ETIOLOGY

The etiology of CLP is extremely complex with both genetic and environmental factors playing their respective roles. With advancement of genomic studies, several genes associated with CLP have been identified. CLP has a complex inheritance pattern with no clear mode of inheritance and a reduced penetrance with a positive family history.

Syndromic Cleft Palate (CP)

It is an X linked disorder characterized by cleft palate and ankyloglosia. High arched palate, bifid uvula or ankyloglosia could be seen in males. Females could either be asymptomatic or express all features of CP. The candidate gene was initially localized to chromosome Xq21. Steinert et al identified T-box transcription factor-22 gene (TBX22) in a large Icelandic family and several small families. Targeted disruption of TBX22 gene in mouse results in wide range of developmental anomalies which show features similar to DiGeorge syndrome.

Syndromic CLP

Single gene disorders can affect both autosomes and X-chromosome and follow Mendelian Inheritance pattern. Van der Woude syndrome (VDWS) is commonly associated with syndromic CLP. This syndrome is characterised by cleft lip with or without cleft palate, pits or mucous cysts on lower lip and hypodontia. Popliteal pterygium syndrome (PPS) includes all features of VDWS plus popliteal pterygium, syndactyly and genitourinary malformation.

The genetic locus for both VDWS and PPS was localized to chromosome 1q32-41. Sequence analysis revealed a point mutation for Interferon regulatory factor 6 gene (IRF6). IRF6 mutations were missense mutations that affected DNA binding domains which caused a domain negative effect resulting in severe phenotype.

CLASSIFICATION

CLP has been broadly divided into cleft palate only and cleft lip with or without cleft palate. Approximately 70% of all cases of CLP and 50% of cases of cleft palate are considered non-syndromic. The remaining 30% of the cases associated with Mendelian Inheritance. Syndromic cleft lip and palate or cleft palate alone can be broadly subdivided into those arising from a characteristic Mendelian disorder, from chromosomal defects and those with obscure etiology.

ETIOLOGICAL FACTORS

Environmental factors such as maternal smoking, alcohol intake, drug use, and exposure to teratogenic agents during pregnancy are known to increase the risk of developing CLP. Genetic factors play a crucial role in the development of CLP, and genetic mutations in genes involved in the development of the face and palate are associated with a higher risk of CLP. Chromosomal abnormalities, such as trisomy 21 (Down syndrome), also increase the risk of developing CLP. The combination of genetic and environmental factors is likely to contribute to the development of CLP.
Non- syndromic CLP (NSCLP)

Blood clotting factor XIII gene (F13A) on chromosome 6p is linked to CLP. Linkage of CLP to endothelin-1 (ET-1) has been observed which is a vaso-active peptide expressed in vascular endothelial cells. In mice studies this association has produced micrognathia, microfossils and cleft palate. In non-syndromic CLP a multifactorial model is proposed since a single major focus could not be isolated.

GENES INVOLVED IN CLEFTING

Transforming Growth Factor (TGF)

Transforming Growth Factor Alpha (TGFA)

TGFA gene is located in chromosome 2p13. Adringer et al. illustrated the association between NSCLP and TGFA alpha in a case control study. The combined effect of TGFA alpha and environmental influence in NSCLP on analysis showed that rare TGFA alpha variant (Taqa1C2 allele) and maternal smoking increase CLP risk by 6-8 times.

Transforming Growth Factor Beta (TGFB)

TGFB gene is located in chromosome 1q41. TGF-β signaling mediates a wide range of biological activities in development and disease. Altered TGF-β signaling causes syndromic and non-syndromic cleft palate. Mutations in TGF-β receptor gene family members cause craniofacial deformities, such as cleft palate. TGF BETA-2 plays an important role in pathogenesis and inactivation of TGF BETA2 in mice resulted in cleft lip and cranial abnormalities. TGF BETA -3 located in chromosome 1q24 induce palatal fusion and secondary palate development. Mice lacking this gene showed cleft palate. A newly discovered SNP of TGF BETA2 (IV AT+104 A>G) increased risk of CLP 16 times in Korean population. In humans TGF BETA2 is associated with non-syndromic CLP in different populations.

Interferon Regulatory Factor (IRF6)

IRF6 is located in chromosome 1q32. It is strongly associated with VDWS and has an autosomal dominant inheritance pattern. An approach that integrated identification of cis-regulatory element using sequence conservation across multiple species analysis of animal models and biochemical studies showed that an etiologic agent (rs642961) was observed which is a vaso-active peptide expressed in vascular endothelial cells. In mice studies this association has produced micrognathia, microfossils and cleft palate. In non-syndromic CLP a multifactorial model is proposed since a single major focus could not be isolated.

Methyltetrahydrofolate Reductase Gene (MTHFR)

MTHFR plays a key role in folic acid metabolism and is responsible for catalyzing conversion of 5, 10- methylene tetrahydrofolate to 5-methyl tetrahydrofolate. It is located in chromosome 1q36. In non-syndromic CLP, MTHF6C677T genotype in mother conferred a fivefold risk of CLP in offspring.

Muscle Segment Homeobox 1 Gene (MSX1)

MSX1 mutation causes an autosomal form of teeth agenesis. In mice studies, mice lacking MSX1 gene developed tooth agenesis, clefting of secondary palate and craniofacial defects. Jezewski et al. performed MSX1 sequential analysis of 917 CLP patients and identified 16 patients with CLP providing evidence in clefting. Neutral polymorphism within MSX1 and TGF alpha has been shown to have a tenfold increase in cleft risk.

Forhead Box El gene (FOXE1)

FOXE1 is located in chromosome 9q22-q33. Moreno et al. analyzed families from Columbia, USA and Philippines for candidate genes within 9q22-q33 and found 32 new variants. 2 SNP’s rs 3758249 and rs4460498 were located inside a 70kb high linkage disequilibrium block containing FOXE1.

Axis Inhibition Protein 2 Gene (AXIN2)

AXIN2 plays important role in craniofacial morphogenesis. Mutations of AXIN2 are responsible severe for oligodontia. Mutations in these genes are seen in families presenting tooth agenesis, cleft lip/palate and gastric cancer.

Poliovirus Receptor Related 1 Gene (PVRL1)

PVRL1 also known as nectin-1 and CDH1 is a member of nectins and is a human protein of immunoglobulin super family. Homogenous PVRL1 loss of function mutation results in autosomal recessive CL/P syndrome. PVRL1 nonsense mutation is associated with sporadic non-syndromic CLP. Rare mutations in sporadic cases and a statistically significant association between common coding variant (G36IV) in PVRL1 and non-syndromic CLP were found in multiple populations.

Sonic Hedgehog Gene (SHH)

SHH is one of the three proteins in the hedgehog family that plays an important role in vertebral organogenesis including odontogenesis. In mice studies, mutations in SHH results in absence of flat bones within skull, open bite, cleft palate and arrested tooth development. In humans mutation of SHH results in cleft palate like features.

Fibroblast Growth Factor Gene (FGF)

Several members of FGF and FGFR families are expressed during craniofacial development and rarely harbor mutations resulting in human clefting syndrome. Bridget et al. sequenced the code region and associated testing of 12 genes. Seven mutations were identified including one nonsense mutation (R609X) in FGF1, a denovo mutation (D73H) in FGF8 and the missense variant in FGFR1, FGFR2 and FGFR3. FGF signaling pathways may contribute to as much as 3-5% of non-syndromic CLP.

Gremlin-1 Gene

Gremlin-1 is a component of BMP4 pathways which is involved in oro-facial development located in chromosome 15q13 and 17q22. Gremlin-1 deficient mouse models have shown that during embryogenesis, Gremlin-1 is crucial for limb/kidney formation. It also plays a role in CLP development.

Special At-Rich Sequence Binding Protein 2 (SATB2)

SATB2 is a DNA binding protein that specifically binds nuclear matrix attachment regions and is specifically involved in chromatin remodeling and transcriptional regulation. Strong expressions of SATB2 was detected during palatal shelves development with maximum expression in the mesenchyme underlying medial edge epithelium. In 2q32q33 micro
deletion syndrome, SATB2 has been implicated as causative in cleft or high palate.

**Midline-1-Gene**

The protein encoded by this gene is also known as RING finger protein. Mutations of this gene on chromosome Xq22 are responsible for Opitz syndrome characterized by cleft lip, laryngeal cleft, heart defects, hypospadias and agenesis of corpus callosum but the exact developmental role of encoded protein remains unclear.

**WNT9B Gene**

Protein Wnt-9b in humans is encoded by WTN 98 gene. WNT pathways has important roles during craniofacial development including face morphogenesis. Loss of function of WNT genes is associated with defects in facial region, cleft lip and kidney morphogenesis in homozygous mice mutants. A Brazilian study by Contours et al. has shown a positive association between non-syndromic CLP and SNP rs1530364 in WTN8 gene further supporting the involvement of WNT9B as a cleft susceptibility gene.

**Paired Box 9 Gene(PAX9)**

Paired box gene 9 is a member of paired box (PAX) family of transcription factors. Mice lacking this gene exhibit impaired development of organs, musculature and skeleton including absent and abnormal teeth. The marker rs4986700 in FGFR3 was associated with oral clefts. Logistic regression analysis provided evidence for gene-gene interaction between FGFR3 (rs4986700) and PAX9 (rs2073242) increases risk for involved CLP.

**Cysteine Rich Secretory Protein Containing Lccl Domain 2 Gene (CRISPLD2)**

CRISPLD2 gene is located in chromosome 1q24.1. Shen et al. in a study in Chinese population found that CRISPLD2 gene is significantly associated with non-syndromic CLP. SNPs rs1546124 is significantly related to non-syndromic CLP and CPO groups while SNPs rs783099 is significantly associated with CPO.

**Dihydrofolate Reductase Gene (DHFR).**

DHFR gene is located in q11-q22 region of chromosome 5 and is involved in DNA synthesis, repair and methylation. DHFR gene is a candidate gene of CL with or without CP. Martelli et al. mapped 4 SNPs on DHFR gene for 400 Italian CLP triads. The rs 1677693 provided association with CLP. This combination of rs 1677693(A)-rs1650713 (G) alleles also showed a significant association.

**MANAGEMENT**

A combination of treatment protocol involving surgical, nutritional, dental, speech, medical and behavioral therapy are required for CLP treatment. Proper elucidation of underlying genetic and molecular mechanism in lip and palate formation could lead to proper treatment protocol for CLP. Stem cell therapy and tissue engineering holds great prospect in unraveling the mystery of CLP.

The problem with surgery is the tissue shortage that accompanied the procedure. Stem cell isolation from amniotic fluid and adult tissues including pulp can provide a sufficient reserve for treating these potential abnormalities. Such cells can be engineered to regenerate appropriate tissue of desired shape and dimensions for treatment.

**CONCLUSION**

Oro-facial clefts consisting of a wide spectrum of clinical presentations ranging from a simple bifid uvula or isolated clefts of lip to extensive defects involving bilateral clefts of lip, palate and alveolar mucosa and in most instances it may cause considerable morbidity to affected children and imposes substantial financial burden for affected families. The formation of cleft is extremely complex with both genetic and environmental factors playing their respective roles. Even though a combination of epidemiologic, phenotypic and genome wide association studies and animal models have helped to extrapolate several genes involved in oro-facial cleft formation, the exact mechanism which cause these anomalies are still obscure in nature. The continuing research on genetic background may help a long way in identifying the etiopathogenesis and for developing future preventive measures.

**References**


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