**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.²³ 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.¹

**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 monzygous twins, compared with dizygous twins. 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 smoking¹⁰, 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 Tgfβ2, 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 Mox1, Pax9 and Lhx8 leading to CP.¹⁵−¹⁷ Furthermore, epithelial-mesenchymal interactions mediated by interrelated gene networks – sonic hedgehog (Shh), bone morphogenetic proteins
### Table 1: Summary of molecular genetic mechanisms in syndromic cleft lip and palate

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Locus</th>
<th>Function</th>
<th>Gene also implicated in non-syndromic CL/P</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleft lip/palate ectodermal dysplasia syndrome (CLPED)</td>
<td>AR</td>
<td>Peri1</td>
<td>11q23.3</td>
<td>Encodes nectin-1 which plays a role in cell adhesion</td>
<td>Yes</td>
<td>28-30</td>
</tr>
<tr>
<td>Acrofrontofacial dysostosis syndrome</td>
<td>AR</td>
<td>Nbas</td>
<td>2p24</td>
<td>Skeletal morphogenesis, mediating Golgi-to-endoplasmic reticulum retrograde traffic.</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>Popliteal pterygium syndrome (PPS)</td>
<td>AD</td>
<td>Irf6</td>
<td>1q32</td>
<td>Mediates TGFβ3 activity in palatal fusion</td>
<td>Yes</td>
<td>25,27</td>
</tr>
<tr>
<td>Van der Woude (VDW) syndrome</td>
<td>AD</td>
<td>Irf6</td>
<td>1q32</td>
<td>Mediates TGFβ3 activity in palatal fusion</td>
<td>Yes</td>
<td>25,27</td>
</tr>
<tr>
<td>Rapp-Hodgkin syndrome (RHS)</td>
<td>AD</td>
<td>Tp63</td>
<td>3q28</td>
<td>Apoptosis. Also in establishment of enhancers needed for expression of genes important in craniofacial development such as Irf6</td>
<td>Yes</td>
<td>32-24</td>
</tr>
<tr>
<td>Roberts syndrome</td>
<td>AR</td>
<td>Eso2</td>
<td>8p21</td>
<td>Acetyltransferase activity necessary for sister chromatid cohesion needed for cell proliferation</td>
<td>-</td>
<td>35,36</td>
</tr>
<tr>
<td>Hay-Wells syndrome</td>
<td>AD</td>
<td>Tp63</td>
<td>3q28</td>
<td>As for RHS</td>
<td>Yes</td>
<td>37</td>
</tr>
<tr>
<td>Blepharochelodontic syndrome</td>
<td>AD</td>
<td>Cdha1</td>
<td>16q22</td>
<td>Cell adhesion molecule involved in the maintenance of epithelial cell morphology during embryonic development</td>
<td>-</td>
<td>38-39</td>
</tr>
<tr>
<td>Thurston syndrome</td>
<td>AR</td>
<td>Ddx59</td>
<td>1q32</td>
<td>Ciliary SHH signaling</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Uvealcoloboma-cleft lip and palate-intellectual disability syndrome</td>
<td>AD</td>
<td>Yap1</td>
<td>11q22</td>
<td>Activation of transcription factors important for apoptosis such as p73</td>
<td>-</td>
<td>41,42</td>
</tr>
<tr>
<td>Varadi-Papp syndrome</td>
<td>AR</td>
<td>Cplane1</td>
<td>5p13</td>
<td>Ciliary SHH signaling</td>
<td>-</td>
<td>43,44</td>
</tr>
<tr>
<td>Cleft palate, cardiac defects and mental retardation (CPCMR)</td>
<td>AD</td>
<td>Meis2</td>
<td>15q14</td>
<td>Palatal fusion. Repression of SHH/FGF feedback loop.</td>
<td>-</td>
<td>45,46</td>
</tr>
<tr>
<td>Vici syndrome</td>
<td>AR</td>
<td>Fipg5</td>
<td>18q12</td>
<td>Autophagy during embryogenesis</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3 (EEC3)</td>
<td>AD</td>
<td>Tp63</td>
<td>3q28</td>
<td>As for RHS</td>
<td>Yes</td>
<td>48</td>
</tr>
<tr>
<td>Branchio-ocular-facial syndrome (BOPS)</td>
<td>AD</td>
<td>Tjap2a</td>
<td>6p24</td>
<td>Transcription activation necessary for formation of neural crest cells during embryogenesis</td>
<td>-</td>
<td>49-51</td>
</tr>
<tr>
<td>Cleft palate with ankyloglossia, X-linked (GX)</td>
<td>X-linked</td>
<td>Tloc22</td>
<td>Xq21</td>
<td>Repressor of transcription, with an important role in horizontal elevation of palatal shelves</td>
<td>-</td>
<td>52,53</td>
</tr>
<tr>
<td>Holoprosencephaly 2</td>
<td>AD</td>
<td>Sic3</td>
<td>2p21</td>
<td>Regulation of SHH expression</td>
<td>-</td>
<td>54,55</td>
</tr>
<tr>
<td>Opitz-Frias syndrome or (Opitz GBBB syndrome type II)</td>
<td>AD</td>
<td>Spec11</td>
<td>22q11.23</td>
<td>Regulates microtubule and actin organization for proper cell adhesion and migration</td>
<td>-</td>
<td>56,57</td>
</tr>
<tr>
<td>Simpson-Golabi-Behmel syndrome type 1</td>
<td>XLR</td>
<td>Gpc3</td>
<td>Xq26.2</td>
<td>Regulation of SHH, FGF, and BMP activities</td>
<td>-</td>
<td>58,59</td>
</tr>
<tr>
<td>Oral-facial-digital syndrome 1</td>
<td>XLD</td>
<td>Ojd1</td>
<td>Xp22.2</td>
<td>Regulation of microtubule function</td>
<td>-</td>
<td>60,61</td>
</tr>
<tr>
<td>Gorlin-Goltz syndrome</td>
<td>AD</td>
<td>Phlb1, Phlb 2, Safa</td>
<td>9q22, 1p32, 10q24</td>
<td>Regulation of SHH signaling</td>
<td>-</td>
<td>62-64</td>
</tr>
<tr>
<td>Waardenburg syndrome, type 1</td>
<td>AD</td>
<td>Pac3</td>
<td>2q36</td>
<td>Transcription factor necessary for skeletal muscle formation</td>
<td>-</td>
<td>65,66</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>AD</td>
<td>Cbl7</td>
<td>8q12</td>
<td>Transcription factor necessary for neural crest cell migration</td>
<td>-</td>
<td>67,68</td>
</tr>
<tr>
<td>DiGeorge syndrome</td>
<td>AD</td>
<td>Tloc1</td>
<td>22q11.21</td>
<td>Regulator of BMP signaling</td>
<td>-</td>
<td>69</td>
</tr>
</tbody>
</table>

(Bmp), and fibroblast growth factors (Fgf) – are essential in normal palatal development. 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. A positive feedback loop also exists.
between the fibroblast growth factor Fgf10 and Shh expression in the palatal mesenchyme and epithelium, respectively. 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, although not all have been linked to specific genes. Cohen 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, are both autosomal dominant conditions secondary to mutations in the interferon regulatory factor-6 (Irf6) gene on chromosome 1q32. Interestingly, mutations in Irf6 have also been found in non-syndromic cleft lip and palate. 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. Orofacial cleft 6 and 8 are also associated with mutations in Irf6.

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.

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

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

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 fetomaternal 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.

REFERENCES

5. Mitchell L, Risch N. Mode of inheritance of nonsyndromic cleft lip with or without cleft palate:


