MOLECULAR GENETICS OF CLEFT LIP AND PALATE: A REVIEW

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INTRODUCTION

Cleft lip with or without cleft palate (CLP) is a common congenital disability. They exist either in combination with one or more other anomalies (syndromic cleft) or in isolation (non-syndromic cleft). Non-syndromic CL/P is more common as it is present in about 70% of cases, out of which 80% are sporadic, and 20% are familial.[1] CLP which is commoner in males, occurs in 1 out of 300 to 2500 births, while isolated cleft palate (CP) which occurs more frequently in females, occurs in 1 out of 1500 births[2,3]. People with cleft lip and palate often require multidisciplinary care involving several surgical repairs commencing in the first year of life, orthodontic interventions for malocclusion, speech therapy, treatment of recurrent middle ear infections, and psychological interventions. These have been noted to contribute a significant burden to the patient, family, and society at large. Thus, an intense effort has been made to unravel its aetiology, which would be important in genetic counselling, risk prediction, and overall prevention of cleft lip and palate[4].

Aetiology of Cleft Lip and Palate

Generally, cleft lip and palate is thought to result from interactions between genetic and environmental factors. Substantial pieces of evidence for the former have arisen from family, and twin studies which revealed high rates of familial aggregation and increased concordance rates in monozygous twins, compared with dizygous twins[5]. For instance, studies by Sivertsen *et al.*[6] and Grosen *et al.*[7] showed that cleft palate has a relative risk of occurrence which is 15 to 56 times higher among first degree relatives. Although environmental factors such as maternal use of alcohol, cigarette and antiepileptic drugs have been identified as risk factors for CLP, recent studies have now revealed important genes either acting alone or within gene networks. Such cases are found as parts of Mendelian monogenic syndromes, chromosomal abnormalities, or otherwise unknown genetic syndromes[8]. These identified genetic risk factors have shed more light on normal craniofacial development with some also implicated in non-syndromic CL/P. As an example of gene-environment interaction, Shaw *et al.*[9] demonstrated a 3 to 8 fold increase in CLP in babies with lack of multivitamins in the first trimester of pregnancy and the TaqI C2 mutation in the *Tgfa* gene. The same mutation was shown to raise the risk of CLP by 6 to 8 times when co-existent with maternal smoking[10], while Jugessur *et al.*[11] found that combined mutations of the *Tgfa* and *Msx1* genes cause an almost ten-fold increase in cleft lip and palate risk as an evidence of gene-gene interaction.

Genetic Regulation of Craniofacial Development

Craniofacial development is a complex event involving several transcription factors and molecular signals. Disruptions in the network of these proteins lead to the development of facial clefts. The diversity in the functions of these genes and their products shows the susceptibility of the craniofacial developmental pathways to form clefts[4].

Facial development in humans begins in the fourth week of intrauterine life with the migration of cranial neural crest cells (CNC) from the rostral part of the neural tube to form the facial primordia and secondary palate[8]. Genes such as *Tgf*b2, *Hoxa2*, *Gli2*, and *Gli3* have been identified to play a role in CNC migration, mutations of which have been shown to contribute to cleft lip and palate in mice[12-14]. Palatal shelves are subsequently derived from the secondary palate and undergo elevation to become horizontally apposed in the midline. Failure of apposition has been linked with mutations in the genes *Msx1*, *Pax9*and *Lhx8* leading to CP[15-17]. Furthermore, epithelial-mesenchymal interactions mediated by interrelated gene networks – sonic hedgehog (*Shh*), bone morphogenetic proteins

Table1: Summary of molecular genetic mechanisms in syndromic cleft lip and palate

Syndrome	Inheritance	Gene	Locus	Function	Gene also implicated in non-syndromic CL/P	References
Cleft lip/palate ectodermal dysplasia syndrome (CLPED)	AR	*Pvrl1*	11q23.3	Encodes nectin-1 which plays a role in cell adhesion	Yes	28-30
Acrofrontofacionasal dysostosis syndrome	AR	*Nbas*	2p24	Skeletal morphogenesis, mediating Golgi-to-endoplasmic reticulum retrograde traffic.	-	31
Popliteal pterygium syndrome (PPS)	AD	*Irf6*	1q32	Mediates TGFβ3 activity in palatal fusion	Yes	25,27
Van der Woude (VDW) syndrome	AD	*Irf6*	1q32	Mediates TGFβ3 activity in palatal fusion	Yes	25,27
Rapp-Hodgkin syndrome (RHS)	AD	*Tp63*	3q28	Apoptosis. Also in establishment of enhancers needed for expression of genes important in craniofacial development such as *Irf6*	Yes	32-24
Roberts syndrome	AR	*Esco2*	8p21	Acetyltransferase activity necessary for sister chromatid cohesion needed for cell proliferation	-	35,36
Hay-Wells syndrome	AD	*Tp63*	3q28	As for RHS	Yes	37
Blepharocheilodontic syndrome	AD	*Cdh1*	16q22	Cell adhesion molecule involved in the maintenance of epithelial cell morphology during embryonic development	-	38-39
Thurston syndrome	AR	*Ddx59*	1q32	Ciliary SHH signaling	-	40
Uvealcoloboma-cleft lip and palate-intellectual disability syndrome	AD	*Yap1*	11q22	Activation of transcription factors important for apoptosis such as p73	-	41,42
Varadi-Papp syndrome	AR	*Cplane1*	5p13	Ciliary SHH signaling	-	43,44
Cleft palate, cardiac defects and mental retardation (CPCMR)	AD	*Meis2*	15q14	Palatal fusion. Repression of SHH/FGF feedback loop.	-	45,46
Vici syndrome	AR	*Epg5*	18q12	Autophagy during embryogenesis	-	47
Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3 (EEC3)	AD	*Tp63*	3q28	As for RHS	Yes	48
Branchiooculofacial syndrome (BOFS)	AD	*Tfap2a*	6p24	Transcription activation necessary for formation of neural crest cells during embryogenesis	-	49-51
Cleft palate with ankyloglossia, X-linked (CPX)	X-linked	*Tbx22*	Xq21	Repressor of transcription, with an important role in horizontal elevation of palatal shelves	-	52,53
Holoprosencephaly 2	AD	*Six3*	2p21	Regulation of SHH expression	-	54,55
Opitz-Frias syndrome or (Opitz GBBB syndrome type II)	AD	*Specc1l*	22q11.23	Regulates microtubule and actin organization for proper cell adhesion and migration	-	56,57
Simpson-Golabi-Behmel syndrome type 1	XLR	*Gpc3*	Xq26.2	Regulation of SHH, FGF, and BMP activities	-	58,59
Oral-facial-digital syndrome 1	XLD	*Ofd1*	Xp22.2	Regulation of microtubule function	-	60,61
Gorlin-Goltz syndrome	AD	*Ptch1, Ptch 2, Sufu*	9q22, 1p32, 10q24	Regulation of SHH signaling	-	62-64
Waardenburg syndrome, type 1	AD	*Pax3*	2q36	Transcription factor necessary for skeletal muscle formation	-	65,66
CHARGE syndrome	AD	*Chd7*	8q12	Transcription factor necessary for neural crest cell migration		67,68
DiGeorge syndrome	AD	*Tbx1*	22q11.21	Regulator of BMP signaling	-	69

(*Bmp*), and fibroblast growth factors (*Fgf*) – are essential in normal palatal development[18]. For example, expression of *Shh* in the palatal epithelium is regulated by *Bmp4* in the mesenchyme. *Shh* then regulates *Bmp2* in the mesenchyme, which is essential for mesenchymal proliferation[19,20]. A positive feedback loop also exists

Table 2: Summary of molecular genetic mechanisms in non-syndromic cleft lip and palate

Non-syndromic CLP	Inheritance	Gene	Locus	Function	References
Orofacial cleft 1	AD	*Ofc1*	6p24	-	71
Orofacial cleft 5	-	*Msx1*	4p16	Homeobox gene controlling expression of downstream genes, involved in patterning of the face and palatal midline apposition.	72-73
Orofacial cleft 6	AD	*Irf6*	1q32	As in VDW and PPS	74
Orofacial cleft 8	-	*Tp63*	3q28	Apoptosis. Also in establishment of enhancers needed for expression of genes important in craniofacial development such as *Irf6*	32-34
Orofacial cleft 11	-	*Bmp4*	14q22	Encodes BMP4 which up-regulates MSX1 and SHH for palatal fusion.	19,75,76

between the fibroblast growth factor *Fgf10* and *Shh* expression in the palatal mesenchyme and epithelium, respectively[19,21]. Also, the homeobox gene *Msx1* further modulates the expression of the genes *Bmp4, Shh,* and *Bmp2* above. At week 12, development of the palate is completed in humans.

Genetic Analysis of Cleft Lip and Palate

Almost 500 syndromes have been identified in syndromic cleft lip and palate[22], although not all have been linked to specific genes. Cohen[23] published a review of 154 of these syndromes with their clinical features to aid diagnosis. However, recent molecular genetic analysis has identified the loci of these mutations and functions of the implicated genes. For example, popliteal pterygium and Van der Woude syndromes, the latter being the most common cause of syndromic cleft lip and palate[24], are both autosomal dominant conditions secondary to mutations in the interferon regulatory factor-6 (*Irf6*) gene on chromosome 1q32[25]. Interestingly, mutations in *Irf6* have also been found in non-syndromic cleft lip and palate[26]. The protein product IRF6 is now known to be a transcription factor up-regulated by TGFb3 protein in palatal fusion during embryonic development in humans[27]. In syndromic cleft lip and palate, a given gene may be affected by several different mutations, which accounts for the varied phenotypes that may be observed[4]. For instance, mutations of the C-terminus of the protein TP63 results in cleft lip or cleft palate, whereas mutations of the conserved DNA binding region at the N-terminus results in cleft lip and palate[8].

We conducted a search on the Online Mendelian Inheritance in Man (OMIM) database with keywords 'cleft lip' and 'cleft palate' which produced over 1500 results. Table 1 summarizes genes implicated in some syndromic cleft lip and palate.

Detection of genes in non-syndromic cleft lip and palate (summarized in Table 2) has been done in recent decades by various methods including linkage analysis, candidate gene approach, and genome-wide association studies (GWAS), with the discovery of shared genetic lesions between syndromic and non-syndromic cleft lip and palate[70].

CONCLUSION

There has been some success in elucidating the genetic basis of cleft lip and palate with the identification of numerous susceptibility genes. However, this number is bound to increase, revealing the overall genetic complexity of craniofacial clefts. Given the role of environmental factors, studies that further explore feto-maternal genetics together with exposure to different environmental factors could aid in the development of a weighted genetic risk assessment for cleft lip and palate which in turn would better inform genetic counselling and prescription of preventive measures.

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