reasoned that many proteins involved in basal body assembly correspond to ciliopathies. One example is the oral-facial-digital syndrome (OFDS). The symptoms of OFDS vary in a wide range; however, most of them involve OFCs, such as cleft lip and cleft palate (Franco & Thauvin-Robinet, 2016). The OFDS subtype I (OMIM 311200) is an X-linked disorder, and it is primarily caused by mutations in the *OFD1* gene (Coene et al., 2009; Franco & Thauvin-Robinet, 2016). The corresponding protein is localized at the basal body of primary cilia (Romio et al., 2003). Knocking out of *Ofd1* in mice results in loss of cilia on the surface of cells, indicating defective ciliogenesis in the mutants (Ferrante et al., 2006). Ofd1 may also be involved in the cilia-dependent Hedgehog signaling (Saitsu et al., 2016). Nevertheless, the role of *Ofd1* in orofacial clefts must still be investigated further.

6.3. Axoneme and IFT

The axoneme of the primary cilium contains nine symmetrically arranged microtubule doublets (also known as axoMTs) with the plus ends toward the cilium tip (Satir & Christensen, 2007; Satir, Pedersen, & Christensen, 2010). The 9+0 primary cilium can be found in many cell types in mammals, such as epithelial and endothelial cells (Cano, Sekine, & Hebrok, 2006; Nauli et al., 2008). Since the protein translation does not happen in the cilium, all the components of the cilium are transported by a specialized transport system known as intraflagellar transport (IFT) (Gerdes, Davis, & Katsanis, 2009). The plus end MT motor kinesin-2 (anterograde) and the minus end MT motor dynein (retrograde) mediate the bidirectional transport within the cilium (Ishikawa & Marshall, 2017). Defects in IFT often cause abnormalities in the structure or number of cilia, which potentially lead to developmental disorder (Cortes et al., 2015). Mutations have been found in genes encoding IFT proteins and motor proteins that can cause a subclass of ciliopathies, such as short-rib polydactyly syndrome (SRPS) (OMIM 613091) (Duran et al., 2017), which is characterized by a small rib cage, short limbs, and facial defects, typically cleft lip/palate (Turkmen et al., 2003). Genes responsible for SRPS include *IFT172*, *IFT140*, *NEK1*, *TCTN3*, *DYNC2H1*, *WDR60*, *WDR34*, *IFT80*, and *WDR35* (Cavalcanti et al., 2011; Dagoneau et al., 2009; Halbritter et al., 2013; McInerney-Leo et al., 2013; Mill et al., 2011; Perrault et al., 2012; Schmidts et al., 2013; Thiel et al., 2011; Thomas et al., 2012). Most of these are involved in retrograde IFT; however, it is unclear as to why retrograde IFT is primarily associated with this subclass of ciliopathies. One possibility is that defects in anterograde IFT may be lethal during early gestation (Cortes et al., 2015).

6.4. Transition zone

The transition zone is where the cargo and IFT particles form complexes to transport into the cilium (Deane et al., 2001). Defects in protein complexes localized at the transition zone are also associated with craniofacial ciliopathies, such as Meckel-Grüber syndrome (MKS) (OMIM 613885) (Norris & Grimes, 2012). MKS has the most severe facial defects among ciliopathies, including holoprosencephaly, sloping forehead, and cleft lip/palate (Barisic et al., 2015; Kheir et al., 2012). Genes such as *CEP290*, *MKS1*, *CC2D2A*, *TMEM67*, *TMEM216*, *B9D1*, and *TCTN2* are found to be mutated in MKS (Garcia-Gonzalo et al., 2011). These proteins are localized in one transition zone complex that controls cilia assembly and ciliary membrane components (Garcia-Gonzalo et al., 2011).

6.5. Ciliary membrane

The axoneme is covered by a highly specialized ciliary membrane adjoined to the plasma membrane (S. Sun, Fisher, Bowser, Pentecost, & Sui, 2019). The ciliary membrane also plays an essential role in cilia function. Because it is enriched with receptors and phosphoinositides, the ciliary membrane is essential for transducing extracellular signals (Benmerah, 2013; Hsu, Chuang, & Sung, 2017). The signaling molecules in the ciliary membrane are regulated and concentrated by the Bardet-Biedl Syndrome coat complex, which forms coated vesicles near the basal body (Baldari & Rosenbaum, 2010; H. Jin et al., 2010; Nachury et al., 2007). Thus, defects of the BBS protein complex impair the interaction between cytoplasmic protein and ciliary molecules and may cause craniofacial ciliopathies with OFCs.

6.6. Primary cilia and orofacial signaling transductions

Many transmembrane receptors have been found to localize in the ciliary membrane. A number of signals pertinent to cilia activity are essential for orofacial patterning and development, including Hh, Wnt, Fgf, and Pdgf. The best understood system is the Sonic hedgehog (Shh) signaling pathway, in particular the Smoothened (Smo) and Patched (Ptch1) receptors (Corbit et al., 2005; Rohatgi, Milenkovic, & Scott, 2007). In culture, Smo is not localized in the cilia of resting cells; however, it can be found in cilia when treated the cells with Hh (Corbit et al., 2005). The Gli transcription factors, downstream effectors of Hh signaling, are found enriched at the ciliary tip (Haycraft et al., 2005). One model suggests that without ligands, Smo is inhibited by Ptch, causing the inhibition of Gli by Sufu (suppressor of fused) proteins, a negative Hh signaling regulator enriched at the ciliary tip (Eggenschwiler & Anderson, 2007; Haycraft et al., 2005; Kogerman et al., 1999; C. Wu, Zhu, Liu, Ruan, & Tao, 2017). Consequently, the Hh signaling pathway is inhibited. On the other hand, Hh binds to Ptch, leading to the movement of Smo into cilia, where it forms the Smo-Sufu-Gli complex to activate Hh signaling pathway (Tukachinsky, Lopez, & Salic, 2010). The link between primary cilia and other orofacial developmental signaling pathways is not as clear as the Hh pathway. For example, there is no evidence indicating the presence of Wnt receptors in the cilia. Nevertheless, several studies showed that primary cilia interact with the Wnt signaling pathway by regulating the phosphorylation and degradation of Disheveled (Dvl) (Simons et al., 2005; Veeman, Slusarski, Kaykas, Louie, & Moon, 2003) or the degradation of β-catenin (Gerdes et al., 2007). During zebrafish and Xenopus

development, Fgf signaling has been shown to regulate the length of the primary cilium in diverse epithelial cells (Neugebauer, Amack, Peterson, Bisgrove, & Yost, 2009). In fibroblasts, the Pdgf receptor Pdgfrα is localized in the primary cilia and acts upstream of the Mek1/2-Erk1/2 pathways (Schneider et al., 2005). All of these signaling pathways play critical roles in orofacial development and clefts.

In conclusion, in addition to a sensory function, primary cilia also play essential roles during developmental processes. Disruption of the function of primary cilia affects midfacial formation and causes syndromic phenotypes. New technologies allow us to further investigate the biological function of cilia and ciliopathies. For example, gene function analysis combined with genome-wide copy number and sequence-based analyses could reveal novel genes whose dysfunction may contribute to ciliopathies.

7. Extracellular matrix and cytoskeleton dynamics in orofacial development and OFCs

Cellular function depends largely on the structural components of the extracellular matrix (ECM) and cytoskeleton. During development, the composition and dynamics of these systems control key processes, such as cell migration, differentiation, and tissue morphogenesis. Here, we briefly describe how the ECM and cytoskeleton systems contribute to midfacial development (mainly palatogenesis) and how disruption of these systems may lead to OFCs.

7.1. The extracellular matrix and cytoskeleton systems

The ECM is a three-dimensional mesh of proteins in the pericellular space (Theocharis, Skandalis, Gialeli, & Karamanos, 2016). It constitutes much of the connective tissue in animals and confers structural, adhesive, and signal transducing properties to cells during development and homeostasis (Meng, Bian, Torensma, & Von den Hoff, 2009; Ramirez, Sakai, Rifkin, & Dietz, 2007; Theocharis et al., 2016). In developing orofacial tissues, the major structural constituents of the ECM vary depending on cell type but usually include glycoproteins such as collagen, fibrillin, fibronectin, and laminin. Additionally, proteoglycans associated with certain glycosaminoglycans (GAGs) such as hyaluronan, chondroitin-4-sulfate, and heparan sulfate, may also be prevalent (Ferguson, 1988; Meng et al., 2009; Moxham, 2003). The ECM facilitates signal transduction through a variety of mechanisms including the presence of embedded signaling elements called matricellular proteins (Bornstein & Sage, 2002). Among matricellular proteins in the developing palate are tenascins and periostin (Chiquet, Blumer, Angelini, Mitsiadis, & Katsaros, 2016; Oka et al., 2012; Singh, Johnston, Ma, & Lozanoff, 1998).

In contrast to the mesh-like nature of the ECM, the cytoskeleton consists of filamentous networks found throughout the cytoplasm that serve as structural and signaling scaffolds (Fletcher & Mullins, 2010). Eukaryotic cells consist of three types of cytoskeletal networks: microfilaments, microtubules, and intermediate filaments. Microfilaments are composed primarily of actin subunits, while microtubules are composed of tubulin subunits. There are multiple types of intermediate filaments that may be composed of keratin, desmin, vimentin,

lamin, and other proteins (Traub, 2012). The cytoskeletal network mostly associated with OFCs is microfilament, which is the major force generating machinery of the cell (Logan, Ruest, Benson, & Svoboda, 2019; Svitkina, 2018). Along with microtubules, microfilaments form fibers that generate subcellular structures and link organelles, vesicles, and other components (Fletcher & Mullins, 2010). They also serve as tracks for motor proteins, such as myosins, which transport cellular cargo and modulate cellular tension. Microfilaments operate through a polymerization mechanism called treadmilling, a process by which actin subunits are added to the leading end and removed from the trailing end of the filament (Bugyi & Carlier, 2010). Using treadmilling, microfilaments generate protrusive (pushing) and contractile (pulling) forces, form subcellular structures such as filopodia/lamellipodia, and allow cells to migrate. Cell motility and vesicular trafficking by microfilament dynamics are controlled by signaling through the Rho family of small GTPases (Ridley, 2006).

Together, the ECM and cytoskeleton integrate communication between intra- and intercellular processes through mechanotransduction, which is the conversion of physical stimulus to biochemical or biological response (Paluch et al., 2015). The ECM and cytoskeleton are linked through transmembrane proteins called integrins, which are heterodimers that facilitate signal transduction between the two compartments (Z. Q. Sun, Guo, & Fassler, 2016). Integrins facilitate adhesion and signaling between cells, and the various heterodimer combinations serve as receptors for ECM-related proteins such as collagens, fibronectins, vitronectins, versican, osteopontin, tenascins, laminins, nephronectin, talin, and filamin (Barczyk, Carracedo, & Gullberg, 2010; Brandenberger et al., 2001; Denda, Reichardt, & Muller, 1998; Garcia-Alvarez et al., 2003; Kiema et al., 2006; Y. J. Wu, La Pierre, Wu, Yee, & Yang, 2005; Yokosaki et al., 1998). Current models for palatogenesis rely on tight spatiotemporal control over both ECM and cytoskeletal dynamics, and mutations of certain ECM and cytoskeletal genes are linked with human OFCs (Logan et al., 2019).

7.2. Extracellular matrix composition during orofacial morphogenesis

Aside from intermediate filaments, the compositions of which vary by tissue type, microfilaments and microtubules usually have a standard composition of actin and tubulin, respectively, across cell types. We will therefore focus primarily on ECM composition, which can vary greatly between tissues. Because many ECM-related proteins are glycosylated or have appended GAGs, studies have sought to determine the importance of these proteins in orofacial development. In developing rats, administration of the glycosylation-inhibiting agent tunicamycin on the nasal placode results in cleft lip (Eto, Figueroa, Tamura, & Pratt, 1981). Treatment of rats with 5-fluoro-2-deoxyuridine, which inhibits global GAG biosynthesis, induced cleft palate (Singh, Moxham, Langley, & Embery, 1997). Together, these studies demonstrate that glycoprotein and proteoglycan synthesis are indispensable for orofacial development in mammalian models. A recent review by Logan et al. (2019) listed 67 ECM-related genes that have been associated with human OFCs, among which are Collagen types II, IX, and XI (Logan et al., 2019). It is therefore important to understand the extracellular mechanisms that direct orofacial development and how alterations of these can lead to OFCs.

Cells secrete specialized ECM proteins to confer tissue-specific properties. For example, elastin contributes to elastic properties of the ECM in blood vessels, skin, tendons, *etc*. (Mithieux & Weiss, 2005). ECM proteins that confer adhesive properties include cadherins, integrins, nectins, and desmosomal proteins (Meng et al., 2009). Polymorphism of genes encoding Cadherin1 (Hozyasz et al., 2014) and Nectin1 (Suzuki et al., 2000) have been associated with OFCs. During embryogenesis or tissue regeneration, the ECM specializes to facilitate developmental processes such as growth factor signaling, tissue enlargement, and cell migration (Dutta & Dutta, 2010; Ramirez et al., 2007). In the palatal shelves, ECM proteins likely facilitate morphological changes by increasing shelf volume, directing force during elevation, serving as a scaffold for cytoskeletal contraction, and contributing to cell migration and tissue fusion (Logan et al., 2019). Secreted growth factors such as Tgf-α, Tgf-β3, and Bmp7 have been shown to control ECM protein expression during orofacial development (Kaartinen et al., 1997; Meng et al., 2009; Proetzel et al., 1995; Wyatt, Osborne, Stewart, & Ragge, 2010).

One role of the ECM is to define epithelial versus mesenchymal compartments of palatal shelves. The epithelial layer, consisting of a bilayer of cuboidal oral ectoderm below squamous periderm cells, is separated from the mesenchyme by the basement membrane (Logan et al., 2019). A major component of the basement membrane is the basal lamina, in which laminins, collagen type IV, perlecan, and nidogen are main constituents (Arends & Lieleg, 2016). This structural separation permits epithelial-mesenchymal interactions that facilitate all stages of palatogenesis. When palatal shelves are vertical, the epithelial ECM maintains characteristics of the basal lamina. The mesenchymal ECM at this stage is diverse, composed of collagen types I-III, fibronectin, hyaluronan, tenascins C and W, the proteoglycans decorin and biglycan, and the matricellular protein periostin. When palatal shelves are reoriented horizontally, epithelial ECM incorporates decorin and biglycan, while mesenchymal ECM incorporates fibrillin 2, a component of elastic microfibrils. For more specific details, Logan et al. (2019) provide a comprehensive outline for the matrix composition of epithelial and mesenchymal tissues at different stages of palatogenesis (Logan et al., 2019).

7.3. Extracellular matrix and cytoskeletal mechanisms of palatogenesis

The ECM and cytoskeleton play crucial roles in both cell structure and cell migration throughout all stages of palatogenesis. CNCCs migrate collectively in a process known as cell streaming. This process is ECM-dependent, and cells at the leading edge of the stream are guided by ECM-associated elements and diffusible factors such as C-X-C motif chemokine 12 (Cxcl12). Defining the exact roles of the ECM and cytoskeleton during palatal elevation and fusion has been controversial, but their importance for these processes remains unequivocal. During elevation, an intrinsic force is generated within the palatal tissue, and its source has been contested. Logan et al. (2019) proposed three major hypotheses as to the mechanism for palatal elevation, each with evidence from molecular observations and mechanistic studies (Logan et al., 2019). The first of these is the hydraulic lifting hypothesis, whereby intrinsic force is generated by the swelling of the palatal ECM. GAG components of proteoglycans, which are abundant in mesenchymal ECM, consist of anionic heteropolysaccharide structures that impart critical water-absorbing functionality.

Accumulation and subsequent hydration of GAGs in mesenchymal tissue is believed to drive tissue enlargement as well as the intrinsic force generated during palatal elevation and reorientation (Meng et al., 2009). This is particularly true for hyaluronan, which can absorb up to ten times its weight in water (Ferguson, 1988; Moxham, 2003). Notably, during rat palatogenesis, hyaluronan expression in palatal mesenchyme increases at the onset of palatal shelf elevation and decreases after elevation (Singh, Moxham, Langley, Waddington, & Embery, 1994). Exposure of rat organ culture to the hyaluronan-degrading enzyme hyaluronidase resulted in cleft palate, demonstrating the specific necessity of appropriate hyaluronan synthesis during palatal shelf elevation (Moxham, 2003). Other GAGs are also expressed in the mesenchyme, including chondroitin-4-sulfate (as chondroitin sulfate proteoglycans, or CSPGs) and heparan sulfate, but these do not undergo abundance changes during elevation (Singh et al., 1994). Nonetheless, their presence in mesenchymal ECM is likely important and may contribute to the matrix stiffness required for elevation. During vertical shelf development, one half of the volume increase is derived from cell proliferation, while the other half is derived from the swelling of hydrated GAGs (Brinkley & Bookstein, 1986; Ferguson, 1988).

Another possible basis for the intrinsic force is the contractility hypothesis, which favors cytoskeletal contraction of mesenchymal tissue *versus* differential expression of matrix proteins (Logan et al., 2019). In rodents, actin and myosin fibers in the mesenchyme are oriented in the direction of palatal remodeling, suggesting a relationship between contractile fibers and palatal shelf elevation (Lessard, Wee, & Zimmerman, 1974; Shah, 1979). Decades later, another study found a rapid shift in the location of matrix proteins during vertical-to-horizontal reorientation of the palatal shelves (Chiquet et al., 2016). Over the course of this day-long process, matrix proteins that are present in the medial edge mesenchyme of vertical shelves shift location to the medial portion of the horizontal shelves. Concurrently, matrix proteins on the medial edge epithelium (MEE) of the vertical shelves become ventrally localized in the horizontal shelves. These observations are consistent with a robust contraction of the mesenchyme, which may generate the intrinsic force observed during elevation. They also report that, during palatal shelf elevation, microfilaments reorient themselves in the direction of palatal extrusion, corroborating previous findings (Logan et al., 2019). In support of this, human cases of cleft palate have been associated with mutations in genes encoding microfilament proteins including β-actin (Di Donato et al., 2014; Verloes et al., 2015), and Filamins A (Robertson et al., 2003) and B (Bicknell et al., 2007), which branch actin filaments at wide angle (Kiema et al., 2006). Mutations in genes encoding cytoskeletal regulators such as Cask (Froyen et al., 2007), Cytospin A (Dasouki, Barr, Erickson, & Cox, 1988; Saadi et al., 2011), and possibly Rho GTPase activating protein 29 (Arhgap29) (Leslie et al., 2012), have also been associated with cleft palate. Often these mutations in cytoskeleton related genes are associated with syndromic OFCs.

Logan et al. (2019) posited a third hypothesis, collective cell migration, whereby a column of mesenchymal cells migrates along an ECM wall (Logan et al., 2019). In this model, all cells are held together by adherens junctions while cells on the surface of the column pull the rest along using integrin-associated focal adhesions. The cells at the leading edge of the column generate microfilament tension, which spreads down the column. They also spread

filopodia in search of migratory guidance factors. This model is like the cell streaming mechanism observed during the migration of the CNCCs toward the orofacial primordia.

7.4. Matrix enzymes in palatogenesis

A common requirement for each hypothesis is the formation of a rigid ECM— while the hydraulic lifting hypothesis uses rigidity to push and expand palatal tissue, the other two hypotheses require a rigid ECM for use as a scaffold either for contraction or migration (Logan et al., 2019). Tensile strength of the ECM is positively correlated with the mass average diameter of collagen fibrils (Khan et al., 2019; Parry, Barnes, & Craig, 1978), indicating a key role for collagen properties. During palatal shelf elevation in rats, mesenchymal collagen type I is reinforced with covalent crosslinks within a fiber oriented in the rostral-caudal plane (Hassell & Orkin, 1976). ECM crosslinking and tissue distribution of collagen thickness are typically accomplished by the activities of the lysyl oxidase-like (LOXL) family of enzymes (Herchenhan et al., 2015). A lathyrogenic (sweet pea derived) chemical, β-aminopropionitrile, may induce cleft palate in rat embryos by inhibiting collagen crosslinking (Pratt Jr & King, 1972; Steffek, Watkins, & Verrusio, 1972). In mice that lack Loxl3, collagen fails to crosslink and cleft palate occurs, further suggesting the importance of ECM crosslinking and stiffening during palatogenesis (J. Zhang et al., 2015). Indeed, *LOXL3* mutations have been associated with OFCs in human patients with Stickler syndrome (Alzahrani, Al Hazzaa, Tayeb, & Alkuraya, 2015; Chan, Alkaabi, ElBarky, & El-Hattab, 2019).

In addition to maintaining a rigid structure, the ECM must also undergo remodeling that involves proteolytic degradation. Studies have identified probable roles for matrix metalloproteases (MMPs) (Blavier et al., 2001; Brown, Yarram, Mansell, & Sandy, 2002), a disintegrin-like and metalloprotease domain with thrombospondin type 1 motif enzymes (ADAMTSs) (Enomoto et al., 2010), and tissue inhibitor of metalloproteases (TIMPs) (Morris-Wiman, Burch, & Basco, 2000) throughout palatogenesis. Overlapping functionalities of members within the MMP and ADAMTS families have made it challenging to identify any functional necessity of these enzymes. However, compound mutant mouse models have enabled investigations in the roles of these enzymes. Double mutants for genes encoding the proteoglycanases Adamts9 and Adamts20 exhibit fully penetrant cleft palate in mouse embryos (Enomoto et al., 2010). These enzymes cooperate to degrade versican during palatogenesis, and their mutations result in decreased palatal shelf growth and elevation, leading to cleft palate. Similarly, combined mutations in genes encoding Mmp14 and Mmp16, which activate Mmp2, cause incompletely penetrant cleft palate, but this is likely due to indirect effects mediated by tissues adjacent to the developing palate (Shi et al., 2008).

MMPs and their TIMP regulators are required for palatal fusion likely through contribution to changes observed during epithelial-mesenchymal transition (EMT). Mmp2 is highly expressed in murine mesenchymal cells adjacent the medial edge epithelium (MEE), while Mmp13 and Timp2 are expressed in the MEE (Blavier et al., 2001). Mmp14 is expressed throughout the palate in both mesenchyme and MEE cells. In cultures of developing palate, addition of recombinant Timp2 (an inhibitor of Mmp2 activity) prevents fusion and EMT of

the MEE. Similar results have been shown by adding the MMP inhibitor BB3103. The resultant clefts were phenotypically similar to those observed in TGF-β3 deficient mice, indicating a role for MMP2 and TIMP2 in EMT. In a separate study, palatal epithelial tissue from children with complete bilateral cleft lip and palate has increased expression of *TIMP2*, indicating that altered *TIMP2* expression may contribute to OFCs through inhibited MMP2 activity and/or decreased apoptosis in the MEE (Smane, Pilmane, & Akota, 2013). Further support of the role of Mmp2 in OFCs came from an experiment similar to that of Blavier et al. Using the MMP inhibitor BB3103, Brown et al. (2002) demonstrated that out of 15 palates developing in organ culture, 14 have decreased Mmp2 activity and do not fuse (Brown et al., 2002). The only palate that fused has increased abundance of Mmp3, which they hypothesize could compensate for loss of Mmp2 activity. Polymorphisms of Mmp3 have since been associated with human cases of OFCs (Letra et al., 2007; Letra et al., 2012; Letra, Zhao, Silva, Vieira, & Hecht, 2014).

To summarize, the cytoskeleton and ECM are integrated systems that define cellular structure during developmental and cellular processes. This is particularly true for palatogenesis, where development must accommodate rapid, robust, and coordinated changes to cellular structure during shelf elevation. In addition to controlling structural dynamics, the cytoskeleton and ECM are also integrated with signaling mechanisms within the cell. Although the exact mechanisms of palatal shelf elevation remain to be determined, the consensus of developmental models is that expansion of a rigid ECM is required, possibly contributing as a scaffold for cytoskeletal dynamics. The use of GWAS studies, inhibitors of specific matrix and cytoskeletal elements, and conditional knockout models continues to advance our understanding of this complex process.

8. Conclusions and perspectives

Orofacial clefts are among the most common craniofacial deformities. Decades of research show that OFCs result from disrupted growth or fusion of the facial processes during normal embryonic development. Several orofacial tissues are derived from multipotent CNCCs which rely on their respective microenvironments for appropriate migration, proliferation, and differentiation. These processes are driven by the coordinated expression or repression of several genes which have been shown to impact cellular behavior during midfacial formation. Epithelial cell dynamics, including EMT, are crucial for fusion of bilateral tissues. Coordinated activities of subcellular structures, including primary cilia, ECM, and the cytoskeleton, are required to drive the rapid and complex developmental processes during orofacial development. Disruption of any of these processes impairs orofacial morphogenesis, often leading to OFC in isolation or as a syndrome of phenotypes.

The ability to treat or prevent OFCs is contingent upon the biomedical field’s understanding of the molecular mechanisms involved in craniofacial development; therefore, primary goal of OFC research is to identify and elucidate these mechanisms. Given the complexity of craniofacial development, this is not a simple task and represents a major challenge in the life sciences. Technological improvements in global gene expression analysis and microscopy, along with the generation of animal models and chemical inhibitors/activators, have greatly expanded our understanding of orofacial development. Despite these advances,

there are still mechanisms of craniofacial development that remain poorly understood. A prime example is lip fusion which, in contrast to palatal fusion, lacks robust models for study. However, recent studies offer some promise for this field. For example, the first epigenomic epidemiology study of OFCs provided further evidence that cleft lip only (CLO) has its own etiologies that are distinct from CL/P (Sharp et al., 2017). Elucidating distinct mechanisms of OFC subtypes may lead to the creation of models that would advance our understanding of specific cellular processes during craniofacial development.

Another technology, single cell RNA sequencing, has emerged and allows for unprecedented resolution of molecular activities at the level of individual cells. This approach is particularly useful for studying *in vivo* contexts of developmental mechanisms and has been utilized for investigating cell fate during embryogenesis (Blase, Cao, & Zhong, 2014; Chu et al., 2016; Farrell et al., 2018; Li et al., 2019; Tang et al., 2010; Wagner et al., 2018; Yan et al., 2013) and regeneration (Aztekin et al., 2019; Ba, Wang, Wu, Sun, & Li, 2019; Carr et al., 2019; Johnson, Masias, & Lehoczky, 2020; Leigh et al., 2018; Londono et al., 2020) in various models. Importantly, it will prove invaluable for investigating the contribution of specific cell populations and lineages in the development of OFCs. It may also serve to clarify the ultimate fates of certain cells such as epithelial seam cells during lip fusion, among many other applications.

In conclusion, while past decades have revealed much about the cellular mechanisms underlying craniofacial development, there is still much to be discovered. While the utilization of current technologies and methodologies is still fruitful, advances in global epigenomics and single cell gene expression analysis will fuel the next generation of OFC studies. It is anticipated that these approaches, especially in combined application, will greatly improve our understanding of the cellular mechanisms underlying OFCs.

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References

Abramyan J, & Richman JM (2015). Recent insights into the morphological diversity in the amniote primary and secondary palates. Dev Dyn, 244(12), 1457–1468. doi:10.1002/dvdy.24338 [PubMed: 26293818]

Abramyan J, Thivichon-Prince B, & Richman JM (2015). Diversity in primary palate ontogeny of amniotes revealed with 3D imaging. J Anat, 226(5), 420–433. doi:10.1111/joa.12291 [PubMed: 25904546]

Ads AH, Piddington R, Goldman AS, & Herold R (1983). Cortisol inhibition of development of various lysosomal enzymes in cultured palatal shelves from mouse embryos. Arch Oral Biol, 28(12), 1115–1119. doi:10.1016/0003-9969(83)90167-x [PubMed: 6582815]

Alappat SR, Zhang Z, Suzuki K, Zhang X, Liu H, Jiang R, … Chen Y (2005). The cellular and molecular etiology of the cleft secondary palate in Fgf10 mutant mice. Dev Biol, 277(1), 102–113. doi:10.1016/j.ydbio.2004.09.010 [PubMed: 15572143]

Alzahrani F, Al Hazzaa SA, Tayeb H, & Alkuraya FS (2015). LOXL3, encoding lysyl oxidase-like 3, is mutated in a family with autosomal recessive Stickler syndrome. Hum Genet, 134(4), 451–453. doi:10.1007/s00439-015-1531-z [PubMed: 25663169]

Andl T, Le Bras GF, Richards NF, Allison GL, Loomans HA, Washington MK, … Andl CD (2014). Concerted loss of TGFbeta-mediated proliferation control and E-cadherin disrupts epithelial homeostasis and causes oral squamous cell carcinoma. Carcinogenesis, 35(11), 2602–2610. doi:10.1093/carcin/bgu194 [PubMed: 25233932]

Aoyama G, Kurosaka H, Oka A, Nakatsugawa K, Yamamoto S, Sarper SE, … Yamashiro T (2019). Observation of Dynamic Cellular Migration of the Medial Edge Epithelium of the Palatal Shelf in vitro. Front Physiol, 10, 698. doi:10.3389/fphys.2019.00698 [PubMed: 31244674]

Arends F, & Lieleg O (2016). Biophysical Properties of the Basal Lamina: A Highly Selective Extracellular Matrix. Composition and Function of the Extracellular Matrix in the Human Body, 203.

Ashique AM, Fu K, & Richman JM (2002). Endogenous bone morphogenetic proteins regulate outgrowth and epithelial survival during avian lip fusion. Development, 129(19), 4647–4660. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12223420 [PubMed: 12223420]

Aztekin C, Hiscock TW, Marioni JC, Gurdon JB, Simons BD, & Jullien J (2019). Identification of a regeneration-organizing cell in the Xenopus tail. Science, 364(6441), 653-+. doi:10.1126/science.aav9996 [PubMed: 31097661]

Ba HX, Wang DT, Wu WY, Sun HM, & Li CY (2019). Single-cell transcriptome provides novel insights into antler stem cells, a cell type capable of mammalian organ regeneration. Funct Integr Genomic, 19(4), 555–564. doi:10.1007/s10142-019-00659-2

Badano JL, Mitsuma N, Beales PL, & Katsanis N (2006). The ciliopathies: an emerging class of human genetic disorders. Annu Rev Genomics Hum Genet, 7, 125–148. doi:10.1146/annurev.genom.7.080505.115610 [PubMed: 16722803]

Bailey LJ, Minkoff R, & Koch WE (1988). Relative growth rates of maxillary mesenchyme in the chick embryo. J Craniofac Genet Dev Biol, 8(2), 167–177. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3182971 [PubMed: 3182971]

Baldari CT, & Rosenbaum J (2010). Intraflagellar transport: it's not just for cilia anymore. Curr Opin Cell Biol, 22(1), 75–80. doi:10.1016/j.ceb.2009.10.010 [PubMed: 19962875]

Barczyk M, Carracedo S, & Gullberg D (2010). Integrins. Cell Tissue Res, 339(1), 269–280. doi:10.1007/s00441-009-0834-6 [PubMed: 19693543]

Barisic I, Boban L, Loane M, Garne E, Wellesley D, Calzolari E, … Verellen-Dumoulin C (2015). Meckel-Gruber Syndrome: a population-based study on prevalence, prenatal diagnosis, clinical features, and survival in Europe. Eur J Hum Genet, 23(6), 746–752. doi:10.1038/ejhg.2014.174 [PubMed: 25182137]

Benmerah A (2013). The ciliary pocket. Curr Opin Cell Biol, 25(1), 78–84. doi:10.1016/j.ceb.2012.10.011 [PubMed: 23153502]

Bicknell LS, Farrington-Rock C, Shafeghati Y, Rump P, Alanay Y, Alembik Y, … Robertson SP (2007). A molecular and clinical study of Larsen syndrome caused by mutations in FLNB. Journal of Medical Genetics, 44(2), 89–98. doi:10.1136/jmg.2006.043687 [PubMed: 16801345]

Blase FH, Cao XY, & Zhong S (2014). Cell fate inclination within 2-cell and 4-cell mouse embryos revealed by single-cell RNA sequencing. Genome Res, 24(11), 1787–1796. doi:10.1101/gr.177725.114 [PubMed: 25096407]

Blavier L, Lazaryev A, Groffen J, Heisterkamp N, DeClerck YA, & Kaartinen V (2001). TGF-beta 3-induced palatogenesis requires matrix metalloproteinases. Molecular Biology of the Cell, 12(5), 1457–1466. doi:DOI 10.1091/mbc.12.5.1457 [PubMed: 11359935]

Bornstein P, & Sage EH (2002). Matricellular proteins: extracellular modulators of cell function. Current Opinion in Cell Biology, 14(5), 608–616. doi:Doi 10.1016/S0955-0674(02)00361-7 [PubMed: 12231357]

Brandenberger R, Schmidt A, Linton J, Wang D, Backus C, Denda S, … Reichardt LF (2001). Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha 8 beta 1 in the embryonic kidney. Journal of Cell Biology, 154(2), 447–458. doi:DOI 10.1083/jcb.200103069

Brinkley LL, & Bookstein FL (1986). Cell distribution during mouse secondary palate closure. II. Mesenchymal cells. J Embryol Exp Morphol, 96, 111–130. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3805979 [PubMed: 3805979]

Brown NL, Yarram SJ, Mansell JP, & Sandy JR (2002). Matrix metalloproteinases have a role in palatogenesis. Journal of Dental Research, 81(12), 826–830. doi:Doi 10.1177/154405910208101206 [PubMed: 12454096]

Brugmann SA, Allen NC, James AW, Mekonnen Z, Madan E, & Helms JA (2010). A primary cilia-dependent etiology for midline facial disorders. Hum Mol Genet, 19(8), 1577–1592. doi:10.1093/hmg/ddq030 [PubMed: 20106874]

Brugmann SA, Cordero DR, & Helms JA (2010). Craniofacial ciliopathies: A new classification for craniofacial disorders. Am J Med Genet A, 152A(12), 2995–3006. doi:10.1002/ajmg.a.33727 [PubMed: 21108387]

Brugmann SA, Goodnough LH, Gregorieff A, Leucht P, ten Berge D, Fuerer C, … Helms JA (2007). Wnt signaling mediates regional specification in the vertebrate face. Development, 134(18), 3283–3295. doi:10.1242/dev.005132 [PubMed: 17699607]

Brugmann SA, Tapadia MD, & Helms JA (2006). The molecular origins of species-specific facial pattern. Curr Top Dev Biol, 73, 1–42. doi:10.1016/S0070-2153(05)73001-5 [PubMed: 16782454]

Brunet CL, Sharpe PM, & Ferguson MW (1995). Inhibition of TGF-beta 3 (but not TGF-beta 1 or TGF-beta 2) activity prevents normal mouse embryonic palate fusion. Int J Dev Biol, 39(2), 345–355. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7669547 [PubMed: 7669547]

Bugyi B, & Carlier MF (2010). Control of Actin Filament Treadmilling in Cell Motility. Annual Review of Biophysics, Vol 39, 39, 449–470. doi:10.1146/annurev-biophys-051309-103849

Burdi AR, & Faist K (1967). Morphogenesis of the palate in normal human embryos with special emphasis on the mechanisms involved. American Journal of Anatomy, 120(1), 149–159. doi:10.1002/aja.1001200112

Bürglin TR (1998). The PBC domain contains a MEINOX domain: coevolution of Hox and TALE homeobox genes? Dev Genes Evol, 208(2), 113–116. [PubMed: 9569353]

Bush JO, & Jiang R (2012). Palatogenesis: morphogenetic and molecular mechanisms of secondary palate development. Development, 139(2), 231–243. doi:10.1242/dev.067082 [PubMed: 22186724]

Cano DA, Sekine S, & Hebrok M (2006). Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. Gastroenterology, 131(6), 1856–1869. doi:10.1053/j.gastro.2006.10.050 [PubMed: 17123526]

Carette MJ, & Ferguson MW (1992). The fate of medial edge epithelial cells during palatal fusion in vitro: an analysis by DiI labelling and confocal microscopy. Development, 114(2), 379–388. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1591998 [PubMed: 1591998]

Carmichael SL, Nelson V, Shaw GM, Wasserman CR, & Croen LA (2003). Socio-economic status and risk of conotruncal heart defects and orofacial clefts. Paediatr Perinat Epidemiol, 17(3), 264–271. doi:10.1046/j.1365-3016.2003.00498.x [PubMed: 12839538]

Carr MJ, Toma JS, Johnston APW, Steadman PE, Yuzwa SA, Mahmud N, … Miller FD (2019). Mesenchymal Precursor Cells in Adult Nerves Contribute to Mammalian Tissue Repair and Regeneration. Cell Stem Cell, 24(2), 240-+. doi:10.1016/j.stem.2018.10.024 [PubMed: 30503141]

Casey LM, Lan Y, Cho ES, Maltby KM, Gridley T, & Jiang R (2006). Jag2-Notch1 signaling regulates oral epithelial differentiation and palate development. Dev Dyn, 235(7), 1830–1844. doi:10.1002/dvdy.20821 [PubMed: 16607638]

Cassell CH, Meyer R, & Daniels J (2008). Health care expenditures among Medicaid enrolled children with and without orofacial clefts in North Carolina, 1995–2002. Birth Defects Res A Clin Mol Teratol, 82(11), 785–794. doi:10.1002/bdra.20522 [PubMed: 18985685]

Cavalcanti DP, Huber C, Sang KH, Baujat G, Collins F, Delezoide AL, … Cormier-Daire V (2011). Mutation in IFT80 in a fetus with the phenotype of Verma-Naumoff provides molecular evidence for Jeune-Verma-Naumoff dysplasia spectrum. J Med Genet, 48(2), 88–92. doi:10.1136/jmg.2009.069468 [PubMed: 19648123]

Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA, & Gruss P (1998). Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. Cell, 94(6), 727–737. doi:10.1016/s0092-8674(00)81732-8 [PubMed: 9753320]

Chai Y, & Maxson RE Jr. (2006). Recent advances in craniofacial morphogenesis. Dev Dyn, 235(9), 2353–2375. doi:10.1002/dvdy.20833 [PubMed: 16680722]

Chai Y, Sasano Y, Bringas P Jr., Mayo M, Kaartinen V, Heisterkamp N, … Shuler C (1997). Characterization of the fate of midline epithelial cells during the fusion of mandibular prominences in vivo. Dev Dyn, 208(4), 526–535. doi:10.1002/(SICI)1097-0177(199704)208:4<526::AID-AJA8>3.0.CO;2-K [PubMed: 9097024]

Chalpe AJ, Prasad M, Henke AJ, & Paulson AF (2010). Regulation of cadherin expression in the chicken neural crest by the Wnt/beta-catenin signaling pathway. Cell Adh Migr, 4(3), 431–438. doi:10.4161/cam.4.3.12138 [PubMed: 20523111]

Chan TK, Alkaabi MK, ElBarky AM, & El-Hattab AW (2019). LOXL3 novel mutation causing a rare form of autosomal recessive Stickler syndrome. Clin Genet, 95(2), 325–328. doi:10.1111/cge.13465 [PubMed: 30362103]

Chiquet M, Blumer S, Angelini M, Mitsiadis TA, & Katsaros C (2016). Mesenchymal Remodeling during Palatal Shelf Elevation Revealed by Extracellular Matrix and F-Actin Expression Patterns. Front Physiol, 7, 392. doi:10.3389/fphys.2016.00392 [PubMed: 27656150]

Christensen K, Juel K, Herskind AM, & Murray JC (2004). Long term follow up study of survival associated with cleft lip and palate at birth. BMJ, 328(7453), 1405. doi:10.1136/bmj.38106.559120.7C [PubMed: 15145797]

Chu LF, Leng N, Zhang J, Hou ZG, Mamott D, Vereide DT, … Thomson JA (2016). Single-cell RNA-seq reveals novel regulators of human embryonic stem cell differentiation to definitive endoderm. Genome Biol, 17. doi:ARTN 173 10.1186/s13059-016-1033-x

Clay MR, & Halloran MC (2011). Regulation of cell adhesions and motility during initiation of neural crest migration. Curr Opin Neurobiol, 21(1), 17–22. doi:10.1016/j.conb.2010.09.013 [PubMed: 20970990]

Coene KL, Roepman R, Doherty D, Afroze B, Kroes HY, Letteboer SJ, … de Brouwer AP (2009). OFD1 is mutated in X-linked Joubert syndrome and interacts with LCA5-encoded lebercilin. Am J Hum Genet, 85(4), 465–481. doi:10.1016/j.ajhg.2009.09.002 [PubMed: 19800048]

Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, & Reiter JF (2005). Vertebrate Smoothened functions at the primary cilium. Nature, 437(7061), 1018–1021. doi:10.1038/nature04117 [PubMed: 16136078]

Cordero DR, Brugmann S, Chu Y, Bajpai R, Jame M, & Helms JA (2011). Cranial neural crest cells on the move: their roles in craniofacial development. Am J Med Genet A, 155A(2), 270–279. doi:10.1002/ajmg.a.33702 [PubMed: 21271641]

Cortes CR, Metzis V, & Wicking C (2015). Unmasking the ciliopathies: craniofacial defects and the primary cilium. Wiley Interdiscip Rev Dev Biol, 4(6), 637–653. doi:10.1002/wdev.199 [PubMed: 26173831]

Couly GF, Coltey PM, & Le Douarin NM (1992). The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development, 114(1), 1–15. [PubMed: 1576952]

Couly GF, Coltey PM, & Le Douarin NM (1993). The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Development, 117(2), 409–429. [PubMed: 8330517]

Cox TC (2004). Taking it to the max: the genetic and developmental mechanisms coordinating midfacial morphogenesis and dysmorphology. Clinical genetics, 65(3), 163–176. [PubMed: 14756664]

Cox TC (2004). Taking it to the max: the genetic and developmental mechanisms coordinating midfacial morphogenesis and dysmorphology. Clin Genet, 65(3), 163–176. doi:10.1111/j.0009-9163.2004.00225.x [PubMed: 14756664]

Crane JF, & Trainor PA (2006). Neural crest stem and progenitor cells. Annu Rev Cell Dev Biol, 22, 267–286. doi:10.1146/annurev.cellbio.22.010305.103814 [PubMed: 16803431]

Croen LA, Shaw GM, Wasserman CR, & Tolarova MM (1998). Racial and ethnic variations in the prevalence of orofacial clefts in California, 1983–1992. Am J Med Genet, 79(1), 42–47. doi:10.1002/(sici)1096-8628(19980827)79:1<42::aid-ajmg11>3.0.co;2-m [PubMed: 9738868]

Cuervo R, & Covarrubias L (2004). Death is the major fate of medial edge epithelial cells and the cause of basal lamina degradation during palatogenesis. Development, 131(1), 15–24. doi:10.1242/dev.00907 [PubMed: 14645125]

Cuervo R, Valencia C, Chandraratna RA, & Covarrubias L (2002). Programmed cell death is required for palate shelf fusion and is regulated by retinoic acid. Dev Biol, 245(1), 145–156. doi:10.1006/dbio.2002.0620 [PubMed: 11969262]

Cui XM, Chai Y, Chen J, Yamamoto T, Ito Y, Bringas P, & Shuler CF (2003). TGF-beta3-dependent SMAD2 phosphorylation and inhibition of MEE proliferation during palatal fusion. Dev Dyn, 227(3), 387–394. doi:10.1002/dvdy.10326 [PubMed: 12815624]

Cui XM, Warburton D, Zhao J, Crowe DL, & Shuler CF (1998). Immunohistochemical localization of TGF-beta type II receptor and TGF-beta3 during palatogenesis in vivo and in vitro. Int J Dev Biol, 42(6), 817–820. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9727838 [PubMed: 9727838]

Czabotar PE, Lessene G, Strasser A, & Adams JM (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol, 15(1), 49–63. doi:10.1038/nrm3722 [PubMed: 24355989]

Dagoneau N, Goulet M, Genevieve D, Sznajer Y, Martinovic J, Smithson S, … Cormier-Daire V (2009). DYNC2H1 mutations cause asphyxiating thoracic dystrophy and short rib-polydactyly syndrome, type III. Am J Hum Genet, 84(5), 706–711. doi:10.1016/j.ajhg.2009.04.016 [PubMed: 19442771]

Dasouki M, Barr M, Erickson RP, & Cox B (1988). Translocation (1–22) in a Child with Bilateral Oblique Facial Clefts. Journal of Medical Genetics, 25(6), 427–431. doi:DOI 10.1136/jmg.25.6.427 [PubMed: 3398011]

De Calisto J, Araya C, Marchant L, Riaz CF, & Mayor R (2005). Essential role of noncanonical Wnt signalling in neural crest migration. Development, 132(11), 2587–2597. doi:10.1242/dev.01857 [PubMed: 15857909]

Deane JA, Cole DG, Seeley ES, Diener DR, & Rosenbaum JL (2001). Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. Curr Biol, 11(20), 1586–1590. doi:10.1016/s0960-9822(01)00484-5 [PubMed: 11676918]

DeAngelis V, & Nalbandian J (1968). Ultrastructure of mouse and rat palatal processes prior to and during secondary palate formation. Arch Oral Biol, 13(6), 601–608. doi:10.1016/0003-9969(68)90138-6 [PubMed: 5244284]

del Barrio MG, & Nieto MA (2002). Overexpression of Snail family members highlights their ability to promote chick neural crest formation. Development, 129(7), 1583–1593. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11923196 [PubMed: 11923196]

Denda S, Reichardt LF, & Muller U (1998). Identification of osteopontin as a novel ligand for the integrin alpha 8 beta 1 and potential roles for this integrin-ligand interaction in kidney morphogenesis. Molecular Biology of the Cell, 9(6), 1425–1435. doi:DOI 10.1091/mbc.9.6.1425 [PubMed: 9614184]

Depew MJ, & Compagnucci C (2008). Tweaking the hinge and caps: testing a model of the organization of jaws. J Exp Zool B Mol Dev Evol, 310(4), 315–335. doi:10.1002/jez.b.21205 [PubMed: 18027841]

Di Donato N, Rump A, Koenig R, Der Kaloustian VM, Halal F, Sonntag K, … Verloes A (2014). Severe forms of Baraitser-Winter syndrome are caused by ACTB mutations rather than ACTG1 mutations. Eur J Hum Genet, 22(2), 179–183. doi:10.1038/ejhg.2013.130 [PubMed: 23756437]

Dixon J, Jones NC, Sandell LL, Jayasinghe SM, Crane J, Rey JP, … Trainor PA (2006). Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. Proc Natl Acad Sci U S A, 103(36), 13403–13408. doi:10.1073/pnas.0603730103 [PubMed: 16938878]

Dudas M, Kim J, Li WY, Nagy A, Larsson J, Karlsson S, … Kaartinen V (2006). Epithelial and ectomesenchymal role of the type I TGF-beta receptor ALK5 during facial morphogenesis and palatal fusion. Dev Biol, 296(2), 298–314. doi:10.1016/j.ydbio.2006.05.030 [PubMed: 16806156]

Dudas M, Li WY, Kim J, Yang A, & Kaartinen V (2007). Palatal fusion - where do the midline cells go? A review on cleft palate, a major human birth defect. Acta Histochem, 109(1), 1–14. doi:10.1016/j.acthis.2006.05.009 [PubMed: 16962647]

Dudas M, Nagy A, Laping NJ, Moustakas A, & Kaartinen V (2004). Tgf-beta3-induced palatal fusion is mediated by Alk-5/Smad pathway. Dev Biol, 266(1), 96–108. doi:10.1016/j.ydbio.2003.10.007 [PubMed: 14729481]

Duran I, Taylor SP, Zhang W, Martin J, Qureshi F, Jacques SM, … Krakow D (2017). Mutations in IFT-A satellite core component genes IFT43 and IFT121 produce short rib polydactyly syndrome with distinctive campomelia. Cilia, 6, 7. doi:10.1186/s13630-017-0051-y [PubMed: 28400947]

Dutta RC, & Dutta AK (2010). Comprehension of ECM-cell dynamics: a prerequisite for tissue regeneration. Biotechnol Adv, 28(6), 764–769. doi:10.1016/j.biotechadv.2010.06.002 [PubMed: 20600786]

Dworkin S, Boglev Y, Owens H, & Goldie SJ (2016). The Role of Sonic Hedgehog in Craniofacial Patterning, Morphogenesis and Cranial Neural Crest Survival. J Dev Biol, 4(3). doi:10.3390/jdb4030024

Eggenschwiler JT, & Anderson KV (2007). Cilia and developmental signaling. Annu Rev Cell Dev Biol, 23, 345–373. doi:10.1146/annurev.cellbio.23.090506.123249 [PubMed: 17506691]

Enomoto H, Nelson CM, Somerville RPT, Mielke K, Dixon LJ, Powell K, & Apte SS (2010). Cooperation of two ADAMTS metalloproteases in closure of the mouse palate identifies a requirement for versican proteolysis in regulating palatal mesenchyme proliferation. Development, 137(23), 4029–4038. doi:10.1242/dev.050591 [PubMed: 21041365]

Eppley BL, van Aalst JA, Robey A, Havlik RJ, & Sadove AM (2005). The spectrum of orofacial clefting. Plast Reconstr Surg, 115(7), 101e–114e. doi:10.1097/01.prs.0000164494.45986.91

Eto K, Figueroa A, Tamura G, & Pratt RM (1981). Induction of cleft lip in cultured rat embryos by localized administration of tunicamycin. J Embryol Exp Morphol, 64(Aug), 1–9. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7310299 [PubMed: 7310299]

Farrell JA, Wang YQ, Riesenfeld SJ, Shekhar K, Regev A, & Schier AF (2018). Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. Science, 360(6392), 979-+. doi:ARTN eaar3131 10.1126/science.aar3131

Ferguson MWJ (1988). Palate Development. Development, 103, 41–60. Retrieved from <Go to ISI>://WOS:A1988Q213300006 [PubMed: 3074914]

Ferrante MI, Zullo A, Barra A, Bimonte S, Messaddeq N, Studer M, … Franco B (2006). Oral-facial-digital type I protein is required for primary cilia formation and left-right axis specification. Nat Genet, 38(1), 112–117. doi:10.1038/ng1684 [PubMed: 16311594]

Ferretti E, Li B, Zewdu R, Wells V, Hebert JM, Karner C, … Selleri L (2011). A conserved Pbx-Wnt-p63-Irf6 regulatory module controls face morphogenesis by promoting epithelial apoptosis. Dev Cell, 21(4), 627–641. doi:10.1016/j.devcel.2011.08.005 [PubMed: 21982646]

Fitchett JE, & Hay ED (1989). Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. Dev Biol, 131(2), 455–474. doi:10.1016/s0012-1606(89)80017-x [PubMed: 2463946]

Fletcher DA, & Mullins D (2010). Cell mechanics and the cytoskeleton. Nature, 463(7280), 485–492. doi:10.1038/nature08908 [PubMed: 20110992]

Franco B, & Thauvin-Robinet C (2016). Update on oral-facial-digital syndromes (OFDS). Cilia, 5, 12. doi:10.1186/s13630-016-0034-4 [PubMed: 27141300]

Frebourg T, Oliveira C, Hochain P, Karam R, Manouvrier S, Graziadio C, … Seruca R (2006). Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. J Med Genet, 43(2), 138–142. doi:10.1136/jmg.2005.031385 [PubMed: 15831593]

Froyen G, Van Esch H, Bauters M, Hollanders K, Frints SGM, Vermeesch JR, … Marynen P (2007). Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: Important role for increased gene dosage of XLMR genes. Human Mutation, 28(10), 1034–1042. doi:10.1002/humu.20564 [PubMed: 17546640]

Gaare JD, & Langman J (1977a). Fusion of nasal swellings in the mouse embryo: Regression of the nasal fin. American Journal of Anatomy, 150(3), 477–499.

Gaare JD, & Langman J (1977b). Fusion of nasal swellings in the mouse embryo: surface coat and initial contact. American Journal of Anatomy, 150(3), 461–475.

Garcia-Alvarez B, de Pereda JM, Calderwood DA, Ulmer TS, Critchley D, Campbell ID, … Liddington RC (2003). Structural determinants of integrin recognition by Talin. Molecular Cell, 11(1), 49–58. doi:Doi 10.1016/S1097-2765(02)00823-7 [PubMed: 12535520]

Garcia-Gonzalo FR, Corbit KC, Sirerol-Piquer MS, Ramaswami G, Otto EA, Noriega TR, … Reiter JF (2011). A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. Nat Genet, 43(8), 776–784. doi:10.1038/ng.891 [PubMed: 21725307]

Gehris AL, & Greene RM (1992). Regulation of murine embryonic epithelial cell differentiation by transforming growth factors beta. Differentiation, 49(3), 167–173. doi:10.1111/j.1432-0436.1992.tb00664.x [PubMed: 1618373]

Gerdes JM, Davis EE, & Katsanis N (2009). The vertebrate primary cilium in development, homeostasis, and disease. Cell, 137(1), 32–45. doi:10.1016/j.cell.2009.03.023 [PubMed: 19345185]

Gerdes JM, Liu Y, Zaghloul NA, Leitch CC, Lawson SS, Kato M, … Katsanis N (2007). Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. Nat Genet, 39(11), 1350–1360. doi:10.1038/ng.2007.12 [PubMed: 17906624]

Glucksmann A (1951). Cell deaths in normal vertebrate ontogeny. Biol Rev Camb Philos Soc, 26(1), 59–86. doi:10.1111/j.1469-185x.1951.tb00774.x [PubMed: 24540363]

Gong SG, & Guo C (2003). Bmp4 gene is expressed at the putative site of fusion in the midfacial region. Differentiation, 71(3), 228–236. doi:10.1046/j.1432-0436.2003.710304.x [PubMed: 12694205]

Graham A (2001). The development and evolution of the pharyngeal arches. J Anat, 199(Pt 1–2), 133–141. doi:10.1046/j.1469-7580.2001.19910133.x [PubMed: 11523815]

Graham A (2003). Development of the pharyngeal arches. Am J Med Genet A, 119A(3), 251–256. doi:10.1002/ajmg.a.10980 [PubMed: 12784288]

Green DR, & Kroemer G (2004). The pathophysiology of mitochondrial cell death. Science, 305(5684), 626–629. doi:10.1126/science.1099320 [PubMed: 15286356]

Greene RM, & Pratt RM (1976). Developmental aspects of secondary palate formation. J Embryol Exp Morphol, 36(2), 225–245. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1033980 [PubMed: 1033980]

Grenier J, Teillet MA, Grifone R, Kelly RG, & Duprez D (2009). Relationship between neural crest cells and cranial mesoderm during head muscle development. PLoS One, 4(2), e4381. doi:10.1371/journal.pone.0004381 [PubMed: 19198652]

Griffith CM, & Hay ED (1992). Epithelial-mesenchymal transformation during palatal fusion: carboxyfluorescein traces cells at light and electron microscopic levels. Development, 116(4), 1087–1099. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1295731 [PubMed: 1295731]

Gul IS, Hulpiau P, Saeys Y, & van Roy F (2017). Evolution and diversity of cadherins and catenins. Exp Cell Res, 358(1), 3–9. doi:10.1016/j.yexcr.2017.03.001 [PubMed: 28268172]

Halbritter J, Bizet AA, Schmidts M, Porath JD, Braun DA, Gee HY, … Hildebrandt F (2013). Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans. Am J Hum Genet, 93(5), 915–925. doi:10.1016/j.ajhg.2013.09.012 [PubMed: 24140113]

Hassell JR, & Orkin RW (1976). Synthesis and distribution of collagen in the rat palate during shelf elevation. Developmental biology, 49(1), 80–88. [PubMed: 1254099]

Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, & Yoder BK (2005). Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. PLoS Genet, 1(4), e53. doi:10.1371/journal.pgen.0010053 [PubMed: 16254602]

Herchenhan A, Uhlenbrock F, Eliasson P, Weis M, Eyre D, Kadler KE, … Kjaer M (2015). Lysyl Oxidase Activity Is Required for Ordered Collagen Fibrillogenesis by Tendon Cells. Journal of Biological Chemistry, 290(26), 16440–16450. doi:10.1074/jbc.M115.641670

Hinrichsen K (1985). The early development of morphology and patterns of the face in the human embryo. Advances in anatomy, embryology, and cell biology, 98, 1–79.

Hinrichsen K (1985). The early development of morphology and patterns of the face in the human embryo. Adv Anat Embryol Cell Biol, 98, 1–79. doi:10.1007/978-3-642-70754-4 [PubMed: 4083112]

Holtgrave EA, & Stoltenburg-Didinger G (2002). Apoptotic epithelial cell death: a prerequisite for palatal fusion. An in vivo study in rabbits. J Craniomaxillofac Surg, 30(6), 329–336. doi:10.1054/jcms.2002.0323 [PubMed: 12425986]

Hong CS, Park BY, & Saint-Jeannet JP (2008). Fgf8a induces neural crest indirectly through the activation of Wnt8 in the paraxial mesoderm. Development, 135(23), 3903–3910. doi:10.1242/dev.026229 [PubMed: 18997112]

Hozyasz KK, Mostowska A, Wojcicki P, Lasota A, Offert B, Balcerek A, … Jagodzinski PP (2014). Nucleotide variants of the cancer predisposing gene CDH1 and the risk of non-syndromic cleft lip with or without cleft palate. Familial Cancer, 13(3), 415–421. doi:10.1007/s10689-014-9727-2 [PubMed: 24838934]

Hsu KS, Chuang JZ, & Sung CH (2017). The Biology of Ciliary Dynamics. Cold Spring Harb Perspect Biol, 9(4). doi:10.1101/cshperspect.a027904

Ingraham CR, Kinoshita A, Kondo S, Yang B, Sajan S, Trout KJ, … Schutte BC (2006). Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (Irf6). Nat Genet, 38(11), 1335–1340. doi:10.1038/ng1903 [PubMed: 17041601]

Ishikawa H, & Marshall WF (2017). Intraflagellar Transport and Ciliary Dynamics. Cold Spring Harb Perspect Biol, 9(3). doi:10.1101/cshperspect.a021998

Iwata J, Parada C, & Chai Y (2011). The mechanism of TGF-beta signaling during palate development. Oral Dis, 17(8), 733–744. doi:10.1111/j.1601-0825.2011.01806.x [PubMed: 21395922]

Jeong J, Mao J, Tenzen T, Kottmann AH, & McMahon AP (2004). Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev, 18(8), 937–951. doi:10.1101/gad.1190304 [PubMed: 15107405]

Ji Y, Hao H, Reynolds K, McMahon M, & Zhou CJ (2019). Wnt Signaling in Neural Crest Ontogenesis and Oncogenesis. Cells, 8(10). doi:10.3390/cells8101173

Jiang R, Bush JO, & Lidral AC (2006). Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn, 235(5), 1152–1166. doi:10.1002/dvdy.20646 [PubMed: 16292776]

Jiang R, Lan Y, Chapman HD, Shawber C, Norton CR, Serreze DV, … Gridley T (1998). Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. Genes Dev, 12(7), 1046–1057. doi:10.1101/gad.12.7.1046 [PubMed: 9531541]

Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, … Nachury MV (2010). The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. Cell, 141(7), 1208–1219. doi:10.1016/j.cell.2010.05.015 [PubMed: 20603001]

Jin JZ, & Ding J (2006). Analysis of cell migration, transdifferentiation and apoptosis during mouse secondary palate fusion. Development, 133(17), 3341–3347. doi:10.1242/dev.02520 [PubMed: 16887819]

Jin YR, Han XH, Taketo MM, & Yoon JK (2012). Wnt9b-dependent FGF signaling is crucial for outgrowth of the nasal and maxillary processes during upper jaw and lip development. Development, 139(10), 1821–1830. doi:10.1242/dev.075796 [PubMed: 22461561]

Johnson GL, Masias EJ, & Lehoczky JA (2020). Cellular Heterogeneity and Lineage Restriction during Mouse Digit Tip Regeneration at Single-Cell Resolution. Dev Cell, 52(4), 525-+. doi:10.1016/j.devcel.2020.01.026 [PubMed: 32097654]

Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, … Trainor PA (2008). Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nat Med, 14(2), 125–133. doi:10.1038/nm1725 [PubMed: 18246078]

Jones NC, & Trainor PA (2005). Role of morphogens in neural crest cell determination. J Neurobiol, 64(4), 388–404. doi:10.1002/neu.20162 [PubMed: 16041760]

Julien O, & Wells JA (2017). Caspases and their substrates. Cell Death Differ, 24(8), 1380–1389. doi:10.1038/cdd.2017.44 [PubMed: 28498362]

Kaartinen V, Cui XM, Heisterkamp N, Groffen J, & Shuler CF (1997). Transforming growth factor-beta3 regulates transdifferentiation of medial edge epithelium during palatal fusion and

associated degradation of the basement membrane. Dev Dyn, 209(3), 255–260. doi:10.1002/(SICI)1097-0177(199707)209:3<255::AID-AJA1>3.0.CO;2-H [PubMed: 9215640]

Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, & Groffen J (1995). Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet, 11(4), 415–421. doi:10.1038/ng1295-415 [PubMed: 7493022]

Kang P, & Svoboda KK (2002). PI-3 kinase activity is required for epithelial-mesenchymal transformation during palate fusion. Dev Dyn, 225(3), 316–321. doi:10.1002/dvdy.10161 [PubMed: 12412014]

Kang P, & Svoboda KK (2003). Nicotine inhibits palatal fusion and modulates nicotinic receptors and the PI-3 kinase pathway in medial edge epithelia. Orthod Craniofac Res, 6(3), 129–142. doi:10.1034/j.1600-0544.2003.02236.x [PubMed: 12962196]

Kaucka M, Ivashkin E, Gyllborg D, Zikmund T, Tesarova M, Kaiser J, … Adameyko I (2016). Analysis of neural crest-derived clones reveals novel aspects of facial development. Sci Adv, 2(8), e1600060. doi:10.1126/sciadv.1600060 [PubMed: 27493992]

Ke FFS, Vanyai HK, Cowan AD, Delbridge ARD, Whitehead L, Grabow S, … Strasser A (2018). Embryogenesis and Adult Life in the Absence of Intrinsic Apoptosis Effectors BAX, BAK, and BOK. Cell, 173(5), 1217–1230 e1217. doi:10.1016/j.cell.2018.04.036 [PubMed: 29775594]

Keeling J, Tsiokas L, & Maskey D (2016). Cellular Mechanisms of Ciliary Length Control. Cells, 5(1). doi:10.3390/cells5010006

Khan MFJ, Little J, Nag TC, Mossey PA, Autelitano L, Meazzini MC, … Rubini M (2019). Ultrastructural analysis of collagen fibril diameter distribution in cleft lip. Oral Diseases, 25(1), 206–214. doi:10.1111/odi.12962 [PubMed: 30144227]

Kheir AE, Imam A, Omer IM, Hassan IM, Elamin SA, Awadalla EA, … Hamdoon TA (2012). Meckel-Gruber syndrome: A rare and lethal anomaly. Sudan J Paediatr, 12(1), 93–96. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27493335 [PubMed: 27493335]

Kiema T, Lad Y, Jiang PJ, Oxley CL, Baldassarre M, Wegener KL, … Calderwood DA (2006). The molecular basis of filamin binding to integrins and competition with talin. Molecular Cell, 21(3), 337–347. doi:10.1016/j.molcel.2006.01.011 [PubMed: 16455489]

Kim S, Lewis AE, Singh V, Ma X, Adelstein R, & Bush JO (2015). Convergence and extrusion are required for normal fusion of the mammalian secondary palate. PLoS Biol, 13(4), e1002122. doi:10.1371/journal.pbio.1002122 [PubMed: 25848986]

Kimmel CB, Miller CT, & Moens CB (2001). Specification and morphogenesis of the zebrafish larval head skeleton. Dev Biol, 233(2), 239–257. doi:10.1006/dbio.2001.0201 [PubMed: 11336493]

Kitase Y, & Shuler CF (2013). Microtubule disassembly prevents palatal fusion and alters regulation of the E-cadherin/catenin complex. Int J Dev Biol, 57(1), 55–60. doi:10.1387/ijdb.120117yk [PubMed: 23585353]

Knight RD, & Schilling TF (2006). Cranial neural crest and development of the head skeleton. Adv Exp Med Biol, 589, 120–133. doi:10.1007/978-0-387-46954-6_7 [PubMed: 17076278]

Knott L, Hartridge T, Brown NL, Mansell JP, & Sandy JR (2003). Homocysteine oxidation and apoptosis: a potential cause of cleft palate. In Vitro Cell Dev Biol Anim, 39(1–2), 98–105. doi:10.1290/1543-706x(2003)039<0098:hoaaap>2.0.co;2 [PubMed: 12892533]

Kogerman P, Grimm T, Kogerman L, Krause D, Unden AB, Sandstedt B, … Zaphiropoulos PG (1999). Mammalian suppressor-of-fused modulates nuclear-cytoplasmic shuttling of Gli-1. Nat Cell Biol, 1(5), 312–319. doi:10.1038/13031 [PubMed: 10559945]

Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, … Murray JC (2002). Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet, 32(2), 285–289. doi:10.1038/ng985 [PubMed: 12219090]

Kumar D, Nitzan E, & Kalcheim C (2019). YAP promotes neural crest emigration through interactions with BMP and Wnt activities. Cell Commun Signal, 17(1), 69. doi:10.1186/s12964-019-0383-x [PubMed: 31228951]

Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, … et al. (1994). Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature, 368(6473), 703–710. doi:10.1038/368703a0 [PubMed: 8152482]

Lan Y, Xu J, & Jiang R (2015). Cellular and Molecular Mechanisms of Palatogenesis. Curr Top Dev Biol, 115, 59–84. doi:10.1016/bs.ctdb.2015.07.002 [PubMed: 26589921]

Le Lievre CS, & Le Douarin NM (1975). Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J Embryol Exp Morphol, 34(1), 125–154. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1185098 [PubMed: 1185098]

Leigh ND, Dunlap GS, Johnson K, Mariano R, Oshiro R, Wong AY, … Whited JL (2018). Transcriptomic landscape of the blastema niche in regenerating adult axolotl limbs at single-cell resolution. Nature Communications, 9. doi:ARTN 5153 10.1038/s41467-018-07604-0

Lemullois M, Boisvieux-Ulrich E, Laine MC, Chailley B, & Sandoz D (1988). Development and functions of the cytoskeleton during ciliogenesis in metazoa. Biol Cell, 63(2), 195–208. doi:10.1016/0248-4900(88)90058-5 [PubMed: 2904829]

Leslie EJ, Mansilla MA, Biggs LC, Schuette K, Bullard S, Cooper M, … Murray JC (2012). Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. Birth Defects Research Part a-Clinical and Molecular Teratology, 94(11), 934–942. doi:10.1002/bdra.23076

Lessard JL, Wee EL, & Zimmerman EF (1974). Presence of contractile proteins in mouse fetal palate prior to shelf elevation. Teratology, 9(1), 113–125. doi:10.1002/tera.1420090114 [PubMed: 4812354]

Letra A, Silva RA, Menezes R, Astolfi CM, Shinohara A, de Souza AP, & Granjeiro JM (2007). MMP gene polymorphisms as contributors for cleft lip/palate: Association with MMP3 but not MMP1. Archives of Oral Biology, 52(10), 954–960. doi:10.1016/j.archoralbio.2007.04.005 [PubMed: 17537400]

Letra A, Silva RM, Motta LG, Blanton SH, Hecht JT, Granjeirol JM, & Vieira AR (2012). Association of MMP3 and TIMP2 promoter polymorphisms with nonsyndromic oral clefts. Birth Defects Research Part a-Clinical and Molecular Teratology, 94(7), 540–548. doi:10.1002/bdra.23026

Letra A, Zhao M, Silva RM, Vieira AR, & Hecht JT (2014). Functional Significance of MMP3 and TIMP2 Polymorphisms in Cleft Lip/Palate. Journal of Dental Research, 93(7), 651–656. doi:10.1177/0022034514534444 [PubMed: 24799419]

Li H, Jones KL, Hooper JE, & Williams T (2019). The molecular anatomy of mammalian upper lip and primary palate fusion at single cell resolution. Development, 146(12). doi:10.1242/dev.174888

Logan SM, & Benson MD (2020). Medial epithelial seam cell migration during palatal fusion. J Cell Physiol, 235(2), 1417–1424. doi:10.1002/jcp.29061 [PubMed: 31264714]

Logan SM, Ruest LB, Benson MD, & Svoboda KKH (2019). Extracellular Matrix in Secondary Palate Development. Anat Rec (Hoboken). doi:10.1002/ar.24263

Londono R, Tighe S, Milnes B, DeMoya C, Quijano LM, Hudnall ML, … Lozito TP (2020). Single cell sequencing analysis of lizard phagocytic cell populations and their role in tail regeneration. Journal of Immunology and Regenerative Medicine, 8, 100029. [PubMed: 32337387]

Losa M, Risolino M, Li B, Hart J, Quintana L, Grishina I, … Selleri L (2018). Face morphogenesis is promoted by Pbx-dependent EMT via regulation of Snail1 during frontonasal prominence fusion. Development, 145(5). doi:10.1242/dev.157628

Lough KJ, Byrd KM, Spitzer DC, & Williams SE (2017). Closing the Gap: Mouse Models to Study Adhesion in Secondary Palatogenesis. J Dent Res, 96(11), 1210–1220. doi:10.1177/0022034517726284 [PubMed: 28817360]

Lumsden A, Sprawson N, & Graham A (1991). Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. Development, 113(4), 1281–1291. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1811942 [PubMed: 1811942]

M'Boneko V, & Merker HJ (1988). Development and morphology of the periderm of mouse embryos (days 9–12 of gestation). Acta Anat (Basel), 133(4), 325–336. doi:10.1159/000146662 [PubMed: 3227794]

Marcusson A, Akerlind I, & Paulin G (2001). Quality of life in adults with repaired complete cleft lip and palate. Cleft Palate Craniofac J, 38(4), 379–385. doi:10.1597/1545-1569_2001_038_0379_qoliaw_2.0.co_2 [PubMed: 11420018]

Marshall WF (2008). Basal bodies platforms for building cilia. Curr Top Dev Biol, 85, 1–22. doi:10.1016/S0070-2153(08)00801-6 [PubMed: 19147000]

Martinez-Alvarez C, Tudela C, Perez-Miguelsanz J, O'Kane S, Puerta J, & Ferguson MW (2000). Medial edge epithelial cell fate during palatal fusion. Dev Biol, 220(2), 343–357. doi:10.1006/dbio.2000.9644 [PubMed: 10753521]

Martinez-Morales PL, Diez del Corral R, Olivera-Martinez I, Quiroga AC, Das RM, Barbas JA, … Morales AV (2011). FGF and retinoic acid activity gradients control the timing of neural crest cell emigration in the trunk. J Cell Biol, 194(3), 489–503. doi:10.1083/jcb.201011077 [PubMed: 21807879]

Mayor R, Morgan R, & Sargent MG (1995). Induction of the prospective neural crest of Xenopus. Development, 121(3), 767–777. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7720581 [PubMed: 7720581]

McInerney-Leo AM, Schmidts M, Cortes CR, Leo PJ, Gener B, Courtney AD, … Wicking C (2013). Short-rib polydactyly and Jeune syndromes are caused by mutations in WDR60. Am J Hum Genet, 93(3), 515–523. doi:10.1016/j.ajhg.2013.06.022 [PubMed: 23910462]

Meng L, Bian Z, Torensma R, & Von den Hoff JW (2009). Biological mechanisms in palatogenesis and cleft palate. J Dent Res, 88(1), 22–33. doi:10.1177/0022034508327868 [PubMed: 19131313]

Mill P, Lockhart PJ, Fitzpatrick E, Mountford HS, Hall EA, Reijns MA, … Amor DJ (2011). Human and mouse mutations in WDR35 cause short-rib polydactyly syndromes due to abnormal ciliogenesis. Am J Hum Genet, 88(4), 508–515. doi:10.1016/j.ajhg.2011.03.015 [PubMed: 21473986]

Miller CT, Schilling TF, Lee K, Parker J, & Kimmel CB (2000). sucker encodes a zebrafish Endothelin-1 required for ventral pharyngeal arch development. Development, 127(17), 3815–3828. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10934026 [PubMed: 10934026]

Millicovsky G, Ambrose LJH, & Johnston MC (1982). Developmental alterations associated with spontaneous cleft lip and palate in CL/Fr mice. American Journal of Anatomy, 164(1), 29–44.

Millicovsky G, & Johnston MC (1981). Active role of embryonic facial epithelium: new evidence of cellular events in morphogenesis. Development, 63(1), 53–66.

Minkoff R, & Kuntz AJ (1978). Cell proliferation and cell density of mesenchyme in the maxillary process and adjacent regions during facial development in the chick embryo. J Embryol Exp Morphol, 46, 65–74. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/702036 [PubMed: 702036]

Mirvis M, Stearns T, & James Nelson W (2018). Cilium structure, assembly, and disassembly regulated by the cytoskeleton. Biochem J, 475(14), 2329–2353. doi:10.1042/BCJ20170453 [PubMed: 30064990]

Mithieux SM, & Weiss AS (2005). Elastin. Fibrous Proteins: Coiled-Coils, Collagen and Elastomers, 70, 437-+. doi:10.1016/S0065-3233(05)70013-9

Miyake T, Cameron AM, & Hall BK (1996). Detailed staging of inbred C57BL/6 mice between Theiler's [1972] stages 18 and 21 (11–13 days of gestation) based on craniofacial development. J Craniofac Genet Dev Biol, 16(1), 1–31. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8675612 [PubMed: 8675612]

Mogass M, Bringas P Jr., & Shuler CF (2000). Characterization of desmosomal component expression during palatogenesis. Int J Dev Biol, 44(3), 317–322. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10853828 [PubMed: 10853828]

Monsoro-Burq AH, Fletcher RB, & Harland RM (2003). Neural crest induction by paraxial mesoderm in Xenopus embryos requires FGF signals. Development, 130(14), 3111–3124. doi:10.1242/dev.00531 [PubMed: 12783784]

Moretti F, Marinari B, Lo Iacono N, Botti E, Giunta A, Spallone G, … Costanzo A (2010). A regulatory feedback loop involving p63 and IRF6 links the pathogenesis of 2 genetically different human ectodermal dysplasias. J Clin Invest, 120(5), 1570–1577. doi:10.1172/JCI40267 [PubMed: 20424325]

Morgan R, El-Tanani M, Hunter KD, Harrington KJ, & Pandha HS (2017). Targeting HOX/PBX dimers in cancer. Oncotarget, 8(19), 32322–32331. doi:10.18632/oncotarget.15971 [PubMed: 28423659]

Morgan R, Plowright L, Harrington KJ, Michael A, & Pandha HS (2010). Targeting HOX and PBX transcription factors in ovarian cancer. BMC Cancer, 10(1), 89. doi:10.1186/1471-2407-10-89 [PubMed: 20219106]

Morgan R, & Sargent MG (1997). The role in neural patterning of translation initiation factor eIF4AII; induction of neural fold genes. Development, 124(14), 2751–2760. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9226446 [PubMed: 9226446]

Mori C, Nakamura N, Okamoto Y, Osawa M, & Shiota K (1994). Cytochemical identification of programmed cell death in the fusing fetal mouse palate by specific labelling of DNA fragmentation. Anat Embryol (Berl), 190(1), 21–28. doi:10.1007/bf00185843 [PubMed: 7527193]

Morris-Wiman J, Burch H, & Basco E (2000). Temporospatial distribution of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinases during murine secondary palate morphogenesis. Anatomy and Embryology, 202(2), 129–141. doi:DOI 10.1007/s004290000098 [PubMed: 10985432]

Morriss-Kay GM, & Wilkie AO (2005). Growth of the normal skull vault and its alteration in craniosynostosis: insights from human genetics and experimental studies. J Anat, 207(5), 637–653. doi:10.1111/j.1469-7580.2005.00475.x [PubMed: 16313397]

Mossey PA, Little J, Munger RG, Dixon MJ, & Shaw WC (2009). Cleft lip and palate. Lancet, 374(9703), 1773–1785. doi:10.1016/S0140-6736(09)60695-4 [PubMed: 19747722]

Mossey PA, Shaw WC, Munger RG, Murray JC, Murthy J, & Little J (2011). Global oral health inequalities: challenges in the prevention and management of orofacial clefts and potential solutions. Adv Dent Res, 23(2), 247–258. doi:10.1177/0022034511402083 [PubMed: 21490237]

Moxham B (2003). The development of the palate-a brief review. European Journal of Anatomy, 7(S1), 53–74.

Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peranen J, Merdes A, … Jackson PK (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell, 129(6), 1201–1213. doi:10.1016/j.cell.2007.03.053 [PubMed: 17574030]

Nakajima A, S. C,F, Gulka AOD, & Hanai JI (2018). TGF-beta Signaling and the Epithelial-Mesenchymal Transition during Palatal Fusion. Int J Mol Sci, 19(11). doi:10.3390/ijms19113638

Nakajima A, Tanaka E, Ito Y, Maeno M, Iwata K, Shimizu N, & Shuler CF (2010). The expression of TGF-beta3 for epithelial-mesenchyme transdifferentiated MEE in palatogenesis. J Mol Histol, 41(6), 343–355. doi:10.1007/s10735-010-9296-0 [PubMed: 20967564]

Nauli SM, Kawanabe Y, Kaminski JJ, Pearce WJ, Ingber DE, & Zhou J (2008). Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. Circulation, 117(9), 1161–1171. doi:10.1161/CIRCULATIONAHA.107.710111 [PubMed: 18285569]

Nawshad A, & Hay ED (2003). TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol, 163(6), 1291–1301. doi:10.1083/jcb.200306024 [PubMed: 14691138]

Nawshad A, LaGamba D, & Hay ED (2004). Transforming growth factor beta (TGFbeta) signalling in palatal growth, apoptosis and epithelial mesenchymal transformation (EMT). Arch Oral Biol, 49(9), 675–689. doi:10.1016/j.archoralbio.2004.05.007 [PubMed: 15275855]

Neugebauer JM, Amack JD, Peterson AG, Bisgrove BW, & Yost HJ (2009). FGF signalling during embryo development regulates cilia length in diverse epithelia. Nature, 458(7238), 651–654. doi:10.1038/nature07753 [PubMed: 19242413]

Nichols DH (1981). Neural crest formation in the head of the mouse embryo as observed using a new histological technique. J Embryol Exp Morphol, 64, 105–120. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7031165 [PubMed: 7031165]

Noden DM (1978). The control of avian cephalic neural crest cytodifferentiation: I. Skeletal and connective tissues. Developmental biology, 67(2), 296–312. [PubMed: 738529]

Noden DM (1983). The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. American Journal of Anatomy, 168(3), 257–276.

Noden DM (1983). The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol, 96(1), 144–165. doi:10.1016/0012-1606(83)90318-4 [PubMed: 6825950]

Noden DM (1988). Interactions and fates of avian craniofacial mesenchyme. Development, 103(Supplement), 121–140. [PubMed: 3074905]

Norris DP, & Grimes DT (2012). Mouse models of ciliopathies: the state of the art. Dis Model Mech, 5(3), 299–312. doi:10.1242/dmm.009340 [PubMed: 22566558]

O'rahilly R (1972). Guide to the staging of human embryos. Anatomischer Anzeiger, 130(5), 556. [PubMed: 5048213]

Oka K, Honda MJ, Tsuruga E, Hatakeyama Y, Isokawa K, & Sawa Y (2012). Roles of collagen and periostin expression by cranial neural crest cells during soft palate development. J Histochem Cytochem, 60(1), 57–68. doi:10.1369/0022155411427059 [PubMed: 22205681]

Paluch EK, Nelson CM, Biais N, Fabry B, Moeller J, Pruitt BL, … Federle W (2015). Mechanotransduction: use the force(s). BMC Biol, 13, 47. doi:10.1186/s12915-015-0150-4 [PubMed: 26141078]

Panamonta V, Pradubwong S, Panamonta M, & Chowchuen B (2015). Global Birth Prevalence of Orofacial Clefts: A Systematic Review. J Med Assoc Thai, 98 Suppl 7, S11–21. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26742364 [PubMed: 26742364]

Parry D, Barnes G, & Craig A (1978). A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. Proceedings of the Royal Society of London. Series B. Biological Sciences, 203(1152), 305–321.

Perrault I, Saunier S, Hanein S, Filhol E, Bizet AA, Collins F, … Rozet JM (2012). Mainzer-Saldino syndrome is a ciliopathy caused by IFT140 mutations. Am J Hum Genet, 90(5), 864–870. doi:10.1016/j.ajhg.2012.03.006 [PubMed: 22503633]

Piotrowski T, Ahn DG, Schilling TF, Nair S, Ruvinsky I, Geisler R, … Ho RK (2003). The zebrafish van gogh mutation disrupts tbx1, which is involved in the DiGeorge deletion syndrome in humans. Development, 130(20), 5043–5052. doi:10.1242/dev.00704 [PubMed: 12952905]

Pratt RM Jr, & King C (1972). Inhibition of collagen cross-linking associated with β-aminopropionitrile-induced cleft palate in the rat. Developmental biology, 27(3), 322–328. [PubMed: 4259741]

Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, … Doetschman T (1995). Transforming growth factor-beta 3 is required for secondary palate fusion. Nat Genet, 11(4), 409–414. doi:10.1038/ng1295-409 [PubMed: 7493021]

Ramirez F, Sakai LY, Rifkin DB, & Dietz HC (2007). Extracellular microfibrils in development and disease. Cell Mol Life Sci, 64(18), 2437–2446. doi:10.1007/s00018-007-7166-z [PubMed: 17585369]

Reiter JF, Blacque OE, & Leroux MR (2012). The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. EMBO Rep, 13(7), 608–618. doi:10.1038/embor.2012.73 [PubMed: 22653444]

Reynolds K, Kumari P, Sepulveda Rincon L, Gu R, Ji Y, Kumar S, & Zhou CJ (2019). Wnt signaling in orofacial clefts: crosstalk, pathogenesis and models. Disease Models & Mechanisms, 12(2), dmm037051. doi:10.1242/dmm.037051 [PubMed: 30760477]

Richardson R, Mitchell K, Hammond NL, Mollo MR, Kouwenhoven EN, Wyatt ND, … Dixon J (2017). p63 exerts spatio-temporal control of palatal epithelial cell fate to prevent cleft palate. PLoS Genet, 13(6), e1006828. doi:10.1371/journal.pgen.1006828 [PubMed: 28604778]

Richardson RJ, Dixon J, Jiang R, & Dixon MJ (2009). Integration of IRF6 and Jagged2 signalling is essential for controlling palatal adhesion and fusion competence. Hum Mol Genet, 18(14), 2632–2642. doi:10.1093/hmg/ddp201 [PubMed: 19439425]

Richardson RJ, Dixon J, Malhotra S, Hardman MJ, Knowles L, Boot-Handford RP, … Dixon MJ (2006). Irf6 is a key determinant of the keratinocyte proliferation-differentiation switch. Nat Genet, 38(11), 1329–1334. doi:10.1038/ng1894 [PubMed: 17041603]

Richardson RJ, Hammond NL, Coulombe PA, Saloranta C, Nousiainen HO, Salonen R, … Dixon MJ (2014). Periderm prevents pathological epithelial adhesions during embryogenesis. J Clin Invest, 124(9), 3891–3900. doi:10.1172/JCI71946 [PubMed: 25133425]

Ridley AJ (2006). Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends in Cell Biology, 16(10), 522–529. doi:10.1016/j.tcb.2006.08.006 [PubMed: 16949823]

Rinon A, Lazar S, Marshall H, Buchmann-Moller S, Neufeld A, Elhanany-Tamir H, … Tzahor E (2007). Cranial neural crest cells regulate head muscle patterning and differentiation during vertebrate embryogenesis. Development, 134(17), 3065–3075. doi:10.1242/dev.002501 [PubMed: 17652354]

Robertson SP, Twigg SR, Sutherland-Smith AJ, Biancalana V, Gorlin RJ, Horn D, … Group, O. P.-s. D. C. C. (2003). Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. Nat Genet, 33(4), 487–491. doi:10.1038/ng1119 [PubMed: 12612583]

Rohatgi R, Milenkovic L, & Scott MP (2007). Patched1 regulates hedgehog signaling at the primary cilium. Science, 317(5836), 372–376. doi:10.1126/science.1139740 [PubMed: 17641202]

Romio L, Wright V, Price K, Winyard PJ, Donnai D, Porteous ME, … Feather SA (2003). OFD1, the gene mutated in oral-facial-digital syndrome type 1, is expressed in the metanephros and in human embryonic renal mesenchymal cells. J Am Soc Nephrol, 14(3), 680–689. doi:10.1097/01.asn.0000054497.48394.d2 [PubMed: 12595504]

Saadi I, Alkuraya FS, Gisselbrecht SS, Goessling W, Cavallesco R, Turbe-Doan A, … Maas RL (2011). Deficiency of the Cytoskeletal Protein SPECC1L Leads to Oblique Facial Clefting. American Journal of Human Genetics, 89(1), 44–55. doi:10.1016/j.ajhg.2011.05.023 [PubMed: 21703590]

Saito T, Cui XM, Yamamoto T, Shiomi N, Bringas P Jr., & Shuler CF (2005). Effect of N'-nitrosonornicotine (NNN) on murine palatal fusion in vitro. Toxicology, 207(3), 475–485. doi:10.1016/j.tox.2004.10.015 [PubMed: 15664274]

Saitsu H, Sonoda M, Higashijima T, Shirozu H, Masuda H, Tohyama J, … Matsumoto N (2016). Somatic mutations in GLI3 and OFD1 involved in sonic hedgehog signaling cause hypothalamic hamartoma. Ann Clin Transl Neurol, 3(5), 356–365. doi:10.1002/acn3.300 [PubMed: 27231705]

Sakai D, & Trainor PA (2009). Treacher Collins syndrome: unmasking the role of Tcof1/treacle. Int J Biochem Cell Biol, 41(6), 1229–1232. doi:10.1016/j.biocel.2008.10.026 [PubMed: 19027870]

San Miguel S, Serrano MJ, Sachar A, Henkemeyer M, Svoboda KK, & Benson MD (2011). Ephrin reverse signaling controls palate fusion via a PI3 kinase-dependent mechanism. Dev Dyn, 240(2), 357–364. doi:10.1002/dvdy.22546 [PubMed: 21246652]

Sasaki Y, Ishida S, Morimoto I, Yamashita T, Kojima T, Kihara C, … Tokino T (2002). The p53 family member genes are involved in the Notch signal pathway. J Biol Chem, 277(1), 719–724. doi:10.1074/jbc.M108080200 [PubMed: 11641404]

Satir P, & Christensen ST (2007). Overview of structure and function of mammalian cilia. Annu Rev Physiol, 69, 377–400. doi:10.1146/annurev.physiol.69.040705.141236 [PubMed: 17009929]

Satir P, Pedersen LB, & Christensen ST (2010). The primary cilium at a glance. J Cell Sci, 123(Pt 4), 499–503. doi:10.1242/jcs.050377 [PubMed: 20144997]

Schmidts M, Frank V, Eisenberger T, Al Turki S, Bizet AA, Antony D, … Bergmann C (2013). Combined NGS approaches identify mutations in the intraflagellar transport gene IFT140 in skeletal ciliopathies with early progressive kidney Disease. Hum Mutat, 34(5), 714–724. doi:10.1002/humu.22294 [PubMed: 23418020]

Schneider L, Clement CA, Teilmann SC, Pazour GJ, Hoffmann EK, Satir P, & Christensen ST (2005). PDGFRalphaalpha signaling is regulated through the primary cilium in fibroblasts. Curr Biol, 15(20), 1861–1866. doi:10.1016/j.cub.2005.09.012 [PubMed: 16243034]

Schupbach PM, & Schroeder HE (1983). Cell release from the palatal shelves and the fusion line. J Biol Buccale, 11(3), 227–241. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6581161 [PubMed: 6581161]

Schutte BC, & Murray JC (1999). The many faces and factors of orofacial clefts. Hum Mol Genet, 8(10), 1853–1859. doi:10.1093/hmg/8.10.1853 [PubMed: 10469837]

Senders CW, Peterson EC, Hendrickx AG, & Cukierski MA (2003). Development of the upper lip. Archives of facial plastic surgery, 5(1), 16–25. [PubMed: 12533133]

Shah RM (1979). A cellular mechanism for the palatal shelf reorientation from a vertical to a horizontal plane in hamster: light and electron microscopic study. J Embryol Exp Morphol, 53(Oct), 1–13. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/575387 [PubMed: 575387]

Sharp GC, Ho K, Davies A, Stergiakouli E, Humphries K, McArdle W, … Relton CL (2017). Distinct DNA methylation profiles in subtypes of orofacial cleft. Clin Epigenetics, 9, 63. doi:10.1186/s13148-017-0362-2 [PubMed: 28603561]

Shears L, Plowright L, Harrington K, Pandha HS, & Morgan R (2008). Disrupting the interaction between HOX and PBX causes necrotic and apoptotic cell death in the renal cancer lines CaKi-2 and 769-P. J Urology, 180(5), 2196–2201.

Shi J, Son MY, Yamada S, Szabova L, Kahan S, Chrysovergis K, … Holmbeck K (2008). Membrane-type MMPs enable extracellular matrix permissiveness and mesenchymal cell proliferation during embryogenesis. Developmental biology, 313(1), 196–209. doi:10.1016/j.ydbio.2007.10.017 [PubMed: 18022611]

Shuler CF, Halpern DE, Guo Y, & Sank AC (1992). Medial edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation in vivo. Dev Biol, 154(2), 318–330. doi:10.1016/0012-1606(92)90071-n [PubMed: 1385235]

Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Kronig C, … Walz G (2005). Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat Genet, 37(5), 537–543. doi:10.1038/ng1552 [PubMed: 15852005]

Singh GD, Johnston J, Ma W, & Lozanoff S (1998). Cleft palate formation in fetal Br mice with midfacial retrusion: tenascin, fibronectin, laminin, and type IV collagen immunolocalization. Cleft Palate Craniofac J, 35(1), 65–76. doi:10.1597/1545-1569_1998_035_0065_cpfifb_2.3.co_2 [PubMed: 9482226]

Singh GD, Moxham BJ, Langley MS, & Embery G (1997). Glycosaminoglycan biosynthesis during 5-fluoro-2-deoxyuridine-induced palatal clefts in the rat. Arch Oral Biol, 42(5), 355–363. doi:10.1016/s0003-9969(97)00031-9 [PubMed: 9233844]

Singh GD, Moxham BJ, Langley MS, Waddington RJ, & Embery G (1994). Changes in the composition of glycosaminoglycans during normal palatogenesis in the rat. Arch Oral Biol, 39(5), 401–407. doi:10.1016/0003-9969(94)90170-8 [PubMed: 8060263]

Sischo L, Wilson-Genderson M, & Broder HL (2017). Quality-of-Life in Children with Orofacial Clefts and Caregiver Well-being. J Dent Res, 96(13), 1474–1481. doi:10.1177/0022034517725707 [PubMed: 28813183]

Smane L, Pilmane M, & Akota I (2013). Apoptosis and MMP-2, TIMP-2 expression in cleft lip and palate. Stomatologija, 15(4), 129–134. [PubMed: 24589636]

Song L, Li Y, Wang K, Wang YZ, Molotkov A, Gao L, … Zhou CJ (2009). Lrp6-mediated canonical Wnt signaling is required for lip formation and fusion. Development, 136(18), 3161–3171. doi:10.1242/dev.037440 [PubMed: 19700620]

Song Y, & Zhang S (2011). Association of CDH1 promoter polymorphism and the risk of non-syndromic orofacial clefts in a Chinese Han population. Arch Oral Biol, 56(1), 68–72. doi:10.1016/j.archoralbio.2010.08.019 [PubMed: 20880515]

Sorrentino A, Thakur N, Grimsby S, Marcusson A, von Bulow V, Schuster N, … Landstrom M (2008). The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. Nat Cell Biol, 10(10), 1199–1207. doi:10.1038/ncb1780 [PubMed: 18758450]

Sozen MA, Suzuki K, Tolarova MM, Bustos T, Fernandez Iglesias JE, & Spritz RA (2001). Mutation of PVRL1 is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. Nat Genet, 29(2), 141–142. doi:10.1038/ng740 [PubMed: 11559849]

Steffek AJ, Watkins CA, & Verrusio AC (1972). Cleft-Palate in Rodents after Maternal Treatment with Various Lathyrogenic Agents. Teratology, 5(1), 33-&. doi:DOI 10.1002/tera.1420050107 [PubMed: 5014450]

Stephan A, Vaughan S, Shaw MK, Gull K, & McKean PG (2007). An essential quality control mechanism at the eukaryotic basal body prior to intraflagellar transport. Traffic, 8(10), 1323–1330. doi:10.1111/j.1600-0854.2007.00611.x [PubMed: 17645436]

Sun D, Baur S, & Hay ED (2000). Epithelial-mesenchymal transformation is the mechanism for fusion of the craniofacial primordia involved in morphogenesis of the chicken lip. Dev Biol, 228(2), 337–349. doi:10.1006/dbio.2000.9946 [PubMed: 11112334]

Sun D, Vanderburg CR, Odierna GS, & Hay ED (1998). TGFbeta3 promotes transformation of chicken palate medial edge epithelium to mesenchyme in vitro. Development, 125(1), 95–105. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9389667 [PubMed: 9389667]

Sun S, Fisher RL, Bowser SS, Pentecost BT, & Sui H (2019). Three-dimensional architecture of epithelial primary cilia. Proc Natl Acad Sci U S A, 116(19), 9370–9379. doi:10.1073/pnas.1821064116 [PubMed: 31004057]

Sun ZQ, Guo SS, & Fassler R (2016). Integrin-mediated mechanotransduction. Journal of Cell Biology, 215(4), 445–456. doi:10.1083/jcb.201609037

Suwa F, Jin Y, Lu H, Li X, Tipoe GL, Lau TY, … Fang YR (2001). Alteration of apoptosis in cleft palate formation and ectomesenchymal stem cells influenced by retinoic acid. Okajimas Folia Anat Jpn, 78(5), 179–186. doi:10.2535/ofaj1936.78.5_179 [PubMed: 11915360]

Suzuki K, Hu D, Bustos T, Zlotogora J, Richieri-Costa A, Helms JA, & Spritz RA (2000). Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nature Genetics, 25(4), 427–430. Retrieved from <Go to ISI>://WOS:000088615000019 [PubMed: 10932188]

Svitkina T (2018). The Actin Cytoskeleton and Actin-Based Motility. Cold Spring Harbor Perspectives in Biology, 10(1). doi:ARTN a018267 10.1101/cshperspect.a018267

Tadeu AM, & Horsley V (2013). Notch signaling represses p63 expression in the developing surface ectoderm. Development, 140(18), 3777–3786. doi:10.1242/dev.093948 [PubMed: 23924630]

Takigawa T, & Shiota K (2004). Terminal differentiation of palatal medial edge epithelial cells in vitro is not necessarily dependent on palatal shelf contact and midline epithelial seam formation. Int J Dev Biol, 48(4), 307–317. doi:10.1387/ijdb.041840tt [PubMed: 15300511]

Tamarin A, & Boyde A (1977). Facial and visceral arch development in the mouse embryo: a study by scanning electron microscopy. J Anat, 124(Pt 3), 563–580. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/604328 [PubMed: 604328]

Tang FC, Barbacioru C, Bao SQ, Lee C, Nordman E, Wang XH, … Surani MA (2010). Tracing the Derivation of Embryonic Stem Cells from the Inner Cell Mass by Single-Cell RNA-Seq Analysis. Cell Stem Cell, 6(5), 468–478. doi:10.1016/j.stem.2010.03.015 [PubMed: 20452321]

Taniguchi K, Sato N, & Uchiyama Y (1995). Apoptosis and heterophagy of medial edge epithelial cells of the secondary palatine shelves during fusion. Arch Histol Cytol, 58(2), 191–203. doi:10.1679/aohc.58.191 [PubMed: 7576871]

Theocharis AD, Skandalis SS, Gialeli C, & Karamanos NK (2016). Extracellular matrix structure. Adv Drug Deliv Rev, 97, 4–27. doi:10.1016/j.addr.2015.11.001 [PubMed: 26562801]

Thiel C, Kessler K, Giessl A, Dimmler A, Shalev SA, von der Haar S, … Rauch A (2011). NEK1 mutations cause short-rib polydactyly syndrome type majewski. Am J Hum Genet, 88(1), 106–114. doi:10.1016/j.ajhg.2010.12.004 [PubMed: 21211617]

Thomas S, Legendre M, Saunier S, Bessieres B, Alby C, Bonniere M, … Attie-Bitach T (2012). TCTN3 mutations cause Mohr-Majewski syndrome. Am J Hum Genet, 91(2), 372–378. doi:10.1016/j.ajhg.2012.06.017 [PubMed: 22883145]

Tinkle CL, Pasolli HA, Stokes N, & Fuchs E (2008). New insights into cadherin function in epidermal sheet formation and maintenance of tissue integrity. Proc Natl Acad Sci U S A, 105(40), 15405–15410. doi:10.1073/pnas.0807374105 [PubMed: 18809908]

Tobin JL, Di Franco M, Eichers E, May-Simera H, Garcia M, Yan J, … Beales PL (2008). Inhibition of neural crest migration underlies craniofacial dysmorphology and Hirschsprung's disease in Bardet-Biedl syndrome. Proc Natl Acad Sci U S A, 105(18), 6714–6719. doi:10.1073/pnas.0707057105 [PubMed: 18443298]

Trainor PA (2010). Craniofacial birth defects: The role of neural crest cells in the etiology and pathogenesis of Treacher Collins syndrome and the potential for prevention. Am J Med Genet A, 152A(12), 2984–2994. doi:10.1002/ajmg.a.33454 [PubMed: 20734335]

Trasler D, & Ohannessian L (1983). Ultrastructure of initial nasal process cell fusion in spontaneous and 6-aminonicotinamide-induced mouse embryo cleft lip. Teratology, 28(1), 91–101. [PubMed: 6227102]

Trasler DG (1968). Pathogenesis of cleft lip and its relation to embryonic face shape in A/J and C57BL mice. Teratology, 1(1), 33–49. [PubMed: 5696816]

Traub P (2012). Intermediate filaments: a review: Springer Science & Business Media.

Tudela C, Formoso MA, Martinez T, Perez R, Aparicio M, Maestro C, … Martinez-Alvarez C (2002). TGF-beta3 is required for the adhesion and intercalation of medial edge epithelial cells during palate fusion. Int J Dev Biol, 46(3), 333–336. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12068957 [PubMed: 12068957]

Tukachinsky H, Lopez LV, & Salic A (2010). A mechanism for vertebrate Hedgehog signaling: recruitment to cilia and dissociation of SuFu-Gli protein complexes. J Cell Biol, 191(2), 415–428. doi:10.1083/jcb.201004108 [PubMed: 20956384]

Tümpel S, Wiedemann LM, & Krumlauf R (2009). Hox genes and segmentation of the vertebrate hindbrain. Curr Top Dev Biol, 88, 103–137. [PubMed: 19651303]

Turkmen M, Temocin K, Acar C, Levi E, Karaman C, Inan G, & Elcioglu N (2003). Short rib-polydactyly syndrome: a case report. Turk J Pediatr, 45(4), 359–362. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14768808 [PubMed: 14768808]

Twigg SR, & Wilkie AO (2015). New insights into craniofacial malformations. Hum Mol Genet, 24(R1), R50–59. doi:10.1093/hmg/ddv228 [PubMed: 26085576]

Vanderas AP (1987). Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J, 24(3), 216–225. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3308178 [PubMed: 3308178]

Vasioukhin V, Degenstein L, Wise B, & Fuchs E (1999). The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc Natl Acad Sci U S A, 96(15), 8551–8556. doi:10.1073/pnas.96.15.8551 [PubMed: 10411913]

Vaziri Sani F, Hallberg K, Harfe BD, McMahon AP, Linde A, & Gritli-Linde A (2005). Fate-mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. Dev Biol, 285(2), 490–495. doi:10.1016/j.ydbio.2005.07.027 [PubMed: 16109396]

Veeman MT, Slusarski DC, Kaykas A, Louie SH, & Moon RT (2003). Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. Curr Biol, 13(8), 680–685. doi:10.1016/s0960-9822(03)00240-9 [PubMed: 12699626]

Verloes A, Di Donato N, Masliah-Planchon J, Jongmans M, Abdul-Raman OA, Albrecht B, … Pilz DT (2015). Baraitser-Winter cerebrofrontofacial syndrome: delineation of the spectrum in 42 cases. Eur J Hum Genet, 23(3), 292–301. doi:10.1038/ejhg.2014.95 [PubMed: 25052316]

Vertii A, Hung HF, Hehnly H, & Doxsey S (2016). Human basal body basics. Cilia, 5, 13. doi:10.1186/s13630-016-0030-8 [PubMed: 26981235]

Wagner DE, Weinreb C, Collins ZM, Briggs JA, Megason SG, & Klein AM (2018). Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo. Science, 360(6392), 981-+. doi:10.1126/science.aar4362 [PubMed: 29700229]

Wang KY, Juriloff DM, & Diewert VM (1995). Deficient and delayed primary palatal fusion and mesenchymal bridge formation in cleft lip-liable strains of mice. J Craniofac Genet Dev Biol, 15(3), 99–116. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8642057 [PubMed: 8642057]

Wang Q, Kurosaka H, Kikuchi M, Nakaya A, Trainor PA, & Yamashiro T (2019). Perturbed development of cranial neural crest cells in association with reduced sonic hedgehog signaling underlies the pathogenesis of retinoic-acid-induced cleft palate. Dis Model Mech, 12(10). doi:10.1242/dmm.040279

Waterman RE, Ross LM, & Meller SM (1973). Alterations in the epithelial surface of A-Jax mouse palatal shelves prior to and during palatal fusion: a scanning electron microscopic study. Anat Rec, 176(3), 361–375. doi:10.1002/ar.1091760311 [PubMed: 4716420]

Wehby GL, & Cassell CH (2010). The impact of orofacial clefts on quality of life and healthcare use and costs. Oral Dis, 16(1), 3–10. doi:10.1111/j.1601-0825.2009.01588.x [PubMed: 19656316]

Westfall MD, Mays DJ, Sniezek JC, & Pietenpol JA (2003). The Delta Np63 alpha phosphoprotein binds the p21 and 14-3-3 sigma promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol Cell Biol, 23(7), 2264–2276. doi:10.1128/mcb.23.7.2264-2276.2003 [PubMed: 12640112]

Wheway G, Nazlamova L, & Hancock JT (2018). Signaling through the Primary Cilium. Front Cell Dev Biol, 6, 8. doi:10.3389/fcell.2018.00008 [PubMed: 29473038]

Wilson NR, Olm-Shipman AJ, Acevedo DS, Palaniyandi K, Hall EG, Kosa E, … Saadi I (2016). SPECC1L deficiency results in increased adherens junction stability and reduced cranial neural crest cell delamination. Sci Rep, 6, 17735. doi:10.1038/srep17735 [PubMed: 26787558]

Wu C, Zhu X, Liu W, Ruan T, & Tao K (2017). Hedgehog signaling pathway in colorectal cancer: function, mechanism, and therapy. Onco Targets Ther, 10, 3249–3259. doi:10.2147/OTT.S139639 [PubMed: 28721076]

Wu YJ, La Pierre DP, Wu J, Yee AJ, & Yang BB (2005). The interaction of versican with its binding partners. Cell Research, 15(7), 483–494. doi:DOI 10.1038/sj.cr.7290318 [PubMed: 16045811]

Wyatt AW, Osborne RJ, Stewart H, & Ragge NK (2010). Bone morphogenetic protein 7 (BMP7) mutations are associated with variable ocular, brain, ear, palate, and skeletal anomalies. Hum Mutat, 31(7), 781–787. doi:10.1002/humu.21280 [PubMed: 20506283]

Xu X, Han J, Ito Y, Bringas P Jr., Deng C, & Chai Y (2008). Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. Dev Cell, 15(2), 322–329. doi:10.1016/j.devcel.2008.06.004 [PubMed: 18694570]

Yakovlev AG, Di Giovanni S, Wang G, Liu W, Stoica B, & Faden AI (2004). BOK and NOXA are essential mediators of p53-dependent apoptosis. J Biol Chem, 279(27), 28367–28374. doi:10.1074/jbc.M313526200 [PubMed: 15102863]

Yan LY, Yang MY, Guo HS, Yang L, Wu J, Li R, … Tang FC (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol, 20(9), 1131-+. doi:10.1038/nsmb.2660 [PubMed: 23934149]

Yang LT, & Kaartinen V (2007). Tgfb1 expressed in the Tgfb3 locus partially rescues the cleft palate phenotype of Tgfb3 null mutants. Dev Biol, 312(1), 384–395. doi:10.1016/j.ydbio.2007.09.034 [PubMed: 17967447]

Yang Y, Pan X, Lei W, Wang J, & Song J (2006). Transforming growth factor-beta1 induces epithelial-to-mesenchymal transition and apoptosis via a cell cycle-dependent mechanism. Oncogene, 25(55), 7235–7244. doi:10.1038/sj.onc.1209712 [PubMed: 16799646]

Yee GW, & Abbott UK (1978). Facial development in normal and mutant chick embryos. I. Scanning electron microscopy of primary palate formation. Journal of Experimental Zoology, 206(3), 307–321.

Yokosaki Y, Matsuura N, Higashiyama S, Murakami I, Obara M, Yamakido M, … Sheppard D (1998). Identification of the ligand binding site for the integrin alpha(theta)beta(1) in the third fibronectin type III repeat of tenascin-C. Journal of Biological Chemistry, 273(19), 11423–11428. doi:DOI 10.1074/jbc.273.19.11423

Yoshida M, Shimono Y, Togashi H, Matsuzaki K, Miyoshi J, Mizoguchi A, … Takai Y (2012). Periderm cells covering palatal shelves have tight junctions and their desquamation reduces the polarity of palatal shelf epithelial cells in palatogenesis. Genes Cells, 17(6), 455–472. doi:10.1111/j.1365-2443.2012.01601.x [PubMed: 22571182]

Yu L, Gu S, Alappat S, Song Y, Yan M, Zhang X, … Chen Y (2005). Shox2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. Development, 132(19), 4397–4406. doi:10.1242/dev.02013 [PubMed: 16141225]

Yu W, Ruest LB, & Svoboda KK (2009). Regulation of epithelial-mesenchymal transition in palatal fusion. Exp Biol Med (Maywood), 234(5), 483–491. doi:10.3181/0812-MR-365 [PubMed: 19234053]

Yu W, Serrano M, Miguel SS, Ruest LB, & Svoboda KK (2009). Cleft lip and palate genetics and application in early embryological development. Indian J Plast Surg, 42 Suppl, S35–50. doi:10.4103/0970-0358.57185 [PubMed: 19884679]

Zaghloul NA, & Brugmann SA (2011). The emerging face of primary cilia. Genesis, 49(4), 231–246. doi:10.1002/dvg.20728 [PubMed: 21305689]

Zhang J, Yang R, Liu ZY, Hou CZ, Zong W, Zhang AZ, … Gao JG (2015). Loss of lysyl oxidase-like 3 causes cleft palate and spinal deformity in mice. Human Molecular Genetics, 24(21), 6174–6185. doi:10.1093/hmg/ddv333 [PubMed: 26307084]

Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, & Chen Y (2002). Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. Development, 129(17), 4135–4146. [PubMed: 12163415]

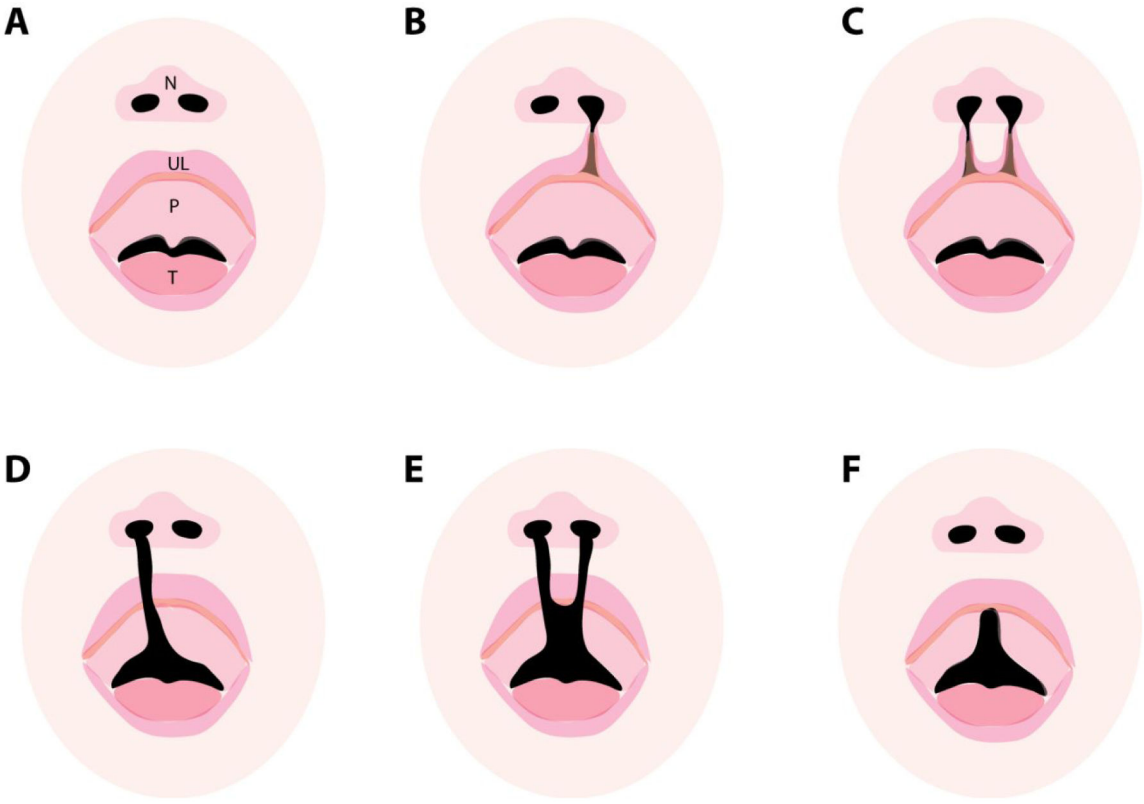

Figure 1. Illustrative diagrams for major types of orofacial clefts in humans.

(**A**) Normally fused orofacial structures. (**B**) Unilateral cleft lip (can be left or right with varied severities). (**C**) Bilateral cleft lip. (**D**) Unilateral cleft lip with cleft palate. (**E**) Bilateral cleft lip with cleft palate. (**F**) Cleft palate only. N, nose; P, palate; T, tongue; UP, upper lip.

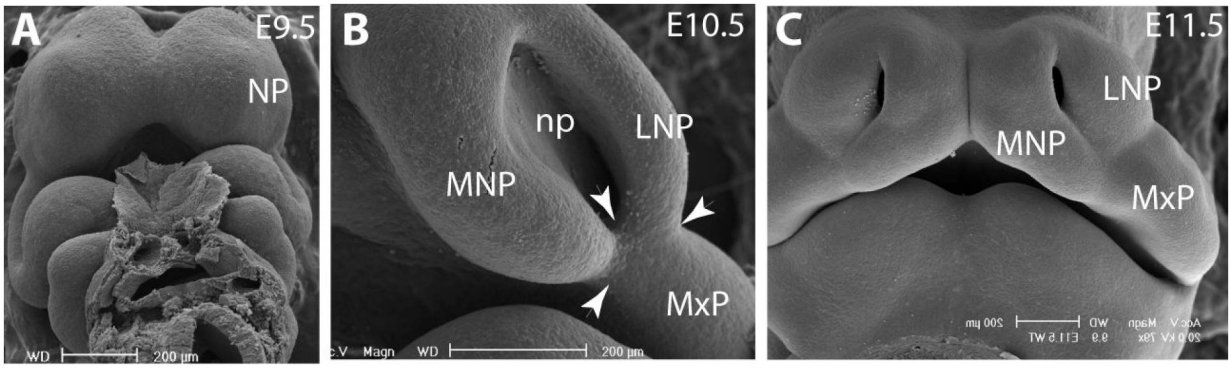

Figure 2. Scanning electron microscopy of the upper lip morphogenesis in mouse embryos (modified from (L. Song et al., 2009)).

Front facial views show the key steps of the upper lip formation from E9.5 to E11.5. (**A**) The nasal placode (NP) can be identified by the formation of rich microvilli (not shown) on the surface at E9.5. (**B**) Following the formation and outgrowth of the medial nasal prominence (MNP) and lateral nasal prominence (LNP), the nasal pit (np) becomes evident at E10.5. The MNP and LNP merge ventrally and will fuse together with the maxillary prominence (MxP) at the lambdoidal (λ) junction (indicated by arrowheads). (**C**) At E11.5, the ventral MNP is widely connected and fused with both the LNP and the MxP, and the paired MNPs are dorsally attached at the facial midline.

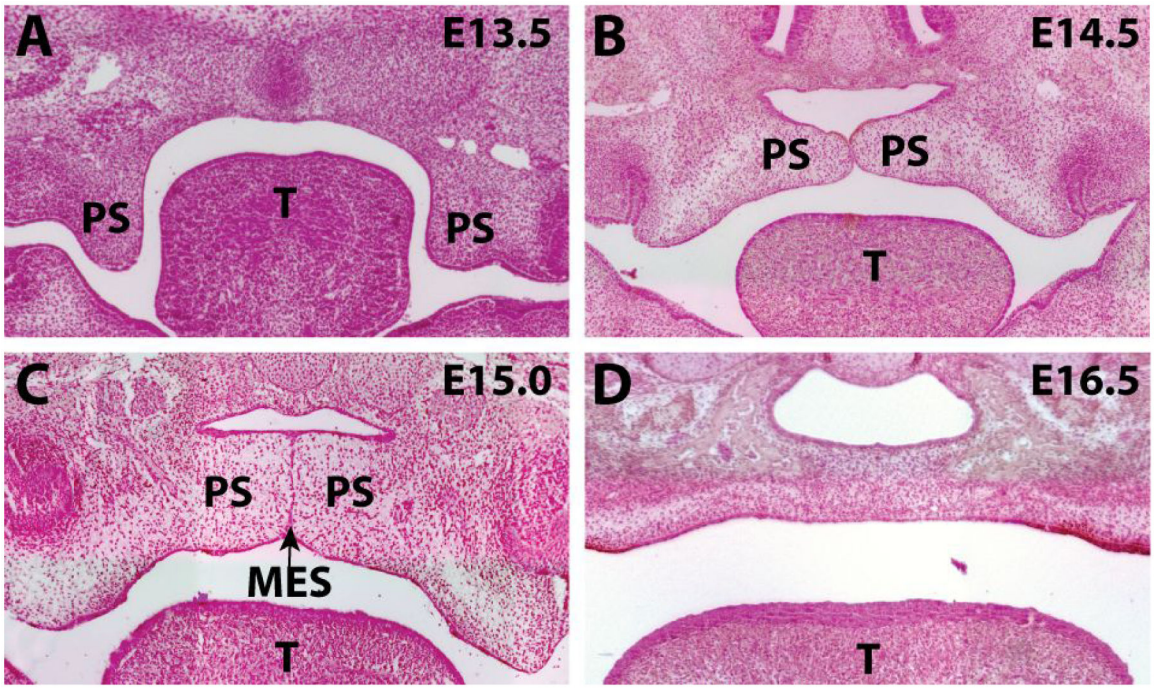

Figure 3. Dynamic morphological changes during palatogenesis in mouse embryos.

Representative coronal sections stained by H&E demonstrate the key steps of palatogenesis, including the vertical growth of the palatal shelves (PS) alongside the tongue (T) at E13.5 (**A**), horizontal elevation and contact at the midline at E14.5 (**B**), the formation of medial epithelial seem (MES) at E15.0 (**C**), and the completion of palatal fusion at E16.5 (**D**).

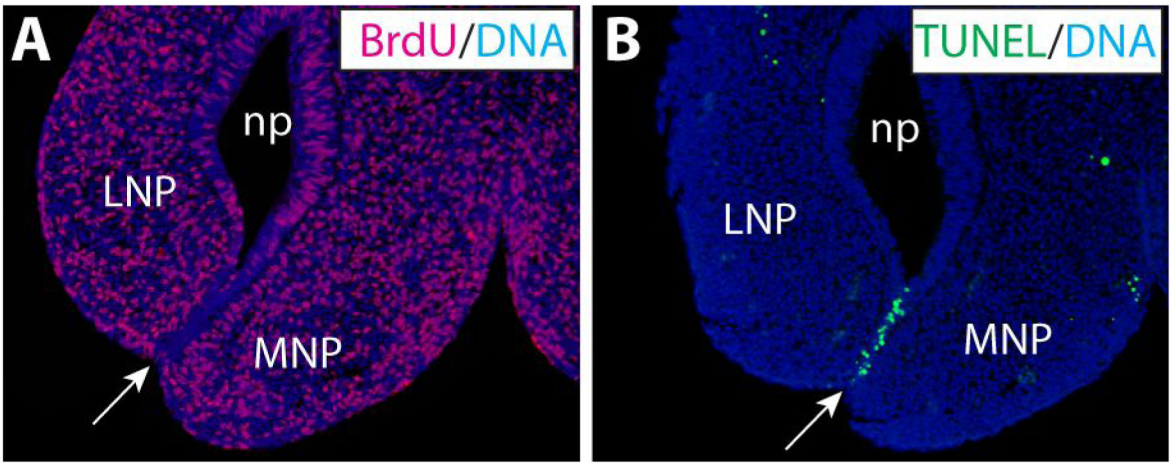

Figure 4. Proliferation and apoptosis during lip fusion at E11.5 in mouse embryos (modified from (L. Song et al., 2009)).

(A) Immunohistochemistry demonstrates robust BrdU incorporation (red, indicating proliferation) in the majority of orofacial primordial cells, except the epithelial seem cells (arrow) at the fusion site. (**B**) TUNEL assay demonstrates the programmed cell death (green) of the epithelial seem at the fusion site (arrow). LNP, lateral nasal prominence; MNP, medial nasal prominence; np, nasal pit.

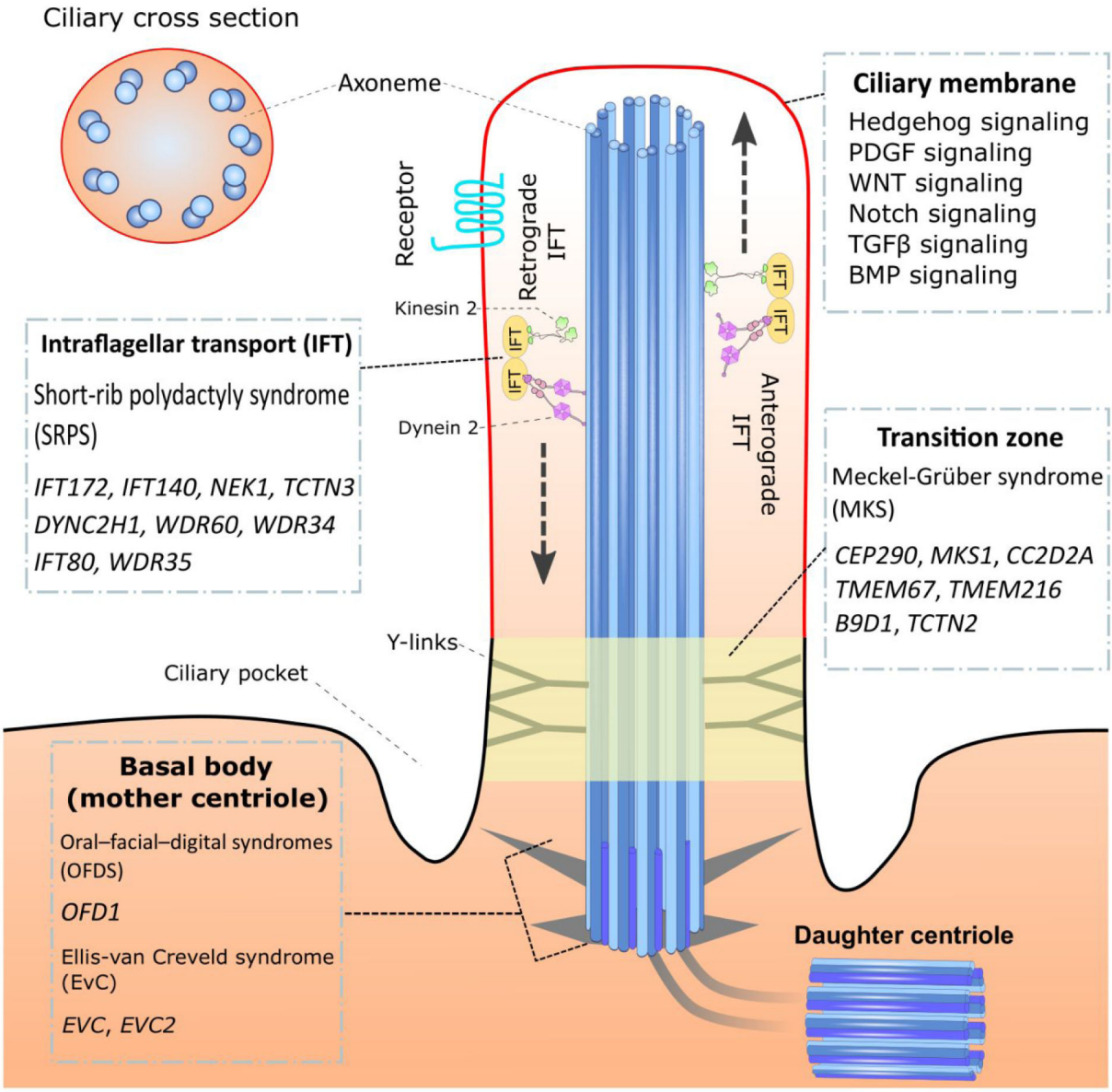

Figure 5. Illustrative diagram of the primary ciliary structure.

The primary cilium is a membrane-bound organelle that extends from the basal body (mother centriole). The axoneme of a primary ciliary contains nine doublet microtubules. The transition zone is localized at the proximal region of the cilium from the distal end of the basal body, defined by the presence of Y-links. The intraflagellar transport (IFT) system mediates the transport of ciliary cargos. The ciliary membrane (red) is enriched with receptors for signaling transduction. Several orofacial developmental signaling pathways that may act through the primary cilia are listed. Craniofacial ciliopathies with orofacial clefts that caused by disrupting the major ciliary components and associated genes are also listed.