Original Article

Molecular Genetics Of Pediatric Orofacial Clefts - An Overview

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Abstract

Orofacial clefts (OFCs) are birth anomalies that occur frequently, affecting the lip and roof of the mouth. The complex etiology includes both genetic and environmental factors that influence their occurrence. OFCs are congenital malformations that are prevalent in the general population and affect the lip, palate, or both. A complex interplay between genetic and environmental factors leads to their development. This review intends to discuss the various molecular pathways involved in OFCs. Advances in genomic technologies have made significant strides in understanding the molecular basis of OFCs. Syndromic OFCs are associated with specific genetic mutations, such as TP63, IRF6, MSX1, and TBX22. Non-syndromic OFCs involve multiple genes and pathways, including PVRL1, FOXE1, MTHFR, and FGFR1. The WNT, TGF-beta, and FGF signaling pathways have also been implicated in OFC pathogenesis. Children with these defects are posed with significant speech, feeding, and hearing challenges, as well as an array of psychosocial problems which warrant multidisciplinary management. Unraveling the etiopathogenesis of the disease has paved the way for personalized medicine and opportunities to improve treatment modalities. An interdisciplinary approach between clinicians, geneticists, and researchers is pivotal in understanding the various aspects of OFCs and in developing effective prevention and treatment protocols.

Keywords: Orofacial clefts, Molecular genetics, Genetic mutations, Signaling pathways

INTRODUCTION

OFCs are the most prevailing birth anomalies occurring in the craniofacial region. It can occur isolated or together, or in different combinations with other congenital deformities. A cleft lip develops when the frontonasal and maxillary processes fail to fuse properly, leading to a cleft that may extend through the lip, alveolus, or nasal floor. A cleft palate occurs as a result of non-fusion of the palatal shelves of the maxillary processes, leading to the development of a cleft of the hard and/or soft palates. These clefts can be either unilateral (on one side) or bilateral (on both sides) and can range from a small notch in the lip to a complete cleft extending into the nose and/or palate. When OFCs appear with other congenital malformations with a discernible pattern, it is classified as syndromic, and if the cleft is seen in isolation without any syndromes, it is non-syndromic. The lifetime cost of treatment is estimated to be $200,000.[1,2] OFCs are relatively common birth defects, with an estimated prevalence

of 1 in 500 to 1 in 700 live births worldwide.[3] Asians and American Indians have the highest rates (1:500), Caucasians have average rates (1:1000), and Africans have the lowest (1:2500).[4]

With the advent of the molecular genetics' era, several studies have highlighted the genetic contribution to OFCs. Blanton et al. (2005)[5] conducted a genome-wide scan for OFC susceptibility loci in affected individuals from 330 multiplex families and identified several significant regions of interest on chromosomes 1, 2, 8, and 17. Rahimov et al.[3] conducted a genome-wide association study (GWAS) of non-syndromic cleft lip with or without cleft palate (NSCL/P) in individuals of European and Asian ancestry. They identified several common variants associated with NSCL/P. Specific genetic mutations have also been identified in syndromic OFCs, such as TP63, IRF6, MSX1, and TBX22. [6-8] Non-syndromic OFCs have also been found to involve multiple genes and pathways, including PVRL1, FOXE1, MTHFR, and FGFR1.[9-12] Signaling pathways such as the WNT, TGF-beta, and FGF pathways have also been implicated in OFC pathogenesis.[13; 14]

A closer look into the genetic causation of OFCs is essential for improving the current treatment modalities and can also help in targeted inventions, including gene therapies. This review paper discusses the genetic mechanisms that underlie both syndromic and non-syndromic forms of OFCs and highlights several key critical advances made in recent years in this area of research.

ETIOLOGY OF OFCs

OFCs are linked with a multifactorial etiology involving genetic as well as environmental factors.

A. Genetic factors: Can be divided into four distinct categories consisting :

1. Inherited genetic mutations

Inherited genetic mutations have been identified as an important factor in the development of OFCs. For example, mutations in the IRF6 gene have been linked with both syndromic and non-syndromic forms of OFCs.[15,16] Evidence points to mutations in the MSX1 gene also being strongly associated with OFCs.[17]

2. Chromosomal abnormalities

Chromosomal abnormalities can also contribute to the development of OFCs. Individuals with trisomy 13 or 18 have an increased risk of OFCs.[18]

3. Epigenetic factors

Epigenetic factors, such as DNA methylation, have also been implicated in the development of OFCs. Alterations in DNA methylation, both hypo-methylation and hypermethylation, can potentially increase the risk of OFCs.[19]

4. Gene-environment interactions

Gene-environment interactions can play a crucial role in the occurrence of OFCs. Maternal smoking during pregnancy has been shown to increase the threat of OFCs in individuals with specific genetic variations.[10]

B. Environmental factors :

Environmental factors significantly contribute to the development of OFCs.

Low maternal intake of folic acid[20], Maternal exposure to environmental toxins such as pesticides[21], use of maternal anticonvulsant medications[22], Smoking, and alcohol consumption during pregnancy [23] were all found to be different environmental factors causing an increased risk of clefts.

GENES IDENTIFIED IN SYNDROMIC OFCs CASES

Syndromic OFCs occur in association with other congenital anomalies or developmental disorders and have a distinct, discernible pattern. Syndromic OFCs account for approximately 30% of all cases of OFCs. Evidence has implicated several genes in the development of syndromic OFCs, which are listed below**.**

TP63:

The TP63 gene encodes for a transcription factor essential for the development of skin, limbs, and other organs. Mutations in the TP63 gene have been connected with several syndromic forms of OFCs. TP63 is known to cause six syndromes with the overlapping expression: ectrodactyly-ectodermal dysplasia clefting syndrome[24], Hay-Wells syndrome[25], Rapp-Hodgkin syndrome[26], split-hand/foot malformation[27], limb-mammary syndrome[28], and ADULT syndrome.[29]

IRF6:

The Interferon regulatory factor 6 (IRF6) gene codes for a transcription factor that takes a key role in the emergence of the lips and palate. Mutations of the IRF6 gene cause Van der Woude syndrome (VDWS), a Mendelian-inherited disorder that accounts for about 2% of all OFCs.[30] This syndrome is defined by the presence of a cleft lip with or without a cleft palate, an isolated cleft palate, or mucous cysts on the lower lip, and hypodontia. All the features of VDWS are also observed in Popliteal pterygium syndrome (PPS) along with additional clinical manifestations such as popliteal pterygium, syngnathia, characteristic toe/nail abnormality, syndactyly, and genitourinary defects. The clinical similarities exhibited by the two syndromes pointed towards VDWS and PPS being caused by the same gene being mutated in different ways. Linkage and chromosomal analysis aided in the localization of VDWS, and it was found to be 1q32-q41. The phenotypic heterogeneity displayed by VDWS and PPS were two discrete types of IRF6 mutation.

MSX1:

The MSX1 gene encodes for a homeobox protein that plays a part in the development of craniofacial structures. There have been a number of resequencing evidence of candidate genes to narrow down specific alternates that may reveal relationships with clefting, and the present credible evidence implicates MSX1.[32] Mutations in the MSX1 gene have been linked with various forms of syndromic OFCs, including Witkop syndrome and tooth agenesis with OFCs.[33]

TBX22:

A transcription factor that contributes to the development of the tongue and palate is encoded by the TBX22 gene. Loss-of-function transmutations in the TBX22 gene have been related to X-linked cleft palate and ankyloglossia (CPX) syndrome.[34] With the aid of in vitro functional assays, Andreou et al.[35] revealed that TBX22 represses transcription and that missense mutations impair its affinity to bind with DNA and, subsequently, damage its ability to repress.[35]

GENES IDENTIFIED IN NON-SYNDROMIC OFCs CASES

OFCs of the non-syndromic variety are clefts that occur in isolation and without conjunction with other identifiable developmental disorders or birth defects. These clefts are caused by an interplay between genetic and environmental factors in various degrees and account for approximately 70% of all cases of OFCs. A number of genes have been implicated in the development of non-syndromic OFCs. This has been proven by several studies and concluded from irrefutable evidence in both the animal model as well as in vitro types of studies. The following genes have been associated with various non-syndromic OFCs cases.

PVRL1:

Nectin-1 is encoded by the Poliovirus receptor-related 1 (PVRL1) gene. It functions as a cell adhesion molecule that initiates a role in the development of the lip and palate. The PVRL1 gene mutation has been strongly linked

with the occurrence of non-syndromic cleft lip, both with cleft palate (CL/P) and without.[16] Mutations in sporadic cases have drawn a noteworthy correlation of a coding variant of PVRL1 known as G361V with non-syndromic orofacial clefting.[36] Furthermore, several groups from South America affected with non-syndromic clefts show mutations in PVRL1 homolog genes - PVR and PVRL2.[37] Individuals hailing from the northern part of Venezuela who was heterozygous carriers of a nonsense type of mutation in PVRL1 (Trp185Stop) were shown to be at significant risk for Non-syndromic OFCs.[38] Homozygosity for the same mutation culminates in the development of ectodermal dysplasia, an autosomal recessive syndrome with clefting among the residents of Margarita Island (Nothern Venezuela), a place notorious for its disproportionately high incidence of clefting (5.4/1000) within the native population.[39]

FOXE1:

FOXE1 is a member of the forkhead/winged helix-domain group of transcription factors that are mainly involved in embryonic development.[3] The FOXE1 gene encodes for one of the transcription factors responsible for the development of both the thyroid gland and the palate. In mice, a targeted disruption of FOXE1 resulted in the development of both cleft palate and thyroid malformation.[40] Mutations within the FOXE1 gene are resolutely linked with non-syndromic cleft palate within several populations despite common coding variants not being narrowed down.[41,42] Similar missense mutations of FOXE1 in three unrelated cases of patients with a non-syndromic type of cleft palate were discovered.[43]

MTHFR:

The methylenetetrahydrofolate reductase (MTHFR) gene encodes for an enzyme that plays a role in folate metabolism, which is important for proper embryonic development. Mutated variants of the MTHFR gene have drawn strong correlations with the occurrence of non-syndromic cleft lip with or without a cleft palate.[44] The maternal MTHFR genotype was shown to contribute to the risk of non-syndromic cleft lip and palate; specifically, the MTHFR C677T genotype in the mother increased the risk by 4.6 times.[45] When compounded by the deficiency of folic acid in the pre-conceptional period, the thermally labile variant of MTHFR conferred a tenfold heightened risk for cleft development.[46]

FGFR:

The Fibroblast Growth Factor Regulator 1 (FGFR1) gene encodes for a receptor tyrosine kinase that contributes to the development of the face and palate. Certain variants of the FGFR1 gene show definitively a connection with the development of non-syndromic cleft lip and palate.[47] A type of missense mutation within the operational frame of FGFR2[12] and microdeletions in FGFR2's genetic locus [48] was detected in patients with the non-syndromic variant of cleft lip. A successful linkage was also established between non-syndromic clefting deformity and a frequently seen genetic variant of FGFR2.[12]

MOLECULAR PATHWAYS IMPLICATED IN OFCs

The face and palate develop from a complex interplay involving multiple molecular pathways. These pathways include WNT, TGF-beta, BMP, and SHH, among others. Dysregulation of these pathways can lead to defective facial and palatal fusion, resulting in OFCs. The pathways identified with a backing of irrefutable evidence in multiple studies include the following:

WNT signaling pathway:

The WNT signaling pathway plays a crucial role in facial and palatal development. Disruption of this pathway can result in cleft lip and/or palate (CL/P). Studies conducted in animal models have revealed that a targeted mutation of WNT 9b leads to CLP. The strain of the animal model, which had a high susceptibility for spontaneous CLP, had the insertion of a retrotransposon of approximately 6.6 kb downstream (a site known as the clf1 locus) in the Wnt9b gene.[49] These findings suggest that WNT 9b plays a key role in the development of the lip.

Mutations in several genes involved in the WNT pathway are implicated in the pathogenesis of CL/P, including APC, AXIN2, and WNT3A.[21]

TGF-beta signaling pathway:

The TGF-beta signaling pathway is involved in the regulation of cell growth and differentiation during embryonic development. Dysregulation of this pathway has been implicated in the pathogenesis of OFCs. The receptors that bind the growth factors belonging to the TGF superfamily play an undeniable role in craniofacial development. Transforming growth factor α (TGFA) has long been linked with clefting and was one of the first genes to be highlighted in conjunction with non-syndromic clefting.[50-52] It plays a pivotal role during the fusion of palatal shelves and also encourages the synthesis of matrix proteins present in the extracellular compartment.[53]

Furthermore, TGFA-associated alleles are among the scarce elements proven to have strong synergy with several environmental factors, including but not limited to maternal smoking and vitamin use.[54-57] Statistically substantial linkages between TGFA and CL/P have been substantiated among individuals across populations through case-control study designs (reviewed by Wyszynski et al. 1996)[58] Mutations involving genes that encode components of the TGF-beta pathway, such as TGFBR1 and SMAD2, have also shown clear correlations with cleft lip and/or palate.[59]

BMP signaling pathway:

The Bone morphogenetic proteins (BMP) modulate critical processes of embryological development, like the proliferation and differentiation of cells, as well as programmed cell death.[60] When the type 1 Bmp receptor gene (Bmpr1a) is inactivated, the catastrophic results entail bilateral CL/P with tooth agenesis, whereas if the ligand Bmp4 is knocked out, it amounts to an isolated cleft lip only.[61] So far, only two studies have been published regarding genetic variants in BMP4 and the subsequent development of NS CL/P. The first study checked the SNPs' impact on the BMP pathway in 150 cases of NS CL/P with no proven relation.[62] An association was found with the haplotypes of the SNP. The other study discussed a relationship of non-synonymous SNP (rs17563) in BMP4 in a group of 184 patients with NS CL/P to 205 control cases from China.[63]

SHH Signaling pathway

The signaling pathway by the name of sonic hedgehog (SHH) is responsible in many ways for embryological evolution and craniofacial morphology, illuminated by a varied range of deformities in the craniofacial region resulting from disruption in the normal functioning of this pathway. The patched receptor gene (PTCH) is one of the downstream effectors of this pathway. A mutation screen was conducted among 220 multiplex families with NS CL/P that involved the PTCH coding sequence, which invariably exposed missense mutations within the SHH-binding domain.[64] Inactivating mutations in GLI2, a transcription factor that conveys intracellular SHH messaging, culminated in features resembling holoprosencephaly in conjunction with cleft lip/palate.[65]

The fact that SNPs found in the GLI2 region are associated with NS CL/P has been reported in multiple populations.[66] In addition, missense mutations involving GLI2 were discovered in patients with NS CL/P.[43] These findings corroborate the fact that the SHH signaling pathway has a role in the etiopathogenesis of NS CL/P.

FUTURE DIRECTIONS IN THE STUDY OF MOLECULAR GENETICS OF OFCs

Recent advances in genomic technologies, including high-throughput sequencing and genome-wide association studies (GWAS), have revolutionized the understanding of the genetic basis of OFCs. These technologies have enabled the identification of new genetic variants linked to cleft lip and/or palate (CL/P), including rare variants and copy number variations (CNVs).[67] Development in molecular genetics also paves the way for personalized medicine in the field of OFCs. Identification of specific genetic variants associated with CL/P can facilitate early diagnosis and targeted therapies early in the course of the disease. For example, individuals with mutations in the IRF6 gene may benefit from early intervention to prevent or reduce the severity of CL/P.[68]

In vitro and animal model studies have proved to be the bedrock in discovering genes and pathways commonly implicated in OFCs. The high frequency of this disorder in the population, along with the intricate interplay of genetic and environmental factors in the pathogenesis of this disorder, has made it one of the most commonly investigated diseases. Most of these studies, however, have assessed the relationship between defects among typically involved genes and pathways in individuals and the subsequent occurrence of OFCs. There is room for analysis and investigation of less common genetic aberrations that culminate in the development of these anomalies, such as low-frequency missense mutations.

Establishing a relationship between less-common genetic variants and clefting would require the sequencing of a considerable number of affected individuals. Efforts directed toward case-control gene resequencing have yielded astonishing results with NS CL/P cases where rare missense variants were noted with statistical significance in affected individuals once an extensive vast number of them were sequenced.[12,69] Defining the role of regulatory RNAs does highlight another untapped avenue for researchers to investigate, the fact that microRNA is associated with the pathogenesis of orofacial cleft, brought to light by various studies in recent times.[70]

CONCLUSION

OFCs are birth defects that occur quite frequently in the general population with a significant accompanying medical, social, and psychological burden. They result from an intricate interplay between genetic and environmental agents. Recent breakthroughs in genomic technologies have expanded our understanding of the molecular pathways implicated in the etiopathogenesis of the defect. Specific genes and pathways, including TP63, IRF6, WNT, and TGF-beta, have been identified as key players in the etiology of OFCs.

Individuals suffering from OFCs are born with a number of impediments, including speech and hearing, along with psychosocial stigmata associated with the disease., Therefore collaboration across multiple disciplines, including genetics, developmental biology, epidemiology, and clinical medicine, is warranted. This interdisciplinary approach is critical to understanding the complex etiology of CL/P and developing effective prevention and treatment strategies. Genetic testing can inform treatment decisions and help identify individuals at increased risk for clefts who may benefit from early intervention. In addition, continued research into the genetic and environmental basis of cleft formation is pivotal in the development of effective prevention strategies and for a marked improvement in clinical outcomes for individuals with clefts, ultimately leading to a heightened quality of life for them.

DECLARATION

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