The impact of developmental genes in non-syndromic cleft lip and/or palate

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Abstract

Non-syndromic cleft lip and/or palate (NSCL/P) is a congenital malformation with a prevalence of 1:700 births. It has a multifactorial etiology. Human craniofacial development takes place during the first 10 weeks of pregnancy. Normal craniofacial development arises from the convergence and fusion of the facial and palatal processes and involves interactions between genes that regulate cell growth, proliferation, differentiation, epithelial-to-mesenchymal transition, and apoptosis. Whole genome/exome analysis, and also genome-wide association studies give us to chance to identify the genetic factors which contribute to the development of NSCL/P. After detecting a cleft lip and/or palate on ultrasonography without associated anomalies, the patient should be evaluated in collaboration with a clinical geneticist, taking into account the many genes and environmental factors involved in NSCL/P etiopathogenesis, and a roadmap for possible genetic diagnosis should be drawn.

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Introduction

Non-syndromic cleft lip and/or palate (NSCL/P) is one of the most common congenital malformations with a prevalence of 1:700 (1,2). NSCL/P is a multifactorial condition caused by an inadequate partition of the nasal and oral cavities with no other anomaly (3,4). Orofacial clefts (OFC) are classified according to their facial position; unilateral or bilateral, involved parts; lip, palate, and lip and palate, and/or their underlying pathogenesis i.e. syndromic and non-syndromic.

Of all cases with cleft lip and/or palate (CLP), 30% are syndromic. The rest of the cases are NSCL/P, and of them 20% are familial and 80% are sporadic (2,5). As an isolated condition, 50% of all CLP patients are syndromic, the rest are sporadic (3). CLP is observed twice as frequently in males than in females (6). Unilateral clefts of the lip account for approximately 75% of all patients and among them the left side is affected twice as frequently as the right side (7).

The development of palate and lip involves a complex process including the organization of cells in tissues through cell growth, migration, differentiation, and apoptosis, which are controlled by gene expression and signaling molecules. Both genes and environmental factors, such as drugs and chemical exposure of the parent, as well as dietary habits, contribute to the occurrence of the disease pathogenesis (8). Hereditary factors are estimated to be 90% effective in the development of NSCL/P (9). Contribution of epigenetic factors and gene-gene/environment interactions make the pathogenesis of NSCL/P complex. This complex nature makes it difficult to understand the exact reason for the clinical condition. To diagnose genetic factors playing a role in disease pathogenesis, prenatally detected cases may be referred to genetic testing and counseling. Due to the complex nature of the disease, it is not always possible to define the exact gene/gene(s) involved. This information should be emphasized during pre- and post-test genetic counseling sessions. The aim of this study was to review the genes involved in NSCL/P.

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Embryogenesis

Human craniofacial development takes place during the first 10 weeks of pregnancy. The fourth and eighth gestational weeks are the time point where normal lip development occurs (10). The early formative systems of vertebrates are firmly controlled and closely monitored biologically, and hereditary and environmental components influence this sensitive interaction (8,11).

Neural crest cells (NCCs) differentiate to cranial processes by migrating (day 21) and differentiating to maxillary, lateral, and medial nasal processes (12). NCCs undergo epithelial-mesenchymal transition before moving to the craniofacial region and constitute the antecedents of the processes that will develop basic facial structures (13).

Head and neck develop from two-sided transient outgrowths called pharyngeal arches, which take 23-24 days and marks the beginning of early facial development. These swellings of tissue are coated by ectodermal epithelial cells, with a center of NCC-determined mesenchyme (14). A medial outgrowth of the first frontonasal prominence (FNP) is involved in the upper lip, roof of the palate, and lower jaw development (14). The FNP is responsible for the development of bilateral nasal pits and extends into the primitive oral cavity (12). The development of the FNP continues by dividing into paired medial and lateral nasal processes. They fuse with the maxillary process to form an intact upper lip (12).

As palate development is an early event during embryogenesis and takes a relatively long time, probable exposure to teratogens increases the risk of CLP formation in mammalian embryos (1). The palate develops in two parts. By the sixth week of development, the primary palate formation occurs from the bilateral medial nasal processes (15). Between the sixth and twelfth weeks, the rest of the palate, termed the secondary palate, develops. At this stage, palatal shelves outgrow from the inner oral side of the maxillary processes (16). The tongue is required for true mammalian hard palate fusion (17). Mammalian palatal bone ossification is followed by bone fusion (16). Palatal bone develops through intramembranous ossification, in which osteoblasts directly lay down the bone matrix. Lip, palate, and nose deformities are caused by a disruption in normal development. The extent of the defect is dependent on the disruption time, severity and amount. For the formation of the primary palate and central lip, rapid cell division is required in the lateral nasal process region. The developing embryo is vulnerable to both genetic and environmental effects during this period (16).

Signaling pathways

With the development of technology, modern techniques including automated analyses have been used to study complex diseases. Whole genome/exome analyses, and also genome-wide association studies (GWAS) provide an opportunity to identify genetic factors that contribute to the development of NSCL/P. Different candidate genes have been reported in recent publications (1,5,18-20). GWAS also helps to identify rare, low-frequency coding variants (21,22). Besides point mutation, copy number variants (CNV), which disrupt the structure and also the regulatory region of the genes, can cause NSCL/P (23).

Signaling molecules and morphogens play a crucial role in mesenchymal proliferation and patterning during craniofacial development. These signaling molecules originate from the epithelial cells of the facial prominences and palate and create reciprocal epithelial-mesenchymal communications that is critical for palatal development (12). Wnt, *TGF/BMP*, Hedgehog, and Fgf-related signaling cascades are involved in these interactions, and mutations of the genes of these signaling pathways may be the underlying reason for CLP and CP (24).

MSX1, BMP4, BMP2, FGF10, and *SHOX2* are among the genes involved in anterior palate development. *BMP4* expression is regulated by a transcription factor called MSX1. *BMP4* regulates sonic hedgehog (Shh) expression in the palatal epithelium, and BMP2 expression takes place downstream of the Shh signaling cascades. Simultaneously, FGF10 uses the FGFR2 receptor to regulate the Shh signaling cascade in the palatal epithelium in a paracrine manner. Activated Shh promotes cell proliferation in the mesenchyme by using the BMP2 signaling cascade. These findings suggest that the growth of the anterior area of the palatal shelf is a very tightly controlled process, and BMP and FGF canonical pathways play a critical role in this process. However, less is known about the function of the genes expressed in the posterior area of the palate but it is understood that FGF8 is the first step in one of the pathways that promote the expression of PAX9 in the posterior area of the palatal mesenchyme. A deficiency in PAX9 results in a developmental defect of the palatal shelf and a cleft palate (CLP) (25).

Palatal shelf development defects are classified into five categories by Chai and Maxson (25): failure of palatal shelf formation due to mutations in *activin-βA* and *FGFR2;* a fusion of the palatal shelf with the tongue or mandible arising from *TBX22* mutations and loss of function mutations in *FGF10*; failure of palatal shelf elevation resulting from mutations in the *PAX9, PITX1,* and *OSR2* genes; failure of palatal shelves to meet after elevation as a consequence of mutations in *MSX1* and *LHX8, TGFBR2* in NCCs, or *Shh* in the epithelium; and persistence of medial edge epithelium caused by *TGFB3* and *EGFR* mutations (25).

Further hereditary investigations identified variants in the *MMP3, MMP25, TIMP2*, and *TIMP3* genes to be causative in the development of NSCL/P. Besides point mutations in coding regions, variants that affect functional promoter activity in *MMP3* and *TIMP2* have also been found to be related to NSCL/P (26,27).

As an oncogene, *FOS* promotes epithelial-to-mesenchymal transition, which is essential for craniofacial development (28). In embryonic development, apoptosis is an important mechanism for maintaining tissue homeostasis. *CASP8* is one of the vital genes involved in craniofacial development (29).

A gene list is provided in Table 1 related to NSCL/P and craniofacial development.

Epigenetics

Epigenetics is described as the regulation of gene expression through reversible chemical modifications without affecting the DNA sequence (61).

Among the best understood epigenetic modifications in animals are histone modifications, which regulate chromatin accessibility during transcription, and DNA methylation, which plays a critical role in many biological processes, and also contributes to the regulation of gene expression during palatal fusion (2,62).

The expression of the several genes that are associated with NSCL/P is controlled by epigenetic modifications. Epigenetically controlled genes include transcription factors (*LHX8, PRDM16, PBX1, GSC, VAX1, MYC*), growth factors and their modulators (*WNT9B, BMP4, EPHB2, BICC1, DHRS2*), and microRNAs (miRNAs) including *MIR140* and *MIR300* (63-67). Xu et al. (66) and Sharp et al. (68) reported methylation position variations in OFC subsets; and emphasized many methylation positions related to genes that differentiated between cleft lip with CLP, cleft lip only, and cleft palate only (CPO).

miRNAs have been reported to regulate the expression of 60% of genes encoding proteins (69), but abnormalities of expression are linked to a variety of diseases, including OFCs (70). An SNP in miR-140 was found to have a significant correlation with NSCL/P (71). Rattanasopha et al. (72) reported a role for miR-140 in PDGFRA regulation in association with human CPO. miR-140 was likewise found to control the expression of *BMP2* and *FGF9* genes in human palatal mesenchyme cells (73). These discoveries highlighted two significant focuses for craniofacial development: (a) Bmp signaling can be carried on by Smad factors and miRNA-17-92, and (b) miR-17-92 can have multiple effects by focusing on a few pathways, including TGF, FGF, Wnt, and others.

Environmental factors influence epigenetic modifications in both cells and organisms, which can result in different developmental outcomes (74). Van Rooij et al. (75) reported that maternal glutathione s-transferase genotype, and smoking as an environmental factor, increased the risk of CLP significantly. Joubert et al. (76) reported that maternal smoking was associated with differential methylation of some of the genes related to OFC, such as *MSX1, PDGFRA, GRHL3, ZIC2*, and *HOXA2*. Jugessur et al. (77) reported that alcohol dehydrogenase gene *ADH1C* variants are associated with clefting.

Human studies have also found that dietary folate plays a role in epigenetic-mediated CL/P (64). Gonseth et al. (64) conducted an epigenome-wide association study to investigate the correlation between epialleles and OFCs in the United States, before setting up mandatory folate treatment in 1998.

Prenatal evaluation

During diagnostic ultrasonography, the defined cleft lip is a direct imperfection stretching out between the lip side and the nostril. CLP with cleft lip might extend between the alveolar side and hard palate, reaching the nasal and oral cavities, and may also extend to the orbits. Diagnosis requires the use of both the transverse and coronal planes. During visualization of cleft and palate, color Doppler might be valuable in showing flow across the palate. The diagnosis of isolated CP is difficult. Even between 11 and 13 gestational weeks, diagnosis of CLP may be possible but mostly CLP is diagnosed by detailed ultrasound examination at 18-22 gestational weeks. Retronasal triangle and maxillary gap views should be obtained during ultrasonographic evaluation of the fetus in screening for OFCs. Magnetic resonance imaging may be an adjunct to prenatal diagnosis of CLP. After the prenatal ultrasound diagnosis of CLP, other system anomalies should be screened and invasive testing for karyotyping and microarray testing should be offered. Prenatal consultation with a multidisciplinary team, including clinical geneticists, should be performed during prenatal evaluation (78). Clinical geneticists take a detailed pedigree and family history followed by reviewing the ultrasonography findings. The finding can be isolated or associated with a specific syndrome (79). After genetic counseling and risk calculation, clinical geneticists decide about appropriate genetic tests (78). Karyotype analysis is necessary to exclude trisomy 13 and other chromosome abnormalities for fetuses with multiple abnormal ultrasonographic findings (80). If there is a suggestion of a specific syndrome due to the associated anomalies, targeted genetic studies including fluorescent in situ hybridization or multiplex ligation-dependent probe amplification can be performed before the microarrayor testing may proceed directly to microarray following the karyotyping. If these test results are normal, whole exome sequencing can be the next step to detect point mutations.

Prognosis relies upon the presence and kind of related abnormalities. If it is isolated, the prognosis is good and normal survival can be achieved with appropriate management. Surgical intervention is frequently performed between postnatal 3-6 months. The recurrence risk can be defined as 5% if one sibling or parent is affected and 10% if two siblings are affected in isolated cases. If the genetic alteration is disclosed

Table 1. A gene list related to NSCL/P and craniofacial in Table 1

Figure 1	Gene name and symbol	Chromosome locus	Mechanism	Phenotype	Reference
1	Orofacial cleft 1 *(OFC1/OFCC1)*	6p24.3		CL ± P CPO	(30)
2	Transforming growth factor-alpha/orofacial cleft 2 *(TGFA/OFC2)*	2p13	*TGF-α* polymorphisms have controversial results in previous studies suggesting the gene as a neighbor gene to the disease locus rather than a disease-causing gene	CL ± P	(31)
3	BCL3 transcription coactivator/orofacial cleft 3 *(BCL3/OFC3)*	19q13	*BCL3* plays a role in cell adhesion. Its downregulation results in disruption of facial development	CL ± P	(32)
4	Orofacial cleft 4 *(OFC4)*	4q21-q31	-	CL ± P	(33)
5	Msh homeobox 1/orofacial cleft 5 *(MSX1/OFC5)*	4p16.1	*MSX1* is a transcriptional repressor playing role in mesenchymal cell proliferation	CL ± P	(34)
6	Interferon regulatory factor 6/orofacial cleft 6 *(IRF6/OFC6)*	1q32.3-q41	Determines the keratinocyte proliferation/ differentiation switch It is assumed that the protein may have a transcriptional activator role Protein play role in midfacial clefting	CL ± P	(35)
7	Poliovirus receptor related1/ orofacial cleft 7 *(PVRL1/OFC7)*	11q23.3	*PVRL1* encodes a cell adhesion family protein and plays role in the development of palatal shelves	CL ± P	(36)
8	Tumor protein p63/orofacial cleft 8 *(TP63/OFC8)*	3q27	As a transcription factor plays a key role in epithelial lineage progression during and after development	CL ± P	(37)
9	Orofacial cleft 9 *(OFC9)*	13q33.1-q34		CL ± P	(38)
10	Small ubiquitin-like modifier 1/orofacial cleft 10 *(SUMO1/OFC10)*	2q33	Plays role in the control of nuclear transport, transcriptional regulation, apoptosis, and protein stability	CL ± P	(39)
11	Bone morphogenetic protein 4 /orofacial cleft 11 *(BMP4/OFC11)*	14q22	A regulatory molecule of several developmental processes including facial development	CL ± P	(40)
12	Orofacial cleft 12 *(OFC12)*	8q24.3	The gene contains cis-acting enhancers that direct Myc expression during facial development	CL ± P	(41)
13	Orofacial cleft 13 *(OFC13)*	1p33		CL ± P	(42)
14	Orofacial cleft 14 *(OFC14)*	1p31		CL ± P	(43)
15	Distal-less homeobox 4/ orofacial cleft 15 *(DLX4/ OFC15)*	17q21	As a transcription factor plays a critical role in the craniofacial development	CL ± P	(44)
16	Methylenetetrahydrofolate reductase *(MTHFR)*	1p36	Folate pathway	CL ± P	(45)
17	Cysteine-rich secretory protein LCCL domain containing 2 *(CRISPLD2)*	16q24.1	It shows its effect on facial development by disrupting the binding of regulatory elements and inhibiting protein expression. The facial developmental processes	CL ± P	(46)
18	CLPTM1 regulator of GABA type A receptor forward trafficking *(CLPTM1)*	19q13.32	Functioning as the regulator of GABA type A receptor	CL ± P	(39)
19	Fibroblast growth factor receptor 2 *(FGFR2)*	10q26.13	Play role in craniofacial osteogenesis and suture homeostasis	CL ± P	(47)
20	Frizzled-related protein *(FRZB)*	2q32	This is a secreted protein. It is involved in the regulation of bone development	CL ± P	(48)

21	Sprouty RTK signaling antagonist 1 *(SPRY1)*	4q28.1	Primary paralog of SPRY2 The encoded protein inhibits the FGF and MAPK pathways	CL ± P	(49)
22	Mitogen-activated protein kinase 3 *[MAPK3 (ERK1)]*	16p11.2	Disruption of this gene leads to OFCs in animal models	CL ± P	(49)
23	TUB-like protein 4 *(TULP4)*	6q25.3		CL ± P	(50)
24	SATB homeobox 2 *(SATB2)*	2q33.1	Communicates with transcription factors that regulate craniofacial development	CP	(51)
25	Meis homeobox 2 *(MEIS2)*	15q14	Transcription regulator contributes to developmental programs	CP	(51)
26	Stearoyl-CoA desaturase 5 *(ACOD4/SCD5)*	4q21.22	Stearoyl-CoA desaturase is located at the integral membrane of the endoplasmic reticulum	CL	(33)
27	Transforming growth factor beta 3 *(TGFB3)*	14q24	Tgf signaling contributes to the formation of secondary palate fusion	CL ± P	(52)
28	ATP binding cassette subfamily A member 4 *(ABCA4)*	1p22	Encodes an ATP-binding cassette transporter	CL ± P	(53)
29	MAF bZIP transcription factor B *(MAFB)*	20q12	Involved in the development and differentiation of keratinocytes	CL ± P	(53)
30	Bone morphogenetic protein 2 *(BMP2)*	20p12.3	Bmp2 and Bmp4 signaling is important for craniofacial patterning	CL ± P	(39)
31	Forkhead box E1 *(FOXE1)*	9q22	It contains a DNA- binding forkhead domain	CL ± P	(54)
32	T-box transcription factor 1 *(TBX1)*	22q11.21	Takes part in early progenitor cells relevant for palate development	CL ± P	(55)
33	Fos proto-oncogene, AP-1 transcription factor subunit *(FOS)*	14q24.3	Control epithelial-to-mesenchymal transition, a critical process during craniofacial development	CL ± P	(28)
34	Matrix metallopeptidase 2 *(MMP2)*	16q12.2	Plays role in extracellular matrix remodeling and subsequent fusion of the palatal shelves	CL ± P	(56)
35	Caspase 8 *(CASP8)*	2q33.1	Takes part in apoptosis, which is crucial to sustaining tissue homeostasis during embryonic development	CL ± P	(29)
36	Ventral anterior homeobox 1 *(VAX1)*	10q25.3	Transcriptional regulator containing a DNA- binding homeobox domain	CL ± P	(1)
37	SMAD-specific E3 ubiquitin-protein ligase 1 *(SMURF1)*	7q22.1	Negative regulator of BMP signaling pathway and controls cell motility, and polarity	CL ± P	(51)
38	Sprouty-related EVH1 domain containing 1 *(SPRED1)*	15q14	Negative regulator of the FGF and MAPK pathways	CL ± P	(51)
39	Grainy head-like transcription factor 3 *(GRHL3)*	1p36.11	Needed for periderm differentiation and assumes a part in palate formation	CP	(57)
40	T-box transcription factor 22 *(TBX22)*	Xq12	Plays role in the adhesion of opposing palatal shelves. It involves palatogenesis and plays a role as a transcriptional repressor	CP	(58)
41	Wnt family member 3 *(WNT3)*	17q21.31-q21.32	Epistatic interaction with COL2A1, as well as interactions with *FGFR1* and *MTHFR* genes in humans	CL ± P	(59)
42	SH3 and PX domains 2A *(SH3PXD2A)*	10q24.33	Involved in cell migration and matrix degradation	CL ± P	(60)
43	Paired box 9 *(PAX9)*	14q13.3	Transcription factor, with the critical role during fetal development	CL ± P	(35)
44	Paired box 7 *(PAX7)*	1p36.13	Transcription factor, with the critical role during fetal development	CL ± P	(41)

NSCL/P: Non-syndromic Cleft lip and/or palate, CL ± P: Cleft lip and/or palate, CPO: Cleft palate only

during genetic work up, genetic counseling should be given to the family about the recurrence risk after trying to determine the inheritance pattern together with the parental genetic evaluation.

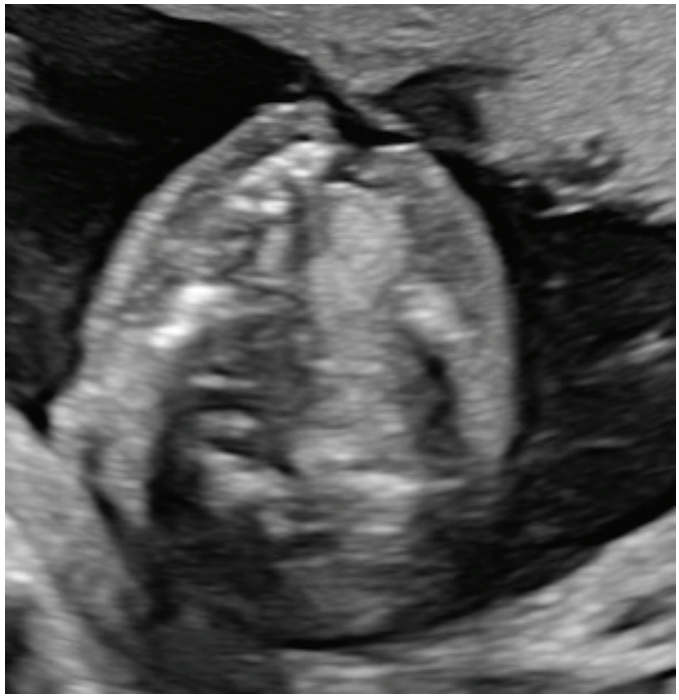

Figure 1. A fetus with a unilateral cleft lip and palate at the sixteenth gestational week

Conclusion

Pathogenesis of OFCs is complex and may frequently include hereditary and environmental interactions that are yet to be fully understood. As the condition is complex, epigenetic modifications may also contribute to the clinical condition if there is no defined genetic reason.

When a cleft lip and/or palate is detected by ultrasonography, in the absence of associated anomalies, the patient should be evaluated in consultation with the clinical geneticist, taking into account many genes and environmental factors involved in NSCL/P etiopathogenesis. A roadmap for possible prenatal genetic diagnosis should be devised, as genetic testing is an important component of pre- and post-natal management of cases.

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