Review

Genetic etiology of cleft lip and cleft palate

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Abstract: Genetic studies in humans have demonstrated that Cleft lip with or without cleft palate (CL/P) have a diverse genetic background and probably environmental factors influencing these malformations. CL/P is one of the most common congenital birth defects in the craniofacial region with complex etiology involving multiple genetic factors, environmental factors and gene-environment interaction. Children born with these defects suffer from various difficulties such as difficulty in speech, hearing, feeding and other psychosocial problems, and their rehabilitation involves a multidisciplinary approach. The article describes the brief introduction of CL/P, epidemiology and general concepts, etiological factors, and the genes implicated in the etiology of nonsyndromic CL/P (NSCL/P) as suggested by different human genetic studies, animal models, and other expression studies.

Keywords: cleft lip; cleft palate; genetics; candidate genes; molecular biology; mutations; genome-wide association study

1. Introduction

Cleft lip with or without cleft palate (CL/P) is one of the most common congenital birth defects in the craniofacial region [1]. The etiology is polygenic and multifactorial, involving various genetic and environmental factors [2–4]. Children born with these defects may suffer from difficulty in speech, hearing, feeding and other psychosocial problems, and their rehabilitation involves a multidisciplinary approach [5]. In 2008, the World Health Organization (WHO) has recognized that birth defects cause
significant infant mortality and childhood morbidity and have included CL/P in their global burden of disease (GBD) initiative [6].

2. Epidemiology and general concepts

The epidemiological data reveal that the prevalence of Cleft lip with or without cleft palate (CL/P) ranges from 1 in 700 to 1000 live births worldwide. It is highest among Asians and American Indians (1:500), average rates in Caucasians (1:1000) and lowest in Africans (1:2500) [7–10]. The incidence of cleft lip and palate varies according to geographical location, ethnicity, race, gender and socioeconomic status [11–14].

Higher prevalence of clefts has been observed in individuals who live in rural areas and lower socioeconomic status [15–17]. The influence of socioeconomic status on the prevalence of orofacial clefts has not been conclusively determined [3,18]. Possible explanations for this geographic variation and socioeconomic statuses include environmental factors such as nutrition, access to the excellent health care system and lifestyle risk factors such as alcohol, smoking.

Several studies have suggested consanguinity is a risk factor for nonsyndromic cleft lip with or without cleft palate (NSCL/P) [19,20], whereas systematic reviews and meta-analyses reported that there is almost twice the risk of a child with NSCL/P being born if parental consanguinity exists [21].

The incidence of clefts in India is around 1:800 to 1:1000 and three infants are born with some type of cleft every hour [22]. Indian Council of Medical Research (ICMR) task force project revealed that 15% of CL/P cases had a familial association of cleft whereas, 85% of cases did not reveal any positive familial history [23]. A 13-year retrospective study from a cleft center suggested consanguinity is a risk factor for NSCL/P in Indian population [24].

In India, majority of these defects are not surgically corrected because of lack of awareness among parents of child born with a cleft have no access to counseling on the care, affordability and availability of experts to provide the quality treatment. Evidence showed the association of MSX1 [25], IRF6 [26] gene with NSCL/P whereas, CRISPLD2 gene did not show any association with clefts in Indian population [27].

Cleft lip with or without cleft palate (CL/P) can be classified into syndromic and nonsyndromic. Most studies suggest that about 70% of the CL/P cases are nonsyndromic and occur as isolated cases without any other physical abnormalities, whereas 30% of are syndromic which are associated with some other developmental anomalies [28–30]. The syndromic cases are significant in number and can be subdivided into chromosomal syndromes, Mendelian disorders, teratogens (e.g. phenytoin or alcohol) and uncategorized syndromes [31,32].

The frequency of occurrence of cleft lip and cleft palate 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 with a ratio of 1:2 male to female [7,33]. Approximately 90% of clefts are unilateral [34]. Among unilateral cases of CL/P, left-sided clefts are common (66%) than right-sided clefts at a 2:1 ratio of left- to right-sided clefts [35]. Figure 1 shows the prevalence of cleft lip and cleft palate.
The epidemiological studies have reported, adult patients with CL/P may suffer from various difficulties such as esthetics, difficulty in speech, hearing, and psychosocial problems. These individuals may have episodes of depression, low self-esteem and low emotional development even after achieving satisfactory cosmetic, functional, and speech therapy. Many adults will reach a point at which they refuse to take the final stages of dental and other surgical treatment. All these problems associated life-long impact on the quality of life of the CL/P patients [36,37].

3. **Etiology of cleft lip with or without cleft palate (CL/P)**

Although the precise etiology of CL/P is unknown, the complex embryogenesis of the lip and palate make these tissues vulnerable to a variety of potential interferences during a critical stage of development. The etiology is believed to be complex and multifactorial, involving various genetic factors, environmental factors and gene-environment interactions.
3.1. Genetic causes

Evidence for a genetic etiology has been available for many years. Scientific literature shows the heritability of NSCL/P (70%), evidence from twin studies and segregation analysis further confirmed the genetic role in the etiology of CL/P [38,39]. The risk of CL/P is more when a positive family history exists, and an affected parent has a 3% to 5% risk of having an affected child, when one child is affected, parents have a 40% chance of having another affected child [2].

3.2. Environmental causes

Several epidemiological studies showed an increased prevalence of CL/P in patients whose mothers were exposed to smoking, alcohol consumption (binge levels), antiepileptic medications, Corticosteroids, nutritional deficiencies (folic acid) and infectious diseases during pregnancy may adversely affect the intrauterine environment during embryogenesis [7,40]. These environmental factors found to increase the risk of NSCL/P. Recently, maternal illnesses such as hyperthermia, parental occupations, diabetes mellitus and obesity [41] have been identified as risk factors.

The preventive effects of maternal folic acid supplementation on NSCL/P are commonly reported, but the evidence remains generally inconsistent. Van Rooij et al. reported a 74% reduction in CL/P risk with using the folic acid supplements in addition to a high folate diet [42] and Wilcox et al. reported a 39% decrease in CL/P risk with using folic acid but no preventive effects were observed for cleft palate only (CPO) cases [43].

The deficiency of enzymes arylamine N-acetyltransferases (NAT1 and NAT2), glutathione S-transferase (GST), and Cytochrome P450 (CYP1A1) may cause greater risk of CL/P if the individuals were exposed to smoking byproducts during pregnancy. These enzymes play an important role in the detoxification and secretion of smoking byproducts and Cytochrome P450 (CYP1A1) is related to the bioactivation of chemicals such as dioxin in cigarette smoke. The increased risk resulting from exposure to maternal smoking during the periconceptual period raises the possibility that deficiencies in detoxification pathways and genes in certain interactions as a cause of NSCL/P [44].

A study reported that homozygous deletions of S-glutathione transferase M1 (GSTM1) and S-glutathione transferase T1 (GSTT1) in mother genome increased the risk of NSCL/P. However, correlation between smoking status, GSTM1/GSTT1 genotypes and risk of CL/P was not very significant [45]. A population-based case-control and family triad study in Norway showed association between a NAT2 gene and isolated cleft lip in case-triads [40] and, there was no association found between TGFA gene and smoking in the etiology CL/P in Indian population [46].

Maternal alcohol intake (binge levels) in short periods of time will increase risk of CL/P has been supported by association with variation in the alcohol dehydrogenase (ADH1C) gene, because the ADH1C involved in the metabolic pathways of many alcohols. Several studies showed an increased risk of nonsyndromic CL/P in women who reported drinking alcohol during the first trimester, compared with women who did not. The mutation of ADH1C gene has suggested its role in the etiology of NSCL/P [47,48].
3.3. Gene-environment (GxE) interaction

Cleft lip and palate is a complex disorder involving the multiple genes and environmental factors. It is essential to consider gene-environment interaction as it helps for a better understanding of the pathogenesis of the disease and analyzing both susceptible and non-susceptible individuals [49]. Several Studies identified gene-environment interactions of maternal smoking, maternal alcohol consumption and folic acid deficiency as a causative factor for NSCL/P [50,51].

Maternal smoking and folic acid intake are the two important factors that appear to modify genetic risks for cleft lip and palate. Studies have found gene-environment interactions between smoking and variants in transforming growth factors (TGF), muscle segment homeobox (MSX) and retinoic acid receptor genes [52]. Two polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C have been shown to decrease MTHFR activity and the C677T polymorphism also decreases circulating folate and increases homocysteine levels and associated with an increased risk of NSCL/P in Asian populations [53]. The observation that drinking high doses of alcohol in short periods of time will increase risk of CL/P has been supported by association with variation in the alcohol dehydrogenase (ADH1C) gene [47]. Genetic variants in TGFA, TGFB3 and MSX1 genes have been investigated for interactions with environmental risk factors such as smoking, alcohol consumption [54].

3.4. Genetic etiology of CL/P

A variety of genetic approaches have been used to identify genes and loci contributing to CL/P which includes animal models expression studies, genomic rearrangements and copy number variants, linkage studies, candidate gene-based association studies, candidate gene sequencing and genome-wide association (GWA) studies [4,55].

In the recent genomic era, advances in genetics and molecular biology techniques have explored the genetic basis of development of these craniofacial defects, and several genes and loci associated with CL/P have been discovered. This article provides an overview of the genes implicated in the etiology of Nonsyndromic CL/P (NSCL/P) as suggested by different human genetic studies, animal models, and other expression studies. Table 1 demonstrates the list of genes involved in the etiology of Nonsyndromic CL/P.
Table 1. Genes involved in the etiology of Nonsyndromic CL/P.

| Gene      | Chromosomal location | OMIM    | Evidence            | References     |
|-----------|----------------------|---------|---------------------|----------------|
| IRF6      | 1q32.2               | 607199  | GWAS, LD, L, M      | [57,60,62,64]  |
| MSX1      | 4p16.2               | 142983  | LD, M, GWAS         | [65,67,69–71]  |
| TGFA      | 2p13.3               | 190170  | LD, GWAS            | [73,75,76,78]  |
| BMP4      | 14q22.2              | 112262  | M                   | [79,81,83]     |
| PAX7      | 1p36.13              | 167410  | GWAS                | [85,93,95]     |
| RUNX2     | 6p21.1               | 600211  | GWAS                | [91,93,95]     |
| MSX1      | 4p16.2               | 142983  | LD, M, GWAS         | [65,67,69–71]  |
| TGFA      | 2p13.3               | 190170  | LD, GWAS            | [73,75,76,78]  |
| BMP4      | 14q22.2              | 112262  | M                   | [79,81,83]     |
| PAX7      | 1p36.13              | 167410  | GWAS                | [85,93,95]     |
| RUNX2     | 6p21.1               | 600211  | GWAS                | [91,93,95]     |
| IRF6      | 1q32.2               | 607199  | GWAS, LD, L, M      | [57,60,62,64]  |
| MSX1      | 4p16.2               | 142983  | LD, M, GWAS         | [65,67,69–71]  |
| TGFA      | 2p13.3               | 190170  | LD, GWAS            | [73,75,76,78]  |
| BMP4      | 14q22.2              | 112262  | M                   | [79,81,83]     |
| PAX7      | 1p36.13              | 167410  | GWAS                | [85,93,95]     |
| RUNX2     | 6p21.1               | 600211  | GWAS                | [91,93,95]     |

Notes: *GWAS: Genome-wide association studies, L: Linkage, M: Mutations, LD: Linkage disequilibrium.

4. Overview of genes involved in the etiology of Nonsyndromic CL/P

4.1. The interferon regulatory factor-6 (IRF6)

The Interferon regulatory factor-6 (IRF6) is one of the most important and consistent gene implicated in the etiology of CLP and belongs to a family of transcription factors that share a highly
conserved helix-turn-helix DNA-binding domain. It is located on the ‘q’ arm of chromosome 1, between positions 32.3 and 41. Van der Woude’s syndrome (VDWS) is the most common form of syndromic CL/P and is characterized by CL/P, isolated CP, pits or mucous cysts on the lower lip, and hypodontia and accounts for 2% of all CL/P cases [56]. Popliteal pterygium syndrome (PPS) has all the features of VDWS plus popliteal pterygium, toe/fingers abnormality, syndactyly, and genital abnormalities. Mutation in the IRF6 causes these two autosomal dominant syndromes in CL/P [57], thereby confirming a common genetic etiology in both syndromes.

Evidence of IRF6 causing CLP/CP, has been confirmed in several populations by the research of Zucchero et al. (2004), Blanton et al. (2005), Jugessur et al. (2008), Huang et al. (2009) and subsequently in GWAS meta-analysis [58–62]. AGenome-wide association (GWA) study consisting of NSCL/P case-parent trios from different populations showed four SNPs of IRF6 having strong genome-wide significance with orofacial clefting [63]. Other studies also reported the substantial contribution of IRF6 in the etiology of nonsyndromic CL/P [25, 64].

4.2. MSH homeobox 1 (MSX1)

Muscle segment homeobox 1 (MSX1) regulates and stimulates the appropriate protein required dedifferentiation process. It is also known as homeobox protein MSX-1/HOX7/MSH Homeo Box Homolog 1 (Drosophila) gene. Human MSX1 gene located at 4p16.1 spans around 4.05 kb and consists of two exons and one intron. MSX1 gene has a role in cyclin D1 up-regulation; thus, it inhibits cell differentiation. It plays a vital role during the development of teeth and craniofacial structures.

In homozygous transgenic mice models, nonfunctioning Msx1 gene showed cleft palate and facial and dental abnormalities [65]. In humans, a nonsense mutation of MSX1 resulted in autosomal dominant tooth agenesis, cleft lip and cleft palate, isolated cleft palate in a Dutch family, suggesting MSX1 involvement in human clefting [66, 67]. Several studies in different populations and a meta-analysis have reported the MSX1 gene as a causative factor in NSCL/P [68–71].

4.3. Transforming growth factor alpha (TGFA)

Transforming Growth Factor Alpha gene encodes a growth factor that binds the epidermal growth factor receptor, which activates a signaling pathway for cell proliferation, differentiation and development, so this gene has been studied extensively in the family of growth factors. TGFA plays an important role in the development of palate and present in the medial edge epithelium of palatal shelves. Several studies have demonstrated a significant association between transforming growth factor-alpha (TGFA) and CL/P [72]. A study showed infants with TGFA genotype whose mothers did not use multivitamins containing folic acid periconceptionally are at a higher risk of being born with CL/P [73]. A meta-analysis and other studies showed inconclusive data and failure of association between TGFA with CL/P due to genetic heterogeneity [74, 75]. A combined case-parent trios and case-control study suggested gene to gene interaction between TGFA and IRF6 influences the risk of developing cleft lip with/without cleft palate [76]. Recently, two meta-analyses confirmed the TGFA Taq 1 polymorphism may be associated with the risk of CL/P [77, 78].
4.4. Bone morphogenetic protein 4 (BMP4)

BMP4 is a member of the BMP family and transforming growth factor beta (TGFβ) superfamily of secretory signaling molecules that play crucial roles during cartilage and bone formation, tooth development and facial development. It is located on chromosome 14q22.2, consists of 5 exons and spans about 7 kb. Mutations in this gene are associated with orofacial cleft and microphthalmia in humans. A study reported BMP4 mutation in a child caused cleft lip and palate [79]. Several studies have suggested the risk of nonsyndromic oral clefts may be influenced by variation in the BMP4 gene [80,81]. Two different meta-analyses also confirmed the association of BMP4 gene SNP (rs17563) with NSCL/P [82,83].

4.5. Paired box protein Pax-7 (PAX7)

Paired box 7 gene is a member of the paired box (PAX) family, encode for specific DNA binding transcription factors and located at 1p36.13. It contains a paired box domain, an octapeptide, and a paired-type homeodomain. The Functions of PAX7 includes Neural crest development, fetal development, expressed in palatal shelves of the maxilla, Meckel’s cartilage, Nasal cavity and Myogenesis. Animal studies showed that mutant mice have a malformation of the maxilla and thus confirming its role in craniofacial development [84].

In humans, a case-parent trio study of PAX7 associated with NSCL/P in four populations (76 from Maryland, 146 from Taiwan, 35 from Singapore, and 40 from Korea) where they assessed the maternal transmission effects of PAX7 genes and they concluded that these genes might influence the risk of CL/P [85], a meta-analysis [86], genome-wide association studies [87,88] and other studies suggested a role of PAX7 in the etiology of NSCL/P [89,90].

4.6. Runt-related transcription factor 2 (RUNX2)

RUNX2 is a member of the RUNX family of transcription factors and encodes a nuclear protein with a Runt DNA-binding domain which is essential for osteoblastic differentiation and skeletal morphogenesis. In humans and mice study showed, the RUNX2 act as a transcriptional regulator for bone formation and tooth development. Mutations in the RUNX2 gene cause a rare autosomal dominant disorder cleidocranial dysplasia, characterized by skeletal defects, supernumerary teeth, and delayed tooth eruption, clefts of the palate or submucous palate [91–93]. A case-parent trio design consists of four populations (Maryland, Taiwan, Singapore, and Korea) were genotyped for 24 single nucleotide polymorphisms (SNPs) of RUNX2 gene, among that three SNPs showed significant excess maternal transmission suggesting RUNX2 may influence the risk of NSCL/P through imprinting effects [94]. Evidence of gene-environment interaction of the RUNX2 gene in a Chinese case-parent trio design showed maternal over-transmission RUNX2 markers and suggested it may influence the susceptibility to NSCL/P through interacting with environmental tobacco smoke [95].

4.7. Small ubiquitin-like modifier 1 (SUMO1)

SUMO1 is a protein coding gene involved in various cellular processes, such as nuclear transport, transcriptional regulation, apoptosis, and protein stability. In Mouse model, haploinsufficiency of
Sumo1 gene resulted in cleft palate [96]. In humans, genetic associations between NSCL/P and SUMO1 variants have been reported in different populations [97,98]. A meta-analysis provided empirical evidence of 4 single-nucleotide polymorphisms (SNPs) in the SUMO1 gene contribute to the risk of nonsyndromic cleft lip with or without palate [99].

4.8. Ventral anterior homeobox 1 (VAX 1)

Ventral anterior homeobox1 is a transcriptional regulator containing a DNA binding homeobox domain. Genes of this family are involved in the regulation of body development and morphogenesis. The homeobox sequences of mouse and human VAX1 are identical. It is expressed in various craniofacial structures, and its deficiency causes cleft palate [100]. A homozygous missense mutation of vax1 was expressed in an Egyptian child from a consanguineous family with bilateral microphthalmia, bilateral CLP, and corpus callosum agenesis [101]. GWAS using case-parent triads from different populations two rare missense mutations in VAX1 replicated previous GWAS findings for markers in VAX1 in the Asian and Saudi population, and identified rare variants of VAX1 that may contribute to the etiology of CL(P) [102–104].

4.9. Forkhead box E1 (FOXE1)

The FOXE1 belongs to a forkhead box (FOX) /winged helix-domain transcription factor family involved in embryogenesis and located at 9q22.33. A mouse model study showed thyroid agenesis, cleft palate [105] similar to previously reported [106]. Mutations within the FOXE1 gene in two siblings resulted in congenital hypothyroidism, athyroidal and Cleft Palate [107]. Genome-wide association studies (GWAS) and meta-analyses also reported the significant association between FOXE1 and nonsyndromic CL/P in different populations [108,109].

4.10. Cysteine-rich secretory protein LCCL domain containing 2 (CRISPLD2)

CRISPLD2 gene located on chromosome 16q24.1 contains 15 exons and extends about 110 kb. In mouse embryos, Crispld2 expressed in nasopharynx, mandible, nasal cartilage, palate, and tooth development [110,111]. Three SNPs of CRISPLD2 in a northern Chinese population [112,113] and Next-generation sequencing of eighteen SNPs of CRISPLD2 confirmed genetic polymorphism with an increased risk of NSCL/P in a Chinese Xinjiang Uyghur population [114], in contrast, three SNPs rs1546124, rs4783099, and rs16974880 of CRISPLD2 gene did not show any association with clefts in Indian population [27].

4.11. The methylenetetrahydrofolate reductase gene (MTHFR)

Methylenetetrahydrofolate reductase (MTHFR) located on 1q36 and is a major enzyme of folic acid metabolism. Mutations in this gene are associated with methylenetetrahydrofolate reductase deficiency [115]. Several associations have been reported between the polymorphisms of the MTHFR gene and the risk of NSCL/P [116–118]. A systematic review and meta-analysis reported the association of MTHFR polymorphism of SNP (rs1801133) as a risk factor for NSCL/P [119].
sequencing study identified the mutation (c.G586A, p.G196S) in the MTHFR gene as a possible cause of NSCL/P [120].

4.12. **Wingless-type MMTV integration site family, member 9B (WNT9B)**

WNT9B is a member of the WNT gene family that encodes extracellular signaling proteins. These genes are required for body axis patterning, cell fate specification, cell proliferation and cell migration during embryonic development. WNT9B directly regulates facial development and expressed in the facial ectoderm at critical stages of midfacial morphogenesis [121]. WNT9B mRNA is expressed in maxillary, medial nasal, and lateral nasal ectoderm. During lip fusion, WNT9B is expressed in the epithelial seam between the fusing medial and lateral nasal processes [122,123]. It also signal through the canonical Wnt signaling pathway to regulate midfacial development and lip fusion and are therefore candidate gene for an etiological role in NSCL/P [124,125].

Several studies have reported the involvement of WNT9B gene in the families of NSCL/P [126–129]. A miRNA study confirmed the role of miR-497-5p and miR-655-3p in the etiology of CL/P by inhibiting cell proliferation during lip fusion [130].

4.13. **Myosin heavy chain 9 (MYH9)**

Myosin heavy chain 9 (MYH9) is located on chromosome 22q13.1 and encodes myosin IIA heavy chain, which participates in a number of cellular functional activities, including the maintenance of cell shape, cytokinesis, migration, and adhesion [131]. It has been reported that polymorphisms of MYH9 are associated with a variety of diseases, including MYH9–related diseases (MYH9-RD), macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss and deafness, and cleft lip and palate [132]. During palatal morphogenesis, MYH9 is abundantly expressed in midline epithelial seam (MES) of palatal shelves before fusion suggesting its role during palate development and contribute to NSCL/P [133].

Several studies have reported the MYH9 role in the etiology of NSCL/P [134–136]. Further, a next-generation sequencing study consisted of 103 cases of nonsyndromic orofacial clefts and 100 normal controls in the Taiwanese population [137] and a case-control study also confirmed MYH9 association with NSCL/P [138].

4.14. **Other candidate genes**

A variety of genetic approaches have identified several genes and loci contributing to etiology of NSCL/P. Candidate genes located on different chromosomes involved in the etiology of NSCL/P is shown in Figure 2.

Advances in molecular and cellular biology techniques have led the way to the discovery of multiple genes involved in the etiology of NSCL/P. A multicenter association study identified various genes significantly associated with NSCL/P; including MSX1, SPRY1, MSX2, PRSS35, TFAP2A, SHH, VAX1, TBX10, WNT11, PAX9, BMP4, JAG2, AXIN2, DVL2, KIF7, and TCBE3, a Stepwise regression analysis revealed that out of 16 genes, 11 genes contributed to 15.5% of the etiology of NSCL/P in a Brazilian population [139]. Different genome-wide association studies have reported
multiple candidate genes such as IRF6, MSX1, SPRY1, SPRY2, CHD7, GABRB3, NOG, NTN1, MMP16, KRT18, DICER1, RAD54B, CREBBP, GADD45G, TFAP2A, VAX1, GSC, PTCH1, MYC, TAF1B, MAFB, OFCC1, ARHGAP29, WNT9B, FGFR1, FGFI0 [140,141] although 26 genes were studied, only 14 genes were associated with clefts in Chinese population. A Microarray hybridization analysis reported COL11A1, TERT, MIR4457, CLPTM1, ESR1, GLI3, OFD1, TBX1, PHF8 and FLNA, POMGNT2, WHSC1, GRM5, ALX1, PAX9, DLK1, FOXC2-FOXL1, MAU2, IRF6, MYCN, VAX1, MAFB [142] association in the etiology of NSCL/P in Chinese population, among these genes, only 14 genes were identified in the pathogenesis of NSCL/P and this evidence suggest that, genetic heterogeneity between the sub-phenotypes of NSCL/P and among different populations.

![Figure 2. Candidate genes located on different chromosomes involved in the etiology of Nonsyndromic CL/P; individual chromosomes have been marked by using a colour scheme in which each gene has been labelled to their parent chromosome by the corresponding colours.]()

The etiology of cleft lip and palate is polygenic and multifactorial, model of inheritance can be influenced by several factors, such as gender of the affected individual, severity of the orofacial cleft, and number of affected relatives. Results from previous studies support the presence of heterogeneity among populations and the presence of multiple genes involved in the etiology of CL/P. Due to genetic heterogeneity associated with NSCL/P, a family with several affected individuals can actually represent the segregation of a single-gene disorder, which would not be promptly recognized based solely on
clinical evaluation. The recurrence risk among families with one first-degree affected relative may vary depending on the population and the identification of other individuals with CL/P in the family should be always interpreted with caution [143,144].

Significant knowledge of the genetic etiology and risk factors of CL/P has already been gained and is being used to reduce the overall health burden of these defects. Identification of specific genetic and environmental causes of CL/P could enable major changes in genetic counseling, improved programs for personalized medicine applications and aid in taking effective preventive measures. With the advances in genomic technology, understanding of the genetic mechanisms leading to CL/P will be achieved in the upcoming years and this will allow more accurate methods of genetic screening, identification high risk individuals and families, and improved prenatal diagnosis.

5. Conclusion

Genetic studies in humans have demonstrated that Cleft lip with or without cleft palate (CL/P) have a diverse genetic background and probably environmental factors influencing these malformations. Several studies have suggested genetic factors play an important role in the etiology of CL/P and by understanding the relative contribution of these candidate genes could be integrated into a genetic test for the weighed risk of CL/P.

Conflict of interest

All authors declare no conflicts of interest in this paper.

Web Resources:

- National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/.
- Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/omim.

References

1. Schutte BC, Murray JC (1999) The many faces and factors of orofacial clefts. *Hum Mol Genet* 8: 1853–1859.
2. Bender PL (2000) Genetics of cleft lip and palate. *J Pediatr Nurs* 15: 242–249.
3. Spritz RA (2001) The genetics and epigenetics of Orofacial clefts. *Curr Opin Pediatr* 13: 556–560.
4. Leslie EJ, Marazita ML (2013) Genetics of Cleft Lip and Cleft Palate. *Am J Med Genet C Semin Med Genet* 163: 246–258.
5. Stanier P, Moore GE (2004) Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. *Hum Mol Genet* 13: R73–81.
6. Mossey P, Little J (2009) Addressing the challenges of cleft lip and palate research in India. *Ind J Plast Surg (supplement 1)* 42: S9–S18.
7. Dixon MJ, Marazita ML, Beaty TH, et al. (2011) Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet* 12: 167–178.
8. Mossey PA, Little J, Munger RG, et al. (2009) Cleft lip and palate. *Lancet* 374: 1773–1785.
9. Croen LA, Shaw GM, Wasserman CR, et al. (1998) Racial and ethnic variations in the prevalence of orofacial clefts in California, 1983–1992. Am J Med Genet 79: 42–47.
10. Ching GHS, Chung CS (1974) A genetic study of cleft lip and palate in Hawaii. 1. Interracial crosses. Am J Hum Genet 26: 162–172.
11. Murray JC, Daack-Hirsch S, Buetow KH, et al. (1997) Clinical and epidemiologic studies of cleft lip and palate in the Philippines. Cleft Palate Craniofac J 34: 7–10.
12. Vandersas AP (1987) Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J 24: 216–225.
13. Hagberg C, Larson O, Milerad J (1997) Incidence of cleft lip and palate and risks of additional malformations. Cleft Palate Craniofac J 35: 40–45.
14. Ogle OE (1993) Incidence of cleft lip and palate in a newborn Zairian sample. Cleft Palate Craniofac J 30: 250–251.
15. Chung CS, Mi MP, Beechert AM, et al. (1987) Genetic epidemiology of cleft lip with or without cleft palate in the population of Hawaii. Genetic Epidemiol 4: 415–423.
16. Wyszynski DF, Wu T (2002) Prenatal and perinatal factors associated with isolated oral clefting. Cleft Palate Craniofac J 39: 370–375.
17. Mossey PA (2007) Epidemiology underpinning research in the etiology of orofacial clefts. Orthod Craniofac Res 10: 114–120.
18. Yang J, Carmichael SL, Canfield M, et al. (2008) Socioeconomic status in relation to selected birth defects in a large multicentered US case-control study. Am J Epidemiol 167: 145–154.
19. Sabbagh HJ, Innes NP, Sallout BI, et al. (2015) Birth prevalence of non-syndromic orofacial clefts in Saudi Arabia and the effects of parental consanguinity. Saudi Med J 36: 1076–1083.
20. Silva CM, Pereira MCM, Queiroz TB, et al. (2019) Can Parental Consanguinity Be a Risk Factor for the Occurrence of Nonsyndromic Oral Cleft? Early Hum Dev 135: 23–26.
21. Sabbagh HJ, Hassan MH, Innes NP, et al. (2014) Parental Consanguinity and Nonsyndromic Orofacial Clefts in Children: A Systematic Review and Meta-Analyses. Cleft Palate Craniofac J 51: 501–513.
22. Reddy SG, Reddy RR, Bronkhorst EM, et al. (2010) Incidence of cleft lip and palate in the state of Andhra Pradesh, South India. Indian J Plast Surg 43: 184–189.
23. Indian Council of Medical Research (ICMR) Task Force Project. (2016) Division of Non-Communicable Diseases; New Delhi.
24. Neela PK, Reddy SG, Husain A, et al. (2019) Association of cleft lip and/or palate in people born to consanguineous parents: A 13year retrospective study from a very high volume cleft center. J Cleft Lip Palate Craniofac Anoma 16: 33–37.
25. Babu GV, Syed AH, Murthy J, et al. (2018) IRF6 rs2235375 single nucleotide polymorphism is associated with isolated non-syndromic cleft palate but not with cleft lip with or without palate in South Indian population. Braz J Otorhinolaryngol 84: 473–477.
26. Babu GV, Syed AH, Murthy J, et al. (2015) Evidence of the involvement of the polymorphisms near MSX1 gene in non-syndromic cleft lip with or without cleft palate. Int J Pediatr Otorhinolaryngol 79: 1081–1084.
27. Neela PK, Reddy SG, Husain A, et al. (2020) CRISPLD2 Gene Polymorphisms with Nonsyndromic Cleft Lip and Palate in Indian Population. Global Med Genet 7: 22–25.
28. Tolarova MM, Cervenka J (1998) Classification and birth prevalence of Orofacial clefts. *Am J Med Genet* 75: 126–137.
29. Wong FK, Hagg U (2004) An update on the aetiology of orofacial clefts. *Hong Kong Med J* 10: 331–336.
30. Rahimov F, Jugessur A, Murray JC (2012) Genetics of Nonsyndromic Orofacial Clefts. *Cleft Palate Craniofac J* 49: 73–91.
31. Jones MC (1988) Etiology of facial clefts. Prospective evaluation of 428 patients. *Cleft Palate J* 25: 16–20.
32. Kohli SS, Kohli VS (2012) A comprehensive review of the genetic basis of cleft lip and palate. *J Oral Maxillofac Pathol* 16: 64–72.
33. Wyszynski DF, Wu T (2002) Prenatal and perinatal factors associated with isolated oral clefting. *Cleft Palate Craniofac J* 39: 370–375.
34. Cobourne MT (2012) Cleft Lip and Palate. Epidemiology, Aetiology and Treatment. London: Karger. *Front Oral Biol* 60–70.
35. Gundlach KK, Maus C (2006) Epidemiological studies on the frequency of clefts in Europe and world-wide. *J Craniomaxillofac Surg* 34: 1–2.
36. Hobbs J (1991) The adult patient. *Clin Commun Disord* 1: 48–52.
37. Murthy J (2009) Management of cleft lip and palate in adults. *Indian J Plast Surg* 42: S116–S122.
38. Murray JC (2002) Gene/environment causes of cleft lip and/or palate. *Clin Genet* 61: 248–256.
39. Funato N, Nakamura M (2017) Identification of shared and unique gene families associated with oral clefts. *Int J Oral Sci* 9: 104–109.
40. Lie RT, Wilcox AJ, Taylor J, et al. (2008) Maternal smoking and oral clefts: the role of detoxification pathway genes. *Epidemiology* 19: 606–615.
41. Stothard KJ, Tennant PW, Bell R, et al. (2009) Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA* 301: 636–650.
42. Van Rooij IA, Ocké MC, Straatman H, et al. (2004) Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. *Prev Med* 39: 689–694.
43. Wilcox AJ, Lie RT, Solvoll K, et al. (2007) Folic acid supplements and risk of facial clefts: national population based case-control study. *BMJ* 334: 464.
44. Martyn TC (2004) The complex genetics of cleft lip and palate. *Eur J Orthod* 26: 7–16.
45. Hozyasz KK, Mostowska A, Surowiec Z, et al. (2005) Genetic polymorphisms of GSTM1 and GSTT1 in mothers of children with isolated cleft lip with or without cleft palate. *Przegl Lek* 62: 1019–1022.
46. Junaid M, Narayanan MBA, Jayanthi D, et al. (2018) Association between maternal exposure to tobacco, presence of TGFA gene, and the occurrence of oral clefts. A case control study. *Clin Oral Investig* 22: 217–223.
47. Chevrier C, Perret C, Bahuau M, et al. (2005) Interaction between the ADH1C polymorphism and maternal alcohol intake in the risk of nonsyndromic oral clefts: an evaluation of the contribution of child and maternal genotypes. *Birth Defects Res A Clin Mol Teratol* 73: 114–122.
48. Jugessur A, Shi M, Gjessing HK, et al. (2009) Genetic determinants of facial clefting: analysis of 357 candidate genes using two national cleft studies from Scandinavia. *PLoS One* 4: e5385.
49. Mossey PA, Little J (2002) Epidemiology of oral clefts: An international perspective. In: Wyszynski D, editor. Cleft lip and palate: From origin to treatment. Oxford: Oxford University Press, 127–144.

50. Krapels IP, van Rooij IA, Ocke MC, et al. (2004) Maternal nutritional status and the risk for orofacial cleft offspring in humans. *J Nutr* 134: 3106–3113.

51. Hayes C, Werler MM, Willett WC, et al. (1996) Case-control study of periconceptional folic acid supplementation and orofacial clefts. *Am J Epidemiol* 143: 1229–1234.

52. Thomas D (2010) Gene-environment-wide association studies: emerging approaches. *Nat Rev Genet* 11: 259–272.

53. Zhao M, Ren Y, Shen L, et al. (2014) Association between MTHFR C677T and A1298C Polymorphisms and NSCL/P Risk in Asians: A Meta-Analysis. *PLoS One* 9: e88242.

54. Jagomägi T, Nikopensius T, Krjutškov K, et al. (2010) MTHFR and MSX1 contribute to the risk of nonsyndromic cleft lip/palate. *Eur J Oral Sci* 118: 213–220.

55. Borkar AS (1993) Epidemiology of facial clefts in the central province of Saudi Arabia. *Br J Plast Surg* 46: 673–675.

56. Burdick AB (1986) Genetic epidemiology and control of genetic expression in Van der Woude syndrome. *J Craniofac Genet Dev Biol Suppl* 2: 99–105.

57. Kondo S, Schutte BC, Richardson RJ, et al. (2002) Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nat Genet* 32: 285–289.

58. Zucchero TM, Cooper ME, Maher BS, et al. (2004) Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip/palate. *N Engl J Med* 351: 769–780.

59. Blanton SH, Cortez A, Stal S, et al. (2005) Variation in IRF6 contributes to nonsyndromic cleft lip and palate. *Am J Med Genet A* 137: 259–262.

60. Jugessur A, Rahimov F, Lie RT, et al. (2008) Genetic variants in IRF6 and the risk of facial clefts: single-marker and haplotype-based analyses in a population-based case-control study of facial clefts in Norway. *Genet Epidemiol* 32: 413–424.

61. Huang Y, Wu J, Ma J, et al. (2009) Association between IRF6 SNPs and oral clefts in West China. *J Dent Res* 88: 715–718.

62. Grant SF, Wang K, Zhang H, et al. (2009) A Genome-Wide Association Study Identifies a Locus for Nonsyndromic Cleft Lip with or without Cleft Palate on 8q24. *J Pediatr* 155: 909–913.

63. Beaty TH, Murray JC, Marazita ML, et al. (2010) A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet* 42: 525–529.

64. Ibarra-Arce A, García-Álvarez M, Cortés-González D (2015) IRF6 polymorphisms in Mexican patients with non-syndromic cleft lip. *Meta Gene* 4: 8–16.

65. Satokata I, Maas R (1994) Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 6: 348–356.

66. Van den Boogaard MJ, Dorland M, Beemer FA, et al. (2000) MSX1 mutation is associated with Orofacial clefting and tooth agenesis in humans. *Nat Genet* 24: 342–343.

67. Jezewski PA, Vieira AR, Nishimura C, et al. (2003) Complete sequencing shows a role for MSX1 in nonsyndromic cleft lip and palate. *J Med Genet* 40: 399–407.

68. Otero L, Gutierrez S, Chaves M, et al. (2007) Association of MSX1 with nonsyndromic cleft lip and palate in a Colombian population. *Cleft palate craniofac J* 44: 653–656.
69. Salahshourifar I, Halim AS, Sulaiman WA, et al. (2011) Contribution of MSX1 variants to the risk of non-syndromic cleft lip and palate in a Malay population. *J Hum Genet* 56: 755–758.
70. Indencleef K, Roosenboom J, Hoskens H, et al. (2018) Six NSCL/P loci show associations with normal-range craniofacial variation. Front. *Genet* 9: 502.
71. Tasanarong P, Pabalan N, Tharabenjasin P, et al. (2019) MSX1 gene polymorphisms and non-syndromic cleft lip with or without palate (NSCL/P): A meta-analysis. *Oral Dis* 25: 1492–1501.
72. Mitchell LE (1997) Transforming growth factor alpha locus and nonsyndromic cleft lip with or without cleft palate: a reappraisal. *Genet Epidemiol* 14: 231–240.
73. Shaw GM, Wasserman CR, Murray JC, et al. (1998) Infant TGF-alpha genotype, orofacial clefts, and maternal periconceptional multivitamin use. *Cleft Palate Craniofac J* 35: 366–367.
74. Holder SE, Vintiner GM, Farren B, et al. (1999) Confirmation of an association between RFLPs at the transforming growth factor-alpha locus and nonsyndromic cleft lip and palate. *J Med Genet* 29: 390–392.
75. Passos-Bueno MR, Gaspar DA, Kamiya T, et al. (2004) Transforming growth factor-alpha and non-syndromic cleft lip with or without palate in Brazilian patients: results of large case-control study. *Cleft Palate Craniofac J* 41: 387–391.
76. Letra A, Fakhouri W, Renata F, et al. (2012) Interaction between IRF6 and TGFA Genes Contribute to the Risk of Nonsyndromic Cleft Lip/Palate. *PLoS One* 7: e45441.
77. Feng C, Zhang E, Duan W, et al. (2014) Association Between Polymorphism of TGFA Taq I and Cleft Lip and/or Palate: A Meta-Analysis. *BMC Oral Health* 11: 14–88.
78. Yan C, He DQ, Chen LY, et al. (2018) Transforming Growth Factor Alpha Taq I Polymorphisms and Nonsyndromic Cleft Lip and/or Palate Risk: A Meta-Analysis. *Cleft Palate Craniofac J* 55: 814–820.
79. Suzuki S, Marazita ML, Cooper ME, et al. (2009) Mutations in BMP4 are associated with subepithelial, microform, and overt cleft lip. *Am J Hum Genet* 84: 406–411.
80. Antunes LS, Küchler EC, Tannure PN, et al. (2013) BMP4 Polymorphism is Associated with Nonsyndromic Oral Cleft in a Brazilian Population. *Cleft Palate Craniofac J* 50: 633–638.
81. Li YH, Yang J, Zhang JL, et al. (2017) BMP4 rs17563 polymorphism and nonsyndromic cleft lip with or without cleft palate: a meta-analysis. *Medicine (Baltim)* 96: e7676.
82. Hao J, Gao R, Wua W, et al. (2018) Association between BMP4 gene polymorphisms and cleft lip with or without cleft palate in a population from South China. *Arch Oral Biol* 93: 95–99.
83. Assis Machado R, De Toledo IP, Martelli-Junior H, et al. (2018) Potential genetic markers for nonsyndromic oral clefts in the Brazilian population: a systematic review and meta-analysis. *Birth Defects Res* 110: 827–839.
84. Mansouri A, Stoykova A, Torres M, et al. (1996) Dysgenesis of cephalic neural crest derivatives in Pax7 mutant mice. *Development* 122: 831–838.
85. Sull JW, Liang KY, Hetmanski JB, et al. (2009) Maternal transmission effects of the PAX genes among cleft case-parent trios from four populations. *Eur J Hum Genet* 17: 831–839.
86. Ludwig KU, Mangold E, Herms S, et al. (2012) Genome-wide meta-analyses of non syndromic cleft lip with or without cleft palate identify six new risk loci. *Nat Genet* 44: 968–971.
87. Beaty TH, Taub MA, Scott AF, et al. (2013) confirming genes influencing risk to Cleft lip with or without cleft palate in a case – parent trio study. *Hum Genet* 132: 771–781.
88. Leslie EJ, Taub MA, Liu H, et al. (2015) Identification of Functional Variants for Cleft Lip with or without Cleft Palate in or near PAX7, FGFR2 and NOG by Targeted Sequencing of GWAS Loci. *Am J Hum Genet* 96: 397–411.

89. Duan SJ, Huang N, Zhang BH, et al. (2017) New insights from GWAS for the cleft palate among Han Chinese population. *Med Oral Patol Oral Cir Bucal* 22: e219–e227.

90. Gaczkowska A, Biedziak B, Budner M, et al. (2019) PAX7 nucleotide variants and the risk of nonsyndromic orofacial clefts in the Polish population. *Oral Dis* 25: 1608–1618.

91. Otto F, Kanegane H, Mundlos S (2002) Mutations in the RUNX2 gene in patients with cleidocranial dysplasia. *Hum Mutat* 19: 209–216.

92. Cooper SC, Flaitz CM, Johnston DA, et al. (2001) A natural history of cleidocranial dysplasia. *Am J Med Genet* 104: 1–6.

93. Aberg T, Cavender A, Gaikwad JS, et al. (2004) Phenotypic changes in dentition of Runx2 homozygote-null mutant mice. *J Histochem Cytochem* 52: 131.

94. Sull JW, Liang KY, Hetmanski JB, et al. (2008) Differential parental transmission of markers in RUNX2 among cleft case-parent trios from four populations. *Genet Epidemiol* 32: 505–512.

95. Wu T, Daniele M, Fallin MD, et al. (2012) Evidence of Gene-Environment Interaction for the RUNX2 Gene and Environmental Tobacco Smoke in Controlling the Risk of Cleft Lip with/without Cleft Palate. *Birth Defects Res A Clin Mol Teratol* 94: 76–83.

96. Alkuraya FS, Saadi I, Lund JJ, et al. (2006) SUMO1 haploinsufficiency leads to cleft lip and palate. *Science* 313: 1751.

97. Song T, Li G, Jing G, et al. (2008) SUMO1 polymorphisms are associated with non-syndromic cleft lip with or without cleft palate. *Biochem Biophys Res Commun* 377: 1265–1268.

98. Carter TC, Molloy AM, Pangilinan F, et al. (2010) Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. *Birth Defects Res A Clin Mol Teratol* 88: 84–93.

99. Tang MR, Wang YX, Han SY, et al. (2014) SUMO1 genetic polymorphisms may contribute to the risk of nonsyndromic cleft lip with or without palate: a meta-analysis. *Genet Test Mol Biomarkers* 18: 616–624.

100. Hallonet M, Hollemann T, Pieler T, et al. (1999) VAX1, a novel homeobox-containing gene, directs development of the basal forebrain and visual system. *Genes Dev* 13: 3106–3114.

101. Slavotinek AM, Chao R, Vacik T, et al. (2012) VAX1 mutation associated with microphthalmia, corpus callosum agenesis, and orofacial clefting: the first description of a VAX1 phenotype in humans. *Hum Mutat* 33: 364–368.

102. Butali A, Suzuki S, Cooper ME, et al. (2013) Replication of Genome Wide Association Identified Candidate Genes Confirm the Role of Common and Rare Variants in PAX7 and VAX1 in the Etiology of Non-syndromic CL/P. *Am J Med Genet A* 161: 965–972.

103. Zhang BH, Shi JY, Lin YS, et al. (2018) VAX1 Gene Associated Non-Syndromic Cleft Lip With or Without Palate in Western Han Chinese. *Arch Oral Biol* 95: 40–43.

104. Sabbagh HJ, Innes NPT, Ahmed ES, et al. (2019) Molecular Screening of VAX1 Gene Polymorphisms Uncovered the Genetic Heterogeneity of Nonsyndromic Orofacial Cleft Among Saudi Arabian Patients. *Genet Test Mol Biomarkers* 23: 45–50.

105. De Felice M, Ovitt C, Biffali E, et al. (1998) A mouse model for hereditary thyroid dysgenesis and cleft palate. *Nature Genet* 19: 395–398.
106. Bamforth JS, Hughes IA, Lazarus JH, et al. (1989) Congenital hypothyroidism, spiky hair, and cleft palate. *J Med Genet* 26: 49–60.
107. Castanet M, Park SM, Smith A, et al. (2002) A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis and cleft palate. *Hum Mol Genet* 11: 2051–2059.
108. Nikopensius T, Kempa I, Ambrozaitytė L, et al. (2011) Variation in FGF1, FOXE1, and TIMP2 genes is associated with nonsyndromic cleft lip with or without cleft palate. *Birth Defects Re Part A* 91: 218–225.
109. Leslie EJ, Carlson JC, Shaffer JR, et al. (2017) Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. *Hum Genet* 136: 275–286.
110. Chiquet BT, Lidral AC, Stal S, et al. (2007) CRISPLD2: a novel NSCLP candidate gene. *Hum Mol Genet* 16: 2241–2248.
111. Shi J, Jiao X, Song T, et al. (2010) CRISPLD2 polymorphisms are associated with nonsyndromic cleft lip with or without cleft palate in a northern Chinese population. *Eur J Oral Sci* 118: 430–433.
112. Letra A, Menezes R, Margaret E, et al. (2011) CRISPLD2 Variants Including a C471T Silent Mutation May Contribute to Nonsyndromic Cleft Lip with or without Cleft Palate. *Cleft Palate Craniofac J* 48: 363–370.
113. Mijiti A, Ling W, Maimaiti A, et al. (2015) Preliminary evidence of an interaction between the CRISPLD2 gene and nonsyndromic cleft lip with or without cleft palate (nsCL/P) in Xinjiang Uyghur population, China. *Int J Pediatr Otorhinolaryngol* 79: 94–100.
114. Chiquet BT, Yuan Q, Swindell EC, et al. (2018) Knockdown of Crispld2 in zebrafish identifies a novel network for nonsyndromic cleft lip with or without cleft palate candidate genes. *Eur J Hum Genet* 26: 1441–1450.
115. Greene ND, Dunlevy LP, Copp AJ (2003) Homocysteine Is Embryo toxic but Does Not Cause Neural Tube Defects in Mouse Embryos. *Anat Embryro* 206: 185–191.
116. Gaspar DA, Matioli SR, Pavanello RC, et al. (2004) Maternal MTHFR Interacts With the Offspring's BCL3 Genotypes, but Not With TGFA, in Increasing Risk to Nonsyndromic Cleft Lip With or Without Cleft Palate. *Eur J Hum Genet* 12: 521–526.
117. Rai V (2018) Strong Association of C677T Polymorphism of Methylenetetrahydrofolate Reductase Gene With Nonsyndromic Cleft Lip/Palate (nsCL/P). *Indian J Clin Biochem* 33: 5–15.
118. Bhaskar L, Murthy J, Babu GV (2011) Polymorphisms in Genes Involved in Folate Metabolism and Orofacial Clefts. *Arch Oral Biol* 56: 723–737.
119. Machado AR, De Toledo IP, Martelli-Junior H, et al. (2018) Potential genetic markers for nonsyndromic oral clefts in the Brazilian population: a systematic review and meta-analysis. *Birth Defects Res* 110: 827–839.
120. Lin L, Bu H, Yang Y, et al. (2017) A Targeted, Next-Generation Genetic Sequencing Study on Tetralogy of Fallot, Combined With Cleft Lip and Palate. *J Craniofac Surg* 28: e351–e355.
121. Lan Y, Ryan RC, Zhang Z, et al. (2006) Expression of Wnt9b and activation of canonical Wnt signaling during midfacial morphogenesis in mice. *Dev Dyn* 235: 1448–1454.
122. Carroll TJ, Park JS, Hayashi S, et al. (2005) Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell* 9: 283–292.
123. Karner CM, Chirumamilla R, Aoki S, et al. (2009) Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nature Genet* 41: 793–799.

124. Juriloff DM, Harris MJ, McMahon AP, et al. (2006) Wnt9b is the mutated gene involved in multifactorial nonsyndromic cleft lip with or without cleft palate in A/WySn mice, as confirmed by a genetic complementation test. *Birth Defects Res. A Clin Mol Teratol* 76: 574–579.

125. Chiquet BT, Blanton SH, Burt A, et al. (2008) Variation in WNT genes is associated with nonsyndromic cleft lip with or without cleft palate. *Hum Mol Genet* 17: 2212–2218.

126. Menezes R, Letra A, Kim AH, et al. (2010) Studies with Wnt genes and nonsyndromic cleft lip and palate. *Birth Defects Res A Clin Mol Teratol* 88: 995–1000.

127. Fontoura C, Silva RM, Granjeiro JM, et al. (2015) Association of WNT9B Gene Polymorphisms With Nonsyndromic Cleft Lip With or Without Cleft Palate in Brazilian Nuclear Families. *Cleft Palate Craniofac J* 52: 44–48.

128. Vijayan V, Ummel R, Weber R, et al. (2018) Association of WNT Pathway Genes With Nonsyndromic Cleft Lip With or Without Cleft Palate. *Cleft Palate Craniofac J* 55: 335–341.

129. Marini NJ, Asrani K, Yang W, et al. (2019) Accumulation of rare coding variants in genes implicated in risk of human cleft lip with or without cleft palate. *Am J Med Genet* 179: 1260–1269.

130. Gajera M, Desai N, Suzuki A, et al. (2019) MicroRNA-655-3p and microRNA-497-5p inhibit cell proliferation in cultured human lip cells through the regulation of genes related to human cleft lip. *BMC Med Genomics* 12: 70.

131. Kim SJ, Lee S, Park HJ, et al. (2016) Genetic association of MYH genes with hereditary hearing loss in Korea. *Gene* 591: 177–182.

132. Pazik J, Lewandowski Z, Oldak M, et al. (2016) Association of MYH9 rs3752462 and rs5756168 polymorphisms with transplanted kidney artery stenosis. *Transplant Proc* 48: 1561–1565.

133. Marigo V, Nigro A, Pecci A, et al. (2004) Correlation between the clinical phenotype of MYH9-related disease and tissue distribution of class II nonmuscle myosin heavy chains. *Genomics* 83: 1125–1133.

134. Martinelli M, Di Stazio M, Scapoli L, et al. (2007) Cleft lip with or without cleft palate: implication of the heavy chain of non-muscle myosin II A. *J Med Genet* 44: 387–392.

135. Chiquet BT, Hashmi SS, Henry R, et al. (2009) Genomic screening identifies novel linkages and provides further evidence for a role of MYH9 in nonsyndromic cleft lip and palate. *Eur J Hum Genet* 17: 195–204.

136. Jia ZL, Li Y, Chen CH, et al. (2010) Association among polymorphisms at MYH9, environmental factors, and nonsyndromic orofacial clefts in western China. *DNA Cell Biol* 29: 25–32.

137. Peng HH, Chang NC, Chen KT, et al. (2016) Nonsynonymous variants in MYH9 and ABCA4 are the most frequent risk loci associated with nonsyndromic orofacial cleft in Taiwanese population. *BMC Med Genet* 17: 59.

138. Wang Y, Li D, Xu Y, et al. (2018) Functional Effects of SNPs in MYH9 and Risks of Nonsyndromic Orofacial Clefts. *J Dent Res* 97: 388–394.

139. Araujo TK, Secolin R, Félix TM, et al. (2016) A multicentric association study between 39 genes and nonsyndromic cleft lip and palate in a Brazilian population. *J Craniomaxillofac Surg* 44: 16–20.

140. Yu Y, Zuo X, He M, et al. (2017) Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. *Nat Commun* 8: 14364.
141. Da Silva HPV, Oliveira GHM, Ururahy MAG, et al. (2018) Application of high-resolution array platform for genome-wide copy number variation analysis in patients with nonsyndromic cleft lip and palate. *J Clin Lab Anal* 32: e22428.

142. Huang L, Jia Z, Shi Y, et al. (2019) Genetic factors define CPO and CLO subtypes of nonsyndromicorofacial cleft. *PLoS Genet* 15: e1008357.

143. Gorlin RJ, Cohen MM, Hennekam RCM (2001) Syndromes of the Head and Neck. 4th edition, New York: Oxford University Press.

144. Grosen D, Chevrier C, Skytthe A, et al. (2010) A cohort study of recurrence patterns among more than 54000 relatives of oral cleft cases in Denmark: support for the multifactorial threshold model of inheritance. *J Med Genet* 47: 162–168.

145. Lidal AC, Romitti PA, Basart AM, et al. (1998) Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. *Am J Hum Genet* 63: 557–568.

146. Suazo J, Santos JL, Scapoli L, et al. (2010) Association between TGFB3 and nonsyndromic cleft lip with or without cleft palate in a Chilean population. *Cleft Palate Craniofac J* 47: 513–517.

147. Vieira AR, Avila JR, Daack-Hirsch S, et al. (2005) Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. *PLoS Genet* 1: e64.

148. Choi SJ, Marazita ML, Hart PS, et al. (2009) The PDGF-C regulatory region SNP rs28999109 decreases promoter transcriptional activity and is associated with CL/P. *Eur J Hum Genet* 17: 774–784.

149. François-Fiquet C, Poli-Merol ML, Nguyen P, et al. (2014) Role of angiogenesis-related genes in cleft lip/palate: review of the literature. *Int J Pediatr Otorhinolaryngol* 78: 1579–1585.

150. Carlson JC, Taub MA, Feingold E, et al. (2017) Identifying Genetic Sources of Phenotypic Heterogeneity in Orofacial Clefts by Targeted Sequencing. *Birth Defects Res* 109: 1030–1038.

151. Figueiredo JC, Stephanie LY, Raimondi H, et al. (2014) Genetic risk factors for orofacial clefts in Central Africans and Southeast Asians. *Am J Med Genet A* 164A: 2572–2580.

152. Leslie EJ, Taub MA, Liu H, et al. (2015) Identification of Functional Variants for Cleft Lip with or without Cleft Palate in or near PAX7, FGFR2, and NOG by Targeted Sequencing of GWAS Loci. *Am J Hum Genet* 96: 397–411.

153. Moreno Uribe LM, Fomina T, Munger RG, et al. (2017) A Population-Based Study of Effects of Genetic Loci on Orofacial Clefts. *J Dent Res* 96: 1322–1329.

154. Liu H, Leslie EJ, Carlson JC, et al. (2017) Identification of common non-coding variants at 1p22 that are functional for non-syndromic orofacial clefting. *Nat Commun* 8: 14759.

155. Cox LL, Cox TC, Moreno Uribe LM, et al. (2018) Mutations in the Epithelial Cadherin-p120-Catenin Complex Cause Mendelian Non-Syndromic Cleft Lip with or without Cleft Palate. *Am J Hum Genet* 102: 1143–1157.

156. Du S, Yang Y, Yi P, et al. (2019) A Novel CDH1 Mutation Causing Reduced E-Cadherin Dimerization Is Associated with Nonsyndromic Cleft Lip With or Without Cleft Palate. *Genet Test Mol Biomarkers* 23: 759–765.

157. Beaty TH, Taub MA, Scott AF, et al. (2013) Confirming genes influencing risk to cleft lip with/without cleft palate in a case-parent trio study. *Hum Genet* 132: 771–781.

158. Moreno Uribe LM, Fomina T, Munger RG, et al. (2017) A Population-Based Study of Effects of Genetic Loci on Orofacial Clefts. *J Dent Res* 96: 1322–1329.
159. Ghazali N, Rahman NA, Kannan TP, et al. (2015) Screening of Transforming Growth Factor Beta 3 and Jagged2 Genes in the Malay Population With Nonsyndromic Cleft Lip With or Without Cleft Palate. *Cleft Palate Craniofac J* 52: e88–94.

160. Simioni M, Araujo TK, Monlleo IL, et al. (2014) Investigation of genetic factors underlying typical orofacial clefts: mutational screening and copy number variation. *J Hum Genet* 60: 17–25.

161. Shi M, Christensen K, Weinberg CR, et al. (2007) Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. *Am J Hum Genet* 80: 76–90.

162. Song T, Wu D, Wang Y, et al. (2013). Association of NAT1 and NAT2 genes with nonsyndromic cleft lip and palate. *Mol Med Rep* 8: 211–216.

163. Sull JW, Liang KY, Hetmanski JB, et al. (2009) Maternal transmission effects of the PAX genes among cleft case-parent trios from four populations. *Eur J Hum Genet* 17: 831–839.

164. Laumonnier F, Holbert S, Ronce N, et al. (2005) Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. *J Med Genet* 42: 780–786.

165. Loenarz C, Ge W, Coleman ML, et al. (2010) PHF8, a gene associated with cleft lip/palate and mental retardation, encodes for an Nepsilon-dimethyl lysine demethylase. *Hum Mol Genet* 19: 217–222.

166. Peanchitlertkajorn S, Cooper ME, Liu YE, et al. (2003) Chromosome 17: gene mapping studies of cleft lip with or without cleft palate in Chinese families. *Cleft Palate Craniofac J* 40: 71–79.

167. Letra A, Silva RA, Menezes R, et al. (2007) MMP gene polymorphisms as contributors for cleft lip/palate: association with MMP3 but not MMP1. *Arch Oral Biol* 52: 954–960.

168. Kumari P, Singh SK, Raman R (2019) TGFβ3, MSX1, and MMP3 as Candidates for NSCL±P in an Indian Population. *Cleft Palate Craniofac J* 56: 363–372.

169. Letra A, Silva RM, Motta LG, et al. (2012) Association of MMP3 and TIMP2 promoter polymorphisms with nonsyndromic oral clefts. *Birth Defects Res A Clin Mol Teratol* 94: 540–548.

170. Letra A, Zhao M, Silva RM, et al. (2014) Functional Significance of MMP3 and TIMP2 Polymorphisms in Cleft Lip/Palate. *J Dent Res* 93: 651–656.

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