Genetic Factors in Nonsyndromic Orofacial Clefts

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Abstract

Keywords

- ▶ orofacial clefts
- ▶ nonsyndromic
- ▶ genetics
- ▶ gene mutation
- ▶ genome-wide association study
- ▶ linkage analysis

Orofacial clefts (OFCs) are the most common congenital birth defects in humans and immediately recognized at birth. The etiology remains complex and poorly understood and seems to result from multiple genetic and environmental factors along with gene–environment interactions. It can be classified into syndromic (30%) and nonsyndromic (70%) clefts. Nonsyndromic OFCs include clefts without any additional physical or cognitive deficits. Recently, various genetic approaches, such as genome-wide association studies (GWAS), candidate gene association studies, and linkage analysis, have identified multiple genes involved in the etiology of OFCs.

This article provides an insight into the multiple genes involved in the etiology of OFCs. Identification of specific genetic causes of clefts helps in a better understanding of the molecular pathogenesis of OFC. In the near future, it helps to provide a more accurate diagnosis, genetic counseling, personalized medicine for better clinical care, and prevention of OFCs.

Introduction

Orofacial clefts (OFC) are the most common congenital malformations in the orofacial region causing a significant personal and social encumbrance.^{1–3} The etiology remains complex involving multiple genetic and environmental factors along with gene–environment interactions.^{4–7} Children born with clefts may have difficulties in sucking, speech, hearing, and unaesthetic facial features due to the anatomical deformities.^{8,9} OFCs generally require surgical reconstruction of the lip and palate at different stages from birth to adulthood,¹⁰ and their rehabilitation involves a multidisciplinary approach such as, pediatric care, speech and hearing therapy, dental and orthodontic treatment, genetic counseling, and other mental health therapy.^{11,12}

Epidemiology

The prevalence of OFCs ranges from 1 in 700 to 1,000 newborns worldwide¹³ and include cleft lip only (CLO), cleft palate only (CPO), and cleft lip and palate (CLP).¹⁴ They may occur as unilateral or bilateral, complete, or incomplete, and may involve the lip only, the palate only, or both.¹⁵

The prevalence of OFC varies according to geographical location, ethnicity, race, gender and socioeconomic status,^{16–19} Asians have the highest prevalence rate (1:500), the intermediate prevalence in Europeans (1:1000), and lowest in Africans (1:2500).^{20–22} In India, the incidence of clefts is around 1:800 to 1:1000, and three infants are born with some type of cleft every hour.²³ These differences appear to persist even after migration, suggesting that they are mediated by genetic, rather than environmental

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factors.²⁴ Overall, 70% of the OFCs are nonsyndromic (NS) and occur as isolated cases without any additional physical or cognitive deficits. In contrast, 30% of clefts are syndromic and are associated with a few other developmental anomalies.²⁵

The frequency of occurrence of OFC differs with regard to gender and side of clefting. Cleft lip is more common in males at a 2:1 male to female ratio, whereas a cleft palate is more common in females.²⁶ Approximately 90% of OFCs are unilateral with primarily left-sided involvement.²⁷

Development of Cleft Lip and Palate

The development of lip and palate begins during the fourth week of gestation where migrating neural crest cells combine with mesodermal cells to establish facial primordia which consist of five different processes (medial nasal, lateral nasal, frontonasal, maxillary, and mandibular processes) derived from the first pharyngeal arch.²⁸ Once facial prominences are formed, the nasal placodes invaginate to form the medial nasal process (MNP) and lateral nasal process (LNP). The maxillary processes initially grow medially, pushing the LNP toward the upper side. During the sixth and seventh weeks of gestation fusion of maxillary processes with each other and also with lateral and MNPs takes place to form upper lip and primary palate.²⁹ Defect in fusion or failure in the growth of these processes results in clefts involving the upper lip, alveolus, and/or primary palate.

The secondary palate begins to develop in the seventh week of embryogenesis; the maxillary processes initially outgrow as palatal shelves which move toward each other vertically. After proper growth they settle at a horizontal position above the tongue which entails much extracellular remodeling.^{30,31} The palatal shelves then fuse in the midline both anteriorly and posteriorly like a zipper that forms a midline epithelial seam (MES). The disintegration of MES is required to maintain palatal confluency, which may involve apoptosis, epithelial-mesenchymal transition (EMT), and cell migration.³² Effective fusion of the secondary palate results in complete separation of the oral and nasal cavities. Cleft palate can result from failure at any of the steps, including palatal shelves elevation, cell migration, or fusion.

In summary, a variety of cellular mechanisms such as cell proliferation, cell migration, cell growth, cell fusion, apoptosis, EMT, and extracellular remodeling are involved in a coordinated manner during the development of lip and palate. Therefore, disruption in the gene/s involved in these processes during lip and palate development may lead to an OFC.

Glimpse into the History of Genetic Etiology of Orofacial Clefts

Although the familiarity of OFC has long been noted, Fogh-Andersen was the first to provide the evidence for genetic factors contributing to the etiology of CLP from family-based studies where it was observed that the siblings of CLP patients had an increased frequency of cleft lip with or without cleft palate.³³ This observation was further confirmed by studies on the familial distribution of congenital clefts of the lip and

palate,³⁴ and Dr. Clark Fraser published a review paper highlighting the conclusions of a workshop on CLP sponsored by the National Institutes of Health of the United States, and he mentioned the etiology is indeed multifactorial.³⁵ Later, more evidence in favor of contribution of genetic factors to the etiology of CLP accrued from segregation analysis³⁶ and twin studies^{37,38} where the monozygotic twins showed high prevalence rate (40%) than the dizygotic twins (4%).

Role of Genetic Factors in the Etiology of Orofacial Clefts

The etiology of OFCs involves genetic factors, environmental influences, and gene–environment interactions, all contributing to its susceptibility. Scientific literature evidence suggests that environmental factors such as maternal tobacco smoking and alcohol consumption, antiepileptic medications, maternal folate deficiency, infections, consanguinity, and geographical location are risk factors for NS cleft lip and palate (NSCLP).^{39–42}

Advances in genetics and molecular biology techniques have discovered multiple genes and loci associated with CLP. This article provides an overview of the genes implicated in the etiology of NSCLP. Identification of specific genetic variation contributing to NSCLP has led to an increase in our understanding of the molecular pathogenesis of OFC.

Genes Involved in the Etiology of Nonsyndromic Orofacial Clefts

Special AT-Rich Sequence-Binding Protein

Special AT-rich sequence-binding protein (SATB2) is a DNA binding protein which binds with nuclear matrix attachment regions. It is involved in transcription regulation and chromatin remodeling process. In an animal study, mouse SATB2 is strongly expressed in the developing cleft palate and is similar to the human SATB2 protein.⁴³ The identification of SATB2 gene responsible for the craniofacial dysmorphologies associated with deletions and translocations at 2q32–q33, only one region of the genome has been significantly associated with the development of isolated cleft palate.⁴⁴ Glass syndrome characterized by cleft palate, gum hyperplasia, slight micrognathia, generalized osteoporosis, and mental retardation reported from Thai patient,⁴⁵ was caused by SATB2 gene mutation. Recently, using salivary miRNAs showed that the SATB2 genes are involved in the development of the cleft palate and lip development.⁴⁶ **Table 1** provides the list of genes involved in the etiology of NS OFC in humans.

B-Cell Leukemia/Lymphoma 3

B-cell leukemia/lymphoma 3 (BCL3) is a proto-oncogene, acts as a transcriptional co-activator through NF-kappa-B target genes and located on 19q13.2. BCL3 gene has also shown a strong association with NS orofacial clefts (NSOCs).⁴⁷ A case-parent trio study showed BCL3 influence risk of CL/P through a parent-of-origin effect with the excess maternal transmission.⁴⁸ Several studies in different

Table 1 Genes involved in the etiology of nonsyndromic orofacial clefts in humans

Gene	Gene symbol	Loci	OMIM	Evidence	References
Special AT-rich sequence-binding protein	<i>SATB2</i>	2q33.1	608148	M	43,46
B-cell leukemia/lymphoma 3	<i>BCL3</i>	19q13.32	109560	LD, L	47,49
Distal-less homeobox 4	<i>DLX4</i>	17q21.33	601911	M	53,55,56
Paired box gene 9	<i>PAX9</i>	14q13.3	167416	L, GWAS	6,60,62,64
Netrin 1	<i>NTN1</i>	17p13.1	601614	GWAS	70,71
T-box transcription factor 22	<i>TBX22</i>	Xq21.1	300307	M, LD	75,77,79
Poliovirus receptor like-1	<i>PVRL1</i>	11q23.3	600644	M, LD, GWAS	81,83,85
Cleft lip and palate associated transmembrane protein 1	<i>CLPTM1</i>	19q13.32	604783	M, L, LD	89,90
MAF bZIP transcription factor B	<i>MAFB</i>	20q12	608968	GWAS	98,101
Fibroblast growth factor receptor 1	<i>FGFR1</i>	8p11.23	136350	M, D	102,103
Transcription factor AP2-α	<i>TFAP2A</i>	6p24.3	107580	D, L	104
S-glutathione transferase T1	<i>GSTT1</i>	22q11.2	600436	LD	108,109
Receptor-like tyrosine kinase	<i>RYK</i>	3q22.2	600524	M	110,111
Gamma-aminobutyric acid receptor, Beta-3	<i>GABRB3</i>	15q12	137192	GWAS	112
ATP-binding cassette, subfamily A, member 4	<i>ABCA4</i>	1p22.1	601691	GWAS	115,117,118

Abbreviations: D, deletions; GWAS, genome-wide association studies; L, linkage; LD, linkage disequilibrium; M, mutations; OMIM, Online Mendelian Inheritance in Man.

populations have implicated the role of *BCL3* in development of NSCL/P. It was found that *BCL3* contributes to the regulation of cell proliferation, and cell cycle regulation can cause a disturbance in facial formation.⁴⁹ A study also reported *BCL3* contribution in angiogenesis-related genes in the etiology of CLP.⁵⁰ However, no association of *BCL3* gene with NSCLP was found in multigenerational families of Indian population.⁵¹

Distal-Less Homeobox 4

Distal-Less Homeobox 4 (*DLX4*) belongs to *DLX* gene family containing a homeobox transcription factor which plays an important role in craniofacial development and palatogenesis. It is located on chromosome 17q21.33 and causes orofacial cleft 15 (OFC15) and cleft lip/palate. In an animal study, the *DLX* genes caused cleft palate showing the importance of these genes in craniofacial morphogenesis.⁵² Whole-exome sequencing study in a Hispanic mother and son with bilateral CLP confirmed the *DLX4* as a potential cause of oral clefts.^{53–55} Recently, a study showed that none of the distal-less 4 (*DLX4*) gene SNPs were associated with NSOCs, so it should be interpreted with a caution in the etiology of nonsyndromic orofacial clefts.⁵⁶

Paired Box Gene 9

Paired Box Gene 9 (*PAX9*) is a member of the paired box (*PAX*) family of transcription factors and contains a paired box domain, an octapeptide, and a paired-type homeodomain. It plays a critical role during neural crest and fetal development. *PAX9* is located on chromosome 14q13.3, consists of five exons, and associated with the formation of the teeth and palate.^{57,58} Tooth agenesis and the formation of a cleft palate in *PAX9*-deficient mice have been reported.⁵⁹

Schuffenhauer et al, first reported the role of *PAX9* in a patient presented with bilateral CLP.⁶⁰ A linkage analysis of *PAX9* gene in two large families and four unrelated families showed that the hypodontia primarily involving molars, suggested that the mutant *PAX9* protein acquires functional defects in DNA binding, as well as the loss of function of *PAX9* resulting in haploinsufficiency during the morphogenesis of the dentition and the subsequent tooth agenesis.^{61,62} Several studies identified mutations at *PAX9* may increase the risk of NS cleft lip with or without palate.^{63–66}

A combined genome-wide association study (GWAS) used unmixed NS CPO and NS CLO subtypes suggested that *PAX9* is a strong genetic factor for NS CPO in the Chinese population. Mutation analysis of *PAX9* SNPs rs12885612 and rs12881248 revealed that *PAX9* is a promising susceptible gene for NS CLO in Western Han Chinese population.^{5,6}

Netrin 1 (NTN1)

The netrin 1 (OMIM: 601614) located on chromosome 17p13.1 is a family of laminin-related secreted proteins. Its functions include axon guidance and cell migration in the central nervous system, angiogenesis, and semicircular canal formation.

NTN1 is expressed at high levels in cells that will come together to form a fusion plate, a prerequisite for the formation of semicircular canals. In netrin 1 mutant mice, fusion plate formation is severely affected, and it stimulates proliferation of the periotic mesenchymal cells which then push the epithelial cell walls together to form the fusion plate.^{67,68} It affects the development of the craniofacial region and has been shown to play a vital role in regulating cell migration during embryogenesis, and it is also expressed in the medial edges and oral sides of the palatal shelves.⁶⁹

A GWAS included 1,409 case-parent trios by several research groups with samples of Asian or European ancestry from Europe, the United States, China, and the Philippines found NTN1 role in the etiology of NSCLP.⁷⁰ A case-control study of NTN1, identified SNP (rs9788972) as a risk locus for NSOCs susceptibility in a northern Chinese population.⁷¹

T-Box Transcription Factor 22

The *T-box 22* (TBX22) gene encodes transcription factors involved in the regulation of developmental processes and plays a major role in human palatogenesis. It contains eight coding exons. TBX22 cause X-linked cleft palate and is characterized by isolated cleft palate and ankyloglossia.⁷² It is expressed in the developing palatal shelves and at the base of the tongue prior to elevation to a horizontal position above the tongue.⁷³

Genome-wide linkage analysis showed the association of TBX22 role in the development of NSCLP.⁷⁴ In addition, mutations in TBX22 were found in individuals with isolated CPO.^{75–77} It plays a significant role in tooth and upper lip development and causes hypodontia and cleft lip.⁷⁸ DNA methylation study suggests that cleft palate-susceptible gene *Tbx22* is associated with gene expression and might be responsible for the developmental failure of palatal fusion, eventually resulting in the formation of cleft palate.⁷⁹

Poliovirus Receptor-Like 1

Poliovirus receptor-like 1 (PVRL1), also known as NECTIN1 (nectin cell adhesion molecule 1) belongs to the nectin subfamily of immunoglobulin-like adhesion molecules that involve cell-cell adhesion. It plays a vital role in the organization of adherens junctions and tight junctions in epithelial and endothelial cells.⁸⁰ During the developmental process, the palatal shelves and palatal epithelium come in close contact and fuse together. PVRL1 plays a major role in these developments during palatogenesis and genetic variations reported to have a significant relationship with CLP. Diseases associated with PVRL1 include cleft lip/palate-ectodermal dysplasia syndrome (CLPED) and herpes simplex.

In animal experiments, PVRL1 expressed at the medial edge epithelium of the palatal shelves and the skin surface epithelium locations that corresponded to the clinical phenotypes of CLPED and in humans, mutations of the *PVRL1* gene resulting CLPED in families from Israel and Brazil.⁸¹ Interestingly, heterozygous mutation of PVRL1 (W185X) associated with NSCLP in northern Venezuela^{82,83} and two novel variants of the *PVRL1* gene were identified in Turkish NSCLP patients.⁸⁴ In an experimental animal studies, PVRL1 expressed at the medial edge epithelium of the palatal shelves and the skin surface epithelium locations that corresponded to the clinical phenotypes of CLPED and in humans, mutations of the *PVRL1* genes caused CLPED in Israel and Brazilian population.^{85–88}

Cleft Lip and Palate-Associated Transmembrane Protein 1

Cleft lip and palate-associated transmembrane protein 1 (CLPTM1) is a multipass transmembrane protein that regulates

GABA-A receptors (e.g., GABRA1) and modulates inhibitory synaptic strength. It is located on chromosome 19q13.3 and plays a role in T-cell development. Mutation of *CLPTM1* genes suggested that a regulatory element in this gene region get affected and which is responsible for the development of orofacial clefts.^{89,90} However, some studies contradict the role of CLPTM1 in the etiology of NSCLP as no evidence of an association with oral clefts was found among the SNPs of CLPTM1 selected for testing in Japanese and Irish population.^{91,92}

MAF bZIP Transcription Factor B

The MAF bZIP Transcription Factor B (*MAFB*) gene encodes a basic leucine zipper (bZIP) transcription factor that plays an important role in the regulation of lineage-specific hematopoiesis. It is located on chromosome 20q11.2, consists of a single exon and spans approximately 3 kb.⁹³ In a mouse study, *MAFB* was expressed in the palatal shelves and the medial edge epithelia (or MEE) during palatal fusion.⁹⁴ Mutations in the *MAFB* gene reported causing multicentric carpotarsal osteolysis syndrome and Duane retraction syndrome 3 with or without deafness.^{95,96}

Several GWAS in European and Asian populations identified the role of *MAFB* in NSCLP.^{97,98} Different systematic review and meta-analyses confirmed that the *MAFB* gene SNP (rs13041247) is associated with NSCLP risk in different population; however, this association is not significant in East Asian or Caucasian populations,^{99,100} whereas, the SNPs rs17820943 and rs6072081 of *MAFB* found to be associated with NSCLP in an East Asian population.¹⁰¹

Other Candidate Genes

A variety of genetic approaches have identified several genes located on different chromosomes, contributing to etiology of NSCLP. Advances in genetics and molecular biology techniques have led the way to the discovery of genetic variation involved in NSCLP. Genes such as *FGFR1*, *TFAP2A*, *GSTT1*, receptor-like tyrosine kinase (RYK), and *ABCA4* have also been found to be associated with NSCLP.

Riley et al assessed the genes involved in the fibroblast growth factor (FGF) signaling pathway and identified the functional impairment in the *FGFR1* gene in NSCLP families and suggested that the FGF signaling pathway may contribute to as much as 3 to 5% of NS cleft lip or palate.¹⁰² Xu et al reported mutations of the *FGFR1* gene in Chinese Kallmann syndrome males with cleft lip/palate.¹⁰³ *TFAP2A* located on 6p24 region has been associated with orofacial clefting. Davies et al reported a patient with cleft palate, microretrognathia, frontal bossing, hypertelorism, flat, broad nasal bridge, low set ears, and developmental delay.¹⁰⁴ However, it was found that there was no significant association between *TFAP2A* and NSCLP in this northern Chinese¹⁰⁵ and Indian population.¹⁰⁶ S-glutathione transferase T1 (*GSTT1*) play an important role in the detoxification and secretion of smoking byproducts and deficiency of this enzyme may cause a greater risk of NSCLP if the individuals were exposed to smoking byproducts during pregnancy.^{107,108} Hozyasz et al suggested that homozygous deletion of *GSTT1* in mother genome might increase the risk of having a child with NSCLP.¹⁰⁹

Further, mutations in the *RYK* gene were also found to be associated with orofacial clefting.^{110,111} Significant linkage disequilibrium between Gamma-aminobutyric acid receptor, Beta-3 (*GABRB3*), and *CLP* was reported by Scapoli et al and this finding in humans is in agreement with previously reported data obtained with the murine model.¹¹² In addition, Baroni et al and Carter et al reported the association of *GABRB3* with oral clefts in different populations.^{113,114} *ATP-binding cassette, subfamily A, member 4* (*ABCA4*) encodes an ATP-binding cassette transporter. Several linkages and GWAS showed the role of *ABCA4* in NSCLP with stronger evidence among Asian samples. *ABCA4* is known to cause the autosomal-recessive retinal degenerative disease Stargardt's disease. A GWAS and a case-parent trio approach by Beaty et al identified *ABCA4* gene association with NSCLP^{115,116} and several other studies also reported a potential role of *ABCA4* in the etiology of CL/P in Brazilian and northern Chinese Han population.^{117,118}

Conclusion

Genetic studies provide insight into the multiple genes involved in the etiology of OFCs. Identification of specific genetic causes of clefts helps for better understanding of the molecular pathogenesis of OFC. In the near future, it helps to provide a more accurate diagnosis, genetic counseling, personalized medicine for better clinical care and prevention of OFCs.

Conflict of Interest

None declared.

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