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Revisiting Genetics of Cleft Lip with or without Cleft Palate and Cleft Palate Only: A Narrative Review

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ABSTRACT

Cleft lip with or without cleft palate (CLP) and cleft palate only (CP) are the most common orofacial deformities observed in humans where almost 1 in 700 to 1 in 2,000 babies born each year are affected worldwide. This condition occurs when the specific and independently derived facial primordial fails to fuse together, hence forming the cleft of the lip and palate or the palate alone. These orofacial abnormalities can be divided into syndromic and non-syndromic where the deformities are either associated with other disorders or present on their own, respectively. It is important to understand every step in the lip and palate development during the embryonic stage to pinpoint the exact problem affecting the normal development of the human face. With current technologies, more genes are identified to be associated with and cause CLP and CP in humans. Therefore, this review aims to elaborate on the latest updates on the genetics of CLP and CP. Polymorphism in some of the genes has been associated with the incidence of these anomalies. Identification of these genes provides new knowledge on how these craniofacial abnormalities occur and hopefully will enable earlier treatment of these deformities to be implemented.

Keywords: *Cleft and genetics*; *cleft lip and palate; cleft palate; craniofacial; facial development*

INTRODUCTION

Cleft lip with or without cleft palate (CLP) and cleft palate only (CP) are the most common craniofacial abnormalities observed in human which it is caused by the failure of the independently derived facial primordia to completely fuse with their anatomical counterparts, leading to an abnormality of the orofacial development. These abnormalities have multifactorial aetiology, comprising both genetic and environmental factors (Leslie & Marazita, 2013). CLP and CP can occur with or

without associated malformation or disorder, which are identified as either syndromic or non-syndromic, respectively. The incidence of craniofacial birth defects varies where CLP affects approximately 1/700 individuals (Kohli & Kohli, 2012) while the incidence for CP is approximately 1 in 2,500 births (Atukorala & Ratnayake, 2021) which depends on the geographical locations and ethnicities (Vanderas, 1987). These conditions cause the affected individuals to face some difficulties in feeding, speech, hearing, and dental problems (Leslie & Marazita, 2013). Additionally, the patients also encountered some psychological problems and financial burdens of the treatment process (Al-Namankany & Alhubaishi, 2018; Martin *et al.*, 2020).

CLP is a deformity that occurs when the lip or mouth of a baby does not properly form during pregnancy (Kohli & Kohli, 2012). It causes the upper lip to open for the baby, which could be a small slit or a large opening all the way through to the nose. A cleft palate is another deformity that happens when the tissue that forms the palate fails to fuse during the pregnancy. This condition could either lead to the opening of both the front and back parts of the palate or only one part, front, or back part of the palate. Non-syndromic CLP comprises most of the orofacial cleft. It is a condition where babies are born with CLP as an isolated condition unassociated with any recognisable anomalies (Kohli & Kohli, 2012). In the past, there were a lot of genes that had been identified to cause non-syndromic CLP. With recent technologies, more research has been conducted and more genes have been identified and found to be associated with CLP and CP.

For years, an animal model such as a mouse (*Mus musculus*) has been utilised as means to understand the developmental process of human craniofacial development as the results obtained from the animal study could be extrapolated to the human embryonic developmental process (Lough *et al.*, 2017). Besides the mammalian model, one of the current methods that has been used to increase the robustness of obtaining research data is by using fish as the animal model. Fish models have advantages over mammalian models because of the faster life cycle, transparent body tissue, embryos and their skeletons are only made up of a few layers of cells (Atukorala & Ratnayake, 2021). Fish models are now commonly acknowledged as suitable animal models for researching human diseases, owing to the relatively expensive cost of mammalian research and the significant evolutionary gap between invertebrate and fish models (Ericsson *et al.*, 2013). Skeletogenic research including skull development (medaka, zebrafish, carp), neurogenesis and head development (Mexican cavefish, zebrafish), and comparative analysis of tooth formation mechanisms are increasingly being studied using the fish models (Machado & Eames, 2017; Atukorala *et al.*, 2019). Whole genome sequencing data is available for most fish models, and the genomic data obtained via this technology enhances the ability to identify human illnesses based on the available genetic information extrapolated from fish and other animal models (Atukorala & Ratnayake, 2021).

As far as population genetics is concerned, the advancement in the genetic analysis tools for DNA sequencing such as next generation sequencing (NGS) involving genotyping and sequencing has enabled more association studies between the susceptible genes and craniofacial abnormalities to be highlighted. While studies using exome/genome sequencing (WES/WGS) are primarily concentrated on uncommon or rare genetic alterations, the genome-wide association studies (GWAS) approach offers an indirect mapping technique that can find related polymorphisms and associate it with the incidence of the disease (Martinelli *et al.*, 2020).

Due to the availability of these technologies in the genetic field, more genetic data has been published. Therefore, this review presents the latest updates on the genetics of

CLP and CP. Genes that have been recently associated with the incidence of CLP and CP are described accordingly.

Embryology of Facial Development

The morphology of the face is shaped by a combination of five fundamental facial prominences (Vyas *et al.*, 2020). The basic morphology of the face is established between the fourth and tenth week after fertilisation (Smarius *et al.*, 2017). Development of the secondary palate begins in the sixth week from medial projections of paired maxillary processes, which later will fuse with the primary palate (Worley *et al.*, 2018). The upper lip is formed when the superficial facial prominences merged (Greene & Pisano, 2010).

Formation of the upper lip and primary palate

The upper lip started to form after 24 days of fertilisation and completed by day 37 (Sperber *et al.,* 2010). Neural tubes formed from the ectoderm in the neural plate folds at the fifth week of gestation and then differentiated to form the ectomesenchyme (Cordero *et al.*, 2011). The ectomesenchyme then forms the five fundamental prominences, namely the facial prominence, the paired maxillary prominences, and the paired mandibular prominences. The nasal placodes formed in the fifth week of gestation from the nasal component of frontonasal prominence. The placodes then invaginate to form the nasal pits, thus dividing the frontonasal prominence into the medial and lateral nasal processes. The lip is formed by both medial and lateral nasal processes. The medial upper lip is formed by the medial nasal process; the lateral nasal process forms the lateral aspect of the nose while the maxillary process forms the cheek and the lateral part of the upper lip (Deshpande & Goudy, 2019). Both processes merged during the sixth week of gestation to form the upper lip. Failure to merge both processes will cause the formation of cleft lip (Smarius *et al.*, 2017).

Development of the primary palate begins after the cranial neural crest cells migrate from the dorsal part of the anterior neural tube to the facial region, where they produce five different facial primordia. The medial nasal process will merge and form an intermaxillary segment which later will form the middle part of the upper lip and provide the basis for the primary palate (Ansari & Bordoni, 2022). The primary palate established the premaxillary part of the maxilla which holds the incisor teeth and eventually forms a small part of the hard palate which is anterior to the incisive foramen. The incidence of cleft of the lip affects the normal formation of the upper lip which could extend to the anterior part of the maxilla (Moore *et al.*, 2012).

Formation of the secondary palate

The secondary palate begins its development in the sixth week from medial projections of the paired maxillary process of the palatal shelves. During palatogenesis, the palatal shelves grow medially towards each other on each side of the developing tongue, and then elevate, causing the tongue to lie below it and fuse horizontally with the primary palate (Bush & Jiang, 2012; Jankowski & Márquez, 2016). The epithelium from both palatal shelves adheres together to produce a single line known as medial edge epithelia (MEE) (Paiva *et al.*, 2019). Once this process is completed, intramembranous ossification will take place in the anterior two-thirds of the palate to form a mineralised palate and the posterior third will form a soft palate which is a fibromuscular tissue. This mechanism is under the control of various signalling factors, such as bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), sonic hedgehog (SHH), vascular endothelial growth factor (VEGF), and Wingless and Int-1 (WNT) (Nassif *et al.*, 2014; Iyyanar & Nazarali, 2017; Zhang *et al.*, 2017). The epithelial and mesenchymal cells involved in early embryonic facial development were driven by a complex regulatory mechanism. If this growth mechanism is disturbed, it will

cause the orofacial cleft to occur (Martinelli *et al*., 2020).

As mentioned above, the formation of the primary palate and secondary palate involves two distinct embryonic developmental processes. There are a lot of genes and mechanisms that guide the growth of the lip and palate, and this can be illustrated in Fig. 1, where specific genes and factors had been studied previously to guide the formation of the lip and palate (Stanier & Moore, 2004). These processes are very complex, and they require a lot of interaction between the cells and signalling one another, thus resulting in the formation of the body itself. Any alteration involving the interactions among these cells such as deletion, mutation, excess, or loss of functions, can cause the failure of the process to be normally executed thus resulting in the inability for the tissues to undergo normal developmental process leading to cleft of the lips and/or palate. The introduction of environmental risk factors such as antibiotic drug use, maternal pesticide exposure, paternal smoking, and threatened abortion, also can amplify the risk of failure of these processes (Hong *et al*., 2021).

Fig. 1 illustrates the formation of lip and palate as well as some of the genes that have been identified to regulate the process of palate development. Some of the genes are expressed in the epithelium of the palate and some are expressed in the mesenchyme. Fig. 2 focuses on the normal developmental stages of palatogenesis of mouse embryos. Table 1 highlights some of the functions and roles played by the genes involved during normal craniofacial development leading to the formation of lip and palate.

Table 1 Roles of some of the known identified genes important in the formation of lip and palate development (reviewed in Stanier & Moore, 2004; Bush & Jiang, 2012; Parada & Chai, 2012; Lan *et al*., 2015; Deshpande & Goudy, 2019; Martinelli *et al*., 2020)

Roles	Genes	References
Regulation of epithelial-mesenchymal interactions and initial building of palatal shelf	*TGFB2, GLI2, GLI3, MSX1, SHH, BMP, PAX9, FGF10*	Satokata & Maas (1994); Peters *et al.* (1998); Zhang *et al.* (2002); Rice *et al.* (2006); Veistinen *et al.* (2009)
Formation and elevation of the palatal shelf	*PDGFC, HOXA2, FOXF2*	Wang *et al.* (2003); Ding *et al.* (2004)
Adhesion and fusion of palatal shelves horizontally in midline	*TGFB3, RUNX1*	Fitzpatrick *et al.* (1990); Kaartinen *et al.* (1995); Proetzel *et al.* (1995); Charoenchaikorn *et al.* (2009)

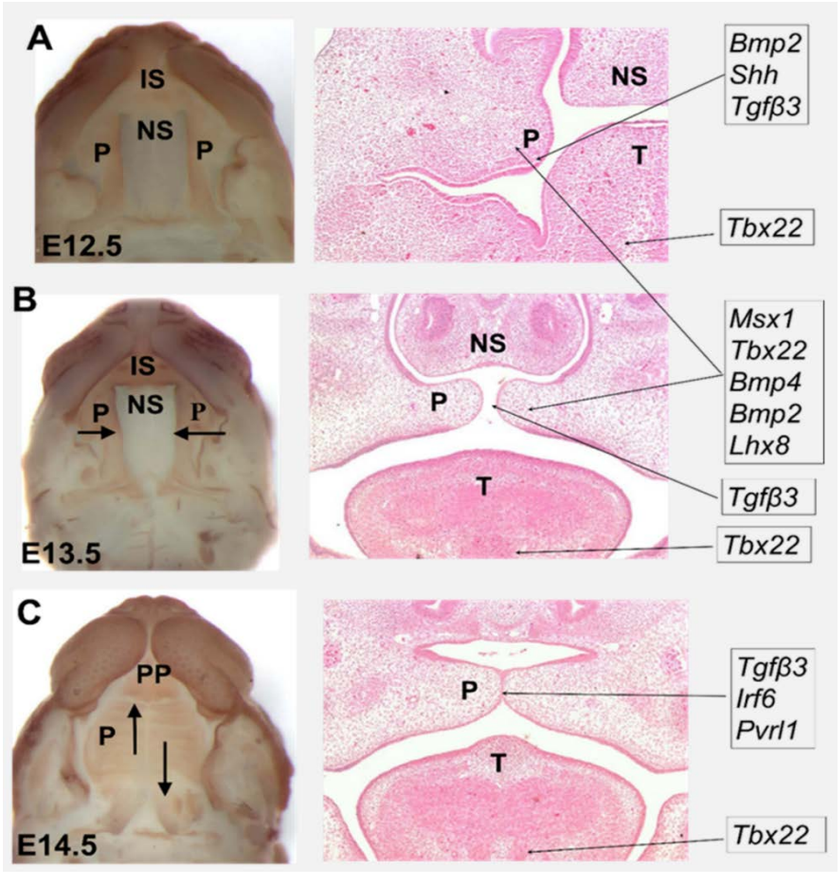

Fig. 1 Palatal view of E12.5-E14.5 wild-type mouse embryos and paired Hematoxylin and Eosin (H&E) stained coronal sections. (A) At E12.5 (human week ~7), the intermaxillary segment (IS) is formed by the merging of the medial nasal prominences internally, while the maxillary prominences give rise to the lateral part of the lips and the budding of the palatal shelves (P), which grow vertically to the tongue (T). The nasal septum (NS) is exposed. (B) At E13.5 (human week ~8), the palatal shelves elevate to a horizontal position above the tongue. Arrows indicate the initial palatal shelf contact and fusion. (C) At E14.5 (human week ~9–10), the intermaxillary segment becomes the philtrum of the upper lip and the primary palate (PP). The palatal shelves fused anteriorly and posteriorly (arrows) together with nasal septum. The disruption of the epithelial seam ensures the complete process of secondary palate development. Some of the genes, such as *Tgfβ3*, are specifically expressed in the oral epithelium of the palatal shelves. Others are expressed in the mesenchymal cells (Stanier & Moore, 2004) with modification.

Source: Figures modified from Mokhtar (2007).

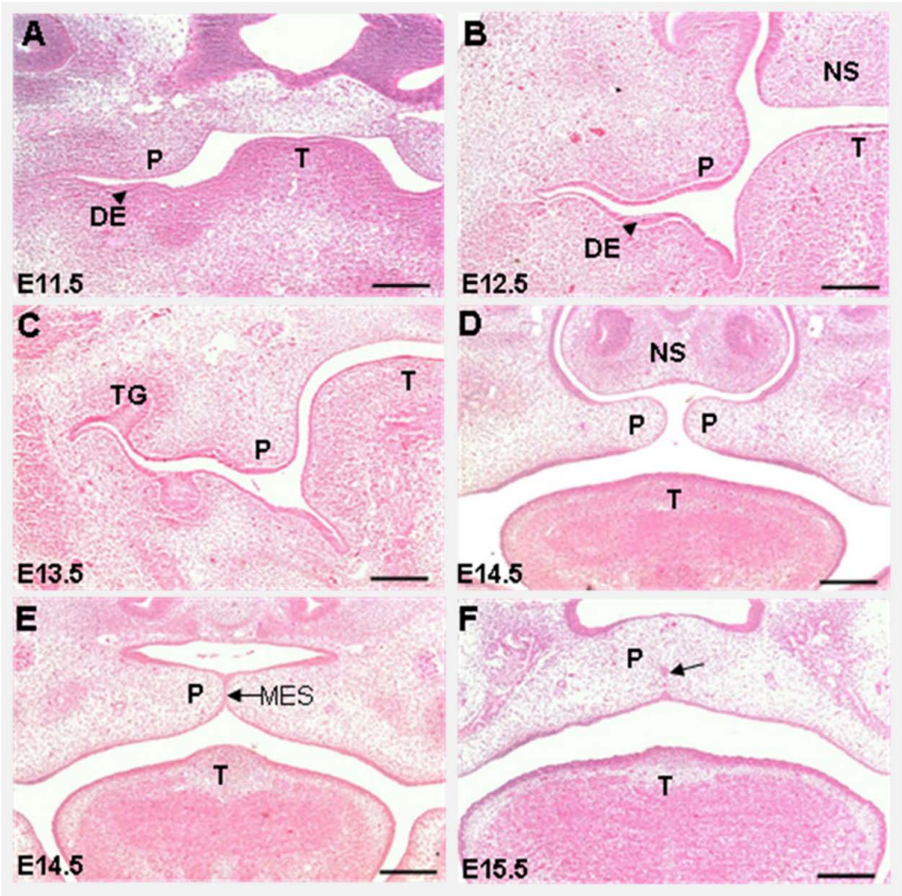

Fig. 2 Haematoxylin and eosin (H&E) stained sections of mouse embryonic heads highlighting the normal phases of palatogenesis. (A) The first outgrowths (P) from the maxillary processes are visible at E11.5. (B and C) At E12.5 and E13.5, the palatal shelves grow vertically downward on either side of the tongue. (D) Around E14.5, the palatal shelves elevate and become horizontally positioned above the tongue. (E) The palatal shelves grow towards each other, adhere, and finally fuse forming the medial edge epithelium seam (E; MES, arrow). (F) By E15.5, the seam degenerates resulting in a complete fusion of the palate mesenchymal cells. Epithelial islands persist in this section (F; arrow). Any molecular disturbances affecting the normal phases of palatogenesis could lead to the incident of secondary cleft palate. (Notes: P = palatal shelves; T = tongue; DE = dental epithelium; TG = tooth germ; NS = nasal septum; Bars, 100 μm).
Source: Modified from Mokhtar (2007).

Genetics of CLP and CP

The aetiology of CLP and CP are generally thought to be caused by the interactions of both genetic and environmental factors. These two main factors play major roles resulting in the formation of orofacial cleft in humans (Martinelli *et al.*, 2020).

More than 300 genes have been implicated in palatal fusion in human and experimental animal models (Deshpande & Goudy, 2019). The manifestation of the disease has been linked to some defects in growth factors and their receptors such as fibroblast growth factor 8 (*FGF8*) and fibroblast growth factor receptor 1 (*FGFR1*) genes. Transforming growth factor beta-1 (*TGFβ1*) is another family gene involved, with the inactivation of its receptor, transforming growth factor beta-3 receptor 2 (*TGFβ3R2*), and the inactivation of bone morphogenetic

protein-7 (*BMP7*). Others also demonstrated the involvement of transcription factors in the pathogenesis of CLP such as mutation in Msh Homeobox 1 (*MSX1*), T-box transcription factor 22 (*TBX22*) and interferon regulatory factor 6 (*IRF6*) (Martinelli *et al.*, 2020).

To determine the genetic determinants of clefts, both association analysis and link analysis were used. Many candidate loci have been implicated in cleft phenotypes, but the results of candidate gene-based association studies conducted on diverse ethnicities and populations have been generally similar or contradictory and genetic heterogeneity is the main cause of inconsistency. *IRF6, MSX1,* Msh Homeobox 2 (*MSX2*), transforming growth factor (*TGF*), transforming growth factor beta-1 (*TGFB1*), transforming growth factor beta-2 (*TGFB2*), transforming growth factor beta-3 (*TGFB3*), T-box transcription factor 1 (*TBX1*), retinoic acid receptor, alpha (*RARA*), tumor protein P63 (*TP63*), myosin heavy chain 9 (*MYH9*), B cell lymphoma 3 (*BCL3*), methylenetetrahydrofolate reductase (*MTHFR*), special AT-rich sequence-binding protein 2 (*SATB2*), forkhead box E1 (*FOXE1*), bone morphogenetic protein-4 (*BMP4*), paired box protein Pax-7 (*PAX7*), poliovirus receptor related-1 (*PVRL1*), and runt-related transcription factor 2 (*RUNX2*) have all been associated to non-syndromic CLP as candidate gene by various researchers (Beaty *et al.*, 2011; Leslie & Marazita, 2013; Bahrami *et al.*, 2021).

In the past, few candidate genes had been identified and known as the cause of CLP and CP. Through these candidate genes, more subtypes have been discovered to broaden the path in identifying the causes of this malformation. The following genes are part of those genes that were recently discovered to cause CLP and CP in humans.

Updates on Genes Associated with CLP

Most of the genetic analysis has been conducted in genes associated with the incidence of CLP since this abnormality represents 65% to 70% of craniofacial abnormalities (Tolarova, 2016). This part of the review will update on some of the latest genes which are linked with CLP when analysed using the recent genetic analysis approach such as GWAS and NGS.

ARHGAP29

ARHGAP29 is a protein coding gene located on chromosome 1p22; encodes Rho GTPase activating protein (GAP) 29, has previously never been described in the face, where it had been first characterised its expression in a murine model (Leslie *et al.*, 2012). In the mouse model, it was strongly detected in the medial and lateral nasal processes and shelves of the secondary palate. *IRF6* is a gene that had been associated with non-syndromic CL/P, and extensive investigation found that *ARHGAP29* expression was decreased in *IRF6* knockout mouse, indicating that it may act downstream of *IRF6* in craniofacial development, further confirming it has an association with cleft lip and palate. Interestingly, recent study has shown that a rare variant of $ARHGAP29^{R551T}$ was also identified to be associated with non-syndromic CLP in Han Chinese population (Li *et al.*, 2022).

CRISPLD2

Cysteine-rich secretory proteins LCCL domain containing 2 (*CRISPLD2*), a member of the cysteine-rich secretory proteins, antigen 5 (Ag5) and pathogenesis-related 1 (Pr-1) (CAP) superfamily, has previously been linked to non-syndromic CLP in human populations and has been demonstrated to be required for normal craniofacial development in zebrafish (Swindell *et al.*, 2015). *CRISPLD2* has a key role in neural crest cells migration, differentiation, and/or survival. *CRISPLD2* is a secreted glycoprotein that interacts with extracellular components that are required for cell migration, and its absence causes cell death and/or aberrant migration of neural crest cells into the craniofacial region (Swindell *et al.*, 2015). *CRISPLD2*

rs4783099 polymorphisms were found to cause a considerably elevated risk of CL/P in a Chinese population (Shen *et al.*, 2011; Ge *et al.*, 2018). However, in Indian population, a population study that used 3 polymorphisms of *CRISPLD2*, namely, rs1546124, rs4783099, and rs16974880, conferred no association with increased risk of non-syndromic CLP (Neela *et al.*, 2020). On the other hand, in case–control studies, a substantial link has been shown between allelic variations at rs1546124 and rs4783099 in the *CRISPLD2* gene and the risk of non-syndromic CP only in Brazilian population (Messetti *et al.*, 2017).

TOX3

TOX high mobility group box family member 3; *TOX3* gene, is located on 16q12 and has never been linked to the development of non-syndromic orofacial clefts (Mohamad Shah *et al.*, 2019). However, it has been suggested that *TOX3* mutations are linked to breast cancer susceptibility (Jones *et al.*, 2013). The specific mechanism of how *TOX3* confers craniofacial deformity is unclear. However, *TOX3* mutations likely interact with defective *FGFs* in the development of this deformity, implying that both are deficient during the predevelopment stage of bone fusion. This was demonstrated in research, which found that *FGF* downregulation interfered with normal embryonic craniofacial development via *FGF* signalling. Reduced *TOX3* induction, thus, would cause *FGF* dysregulation during lip and palate fusion, eventually leading to aberrant growth of these craniofacial structures (Mohamad Shah *et al.*, 2019).

COL21A1

Collagen alpha-1 (XXI) chain is encoded by the *COL21A1* gene located in chromosome 6p12.2 regions. There is a novel finding on *COL21A1* low copy number which had been confirmed as one of the contributing genes to non-syndromic CLP formation. The function of *COL21A1* is known to maintain the integrity of the extracellular matrix. It is a part of the family collagen that is expressed in muscle phenotype. This gene has never been discussed as a candidate gene for cleft lip and palate, and the report from (Mohamad Shah *et al.*, 2019) demonstrated as the first evidence that a low copy number of *COL21A1* at 6p12.2 region might be associated with non-syndromic orofacial cleft. The low concentration of COL21A1 in DNA samples may be the cause of the identification of COL21A1 low copy number in the non-syndromic cleft lip and palate (NSCLP) family. The cleft lip deformity may have been caused by this disorder, which may have interfered with the normal collagen activity required to maintain the tissue integrity of the lip and/or palate muscle (Mohamad Shah *et al.*, 2019).

HYAL2

HYAL2 which encodes hyaluronidase 2, a membrane-localised protein, degrades extracellular hyaluronan (HA), a critical component of the developing heart and palatal shelf matrix. HA is a glycosaminoglycan found in the extracellular matrix that is prevalent during development. HA, acting as a scaffold and a signalling molecule between the matrix and the cells; is important for regulating normal structural integrity and tissue responses during injury, repair, and regeneration (Garantziotis & Savani, 2019). The lack of *HYAL2* expression is a novel cause of syndromic CLP in humans and mice (Muggenthaler *et al.*, 2017). Reduction of *HYAL2* expression affects the normal turnover rates of HA degradation thus affecting the normal development of the craniofacial process, especially palate development. Decreased HA has previously been related with a greater risk for CP in the Tbx1-/- mouse (Goudy *et al.*, 2010) whereas increased HA has been associated with CP in Sim2-/- mice (Shamblott *et al.*, 2002) supporting the idea that changed *HYAL2* levels may impact palatal development. Interestingly, this study also deliberated on the importance of

HYAL2 in heart development (Muggenthaler *et al.*, 2017).

VAX1

In mice and zebrafish, V*ax1* and *Vax2* have been linked to eye development and the closing of the choroid fissure (Slavotinek *et al.*, 2012). Ventral anterior homeobox (VAX) are transcription factors recently discovered to operate as activators of a powerful dominant-negative version of the canonical Wnt signalling mediator *Tcf7l2*, designated as *dnTcf7l2* (Vacik *et al.*, 2011). Mutation in *VAX1* but not *VAX2* has been linked to orofacial cleft in humans. Sequencing of *VAX1* revealed one mutation, with homozygosity for two adjacent nucleotide substitutions, c.453G>A and c.454C>A, that predicted p.Arg152Ser in *VAX1* gene analysed from a single patient having cleft lip and palate and other related abnormalities (Slavotinek *et al.*, 2012). *VAX1* mutation prevents activation of *dnTCF7L2*, leading to derepression of *TCF7L2* target genes and causing hyperactivation of Wnt signalling. This condition indirectly causes accumulation of *Wnt* thus resulting in cleft. It is possible that this mechanism explains at least part of the observed phenotype found with the VAX1p. Arg152Ser mutation (Slavotinek *et al.*, 2012).

GLI2

GLI2 is a zinc finger protein that belongs to the GLI family and is the only known transcriptional effector of the sonic hedgehog (*SHH*) signalling pathway. *GLI2* mutations have been discovered in patients with orofacial clefts. Three potential missense mutations in the coding area of *GLI2* (c.2684C > T_p.Ala895Val, c.4350G > T_p.Gln1450His, and c.4622C > A_p. Ser1541Tyr) which were predicted to be deleterious thus affected the normal function of *GLI2* protein, were discovered in the Chinese non-syndromic CLP family, implying that *GLI2* is involved in the pathogenesis of CLP (Meng *et al.*, 2019). The mutation c.2684C > T was predicted to affect the structure of the *GLI2* protein and hence might lead to misregulation of the *GLI2* (OMIM: 165230) gene (Hui & Angers, 2011; Meng *et al.*, 2019). Given that all three mutations may inhibit the activation of *GLI2*, they are most likely to inhibit or block the action of the *SHH* signalling pathway, which would explain the pathophysiology of cleft lip and palate (Meng *et al.*, 2019).

MSX1–SNP rs3821949 Variant

MSX is a group of genes that are expressed in specific tissues such as teeth and bone (Chandrasekharan & Ramanathan, 2017). *MSX1* is a well-known gene that had been studied for years related to cleft. During early development, the muscle segment homeobox1 (*MSX1*) gene at 4p16.1 encodes a DNA-binding motif that is expressed in spatially restricted regions of the skull (Kim *et al.*, 2013). Several novel mutations had been revealed by complete sequencing of *MSX1* in humans. One of the recent single nucleotide polymorphisms (SNP) that had been discovered to cause cleft in children was rs3821949 suggesting positive association in Korean population (Kim *et al.*, 2013). The *MSX1* polymorphism rs12532 has also been linked to the development of CLP in a recent study (Lancia *et al.*, 2020). Interestingly, Gu *et al.* (2018) found that the *MSX1 SNP rs12532*; which affects mRNA expression and stability, is a putative risk factor for non-syndromic CP only and not for non-syndromic CLP.

Updates on Genes Associated with CP

Genetics of CP usually overlapped with CLP genetics, but some genes are only related to CP and are not observed in cases with CLP.

ROCK1

The two Rho kinase isoforms (*ROCK1* and *ROCK2*) are highly conserved serine/threonine kinases involved in controlling key cellular functions such as cell contraction, adhesion, migration, apoptosis, and proliferation which are crucial processes

in morphogenesis (Phillips *et al.*, 2012). *ROCK1* protein; also known as rho-associated, coiled-coil-containing protein kinase 1, regulates the interaction between non-muscle myosin II and actin A to induce cell contraction which is a critical step in morphogenesis. Previous cohort studies performed in Italy and Iran have demonstrated that polymorphism in *ROCK1* is associated with non-syndromic CP condition. The study showed a significant level of association between *ROCK1 rs35996865-G* and non-syndromic CP in both analysed populations (Palmieri *et al.*, 2020). Additionally, a significant association with a specific haplotype marker for rs288980-T, rs17202375-A, rs2127958-T, and rs35996865-G was observed for the Italian population. The study hypothesised that under transmitted allele of rs35996865-G may be implicated in the non-syndromic CP phenotype. The associated SNP rs35996865 maps in the *ROCK1* promoter region which is upstream to the transcription start site. Nevertheless, it is still unclear how or if this polymorphism can affect the expression of the examined gene. The SNP could be in linkage disequilibrium with another causative polymorphism or a mutation that could affect either the promoter or coding sequence of *ROCK1* which could further affected palatogenesis (Palmieri *et al.*, 2020).

GRHL3

GRHL3; grainyhead like transcription factor 3, appears to be the second candidate gene involved in Van der Woude Syndrome and cleft palate (Peyrard-Janvid *et al.*, 2014). This gene is important in epidermal barrier formation, neural tube closure and wound repair. *IRF6*; a known gene where its mutation is related to Van der Woude Syndrome, targets *GRHL3* in the periderm differentiation pathway (Mangold *et al.*, 2016). Mutation in *GRHL3* abrogated periderm development in mouse embryos with some of the embryos exhibited CP abnormalities (Peyrard-Janvid *et al.*, 2014). Studies by GWAS and sequencing approaches have indicated a missense variant (rs41268753) in *GRHL3* increases non-syndromic CP only cases of European ancestry (Leslie *et al.*, 2016). Mangold *et al.* (2016) collected evidence supporting the non-synonymous polymorphism rs41268753 as a susceptibility factor for non-syndromic CP and identified uncommon mutations of this gene in patients, independently confirming the role of *GRHL3* in non-syndromic CP pathogenesis (Kantaputra *et al.*, 2011).

TBX22

TBX22 is a well-known gene that causes cleft palate by mutation. There have been reports of nonsense, frameshift, splice site, and missense mutations, with the latter generally occurring in the highly conserved 180-amino-acid T-box domain. These cause a loss of function, which is mediated by a decrease in DNA binding, but they may also limit the protein's capacity to undergo post-translational modification (Kantaputra *et al.*, 2011). A study that was done in China revealed novel *TBX22* mutations where the result detected this mutation leading to an abnormal transcription or translation, followed by a loss of function of *TBX22*. It was first reported in a Chinese family where a hemizygous missense mutation, c.874G>A may aggravate effects on the phenotypes of CP (Dai *et al.*, 2018).

DISCUSSION

Facial development involves multiple facial processes which are governed by multiple molecules and signalling pathways. These facial processes must be regulated intricately by molecules that are expressed spatiotemporally whereby misregulation of these molecules could lead to orofacial abnormalities. Local changes in growth factors, extracellular matrix, and cell adhesion molecules together with other signalling pathways have been shown to play significant roles in facial development. As such, any changes or mutations affecting

these developmental genes have been demonstrated to contribute to the incidence of craniofacial abnormalities.

CLP and CP are a very vast topic implicated by various factors in their pathophysiology. Actual aetiology leading to the formation of craniofacial abnormality is still debatable. Nevertheless, the use of advanced technology in varieties of fundamental, applied, and clinical research provides significant findings thus highlighting the possible factors contributing to its occurrence. Utilisation of animal models and genetic analysis techniques such as GWAS and NGS provide excellent genetic tools in extrapolating the available data towards the actual incidence observed in the studied population, hence providing important information regarding the association of certain genes or polymorphisms with the incidence of CLP and CP only.

CONCLUSION

Overall, it is hard to pinpoint specific genes that can be related to only CLP or CP alone since studies have shown that most of these genes can be associated with both cleft conditions and are sometimes inter-related. More genes and polymorphisms are associated with the incidence of orofacial deformities. The variations of the disorder itself make orofacial cleft a difficult yet interesting subject to be discovered. With the advancement of technology, more investigations in searching the genetic causes of these common birth defects have been made possible, hence promising better results in the future.

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