

## REMINISCENCING THE GENETICS OF OROFACIAL CLEFTING

Subhra Mandal <sup>\*1</sup>, Prabir Mandal <sup>2</sup>, Swapan Bhattacharjee <sup>3</sup>.

<sup>\*1</sup> Associate Professor, Dept. of Anatomy, Medical College Kolkata, West Bengal, India.

<sup>2</sup> Post Graduate Resident in DNB (PMR), S.N.P. Hospital, Kolkata, West Bengal, India.

<sup>3</sup> Additional Professor, Dept. of Anatomy, Medical College Kolkata, West Bengal, India.

### ABSTRACT

Cleft lip with or without cleft palate is the most common craniofacial birth defect (1/1000 live births), caused by a complex interaction of chromosome rearrangements, gene mutations as well as environmental influences. The frequent occurrence of orofacial clefting, along with their extensive psychological, surgical, speech and dental involvement emphasize the importance of identifying the underlying etiology. Rare cleft forms, where they occur as a component of multiple congenital anomaly syndromes, have Mendelian or Teratogenic origins; the non-syndromic forms of orofacial clefts are more common and are probably due to secondary gene-environment interactions. The purpose of our review is to provide a short summary of the vast spectrum of genetic architecture of orofacial clefting covering both syndromic as well as nonsyndromic forms of clefting. Although the gene identification process for orofacial clefting in humans is in an early stage (especially in India); the research pace is rapidly accelerating worldwide. Ongoing human genome wide linkage studies have identified regions that are likely to contain genes that when mutated cause orofacial clefting. Our main aim is to bring together a discussion of new and previously identified candidate genes to create a more cohesive picture of interacting pathways that shape the human craniofacial region. In addition, sequencing of protein coding regions in candidate genes and screening for genetic variation in noncoding regulatory elements, will help in the area of advanced research. Furthermore, statistical geneticists are developing new methods to characterize both gene-gene and gene-environment inter-actions, for explaining the pathology of this common birth defect. The ultimate goal of these studies is to provide knowledge for more accurate risk counseling and the development of preventive therapies.

Though India has an estimated population of one million untreated cleft patients, facilities for its treatment have been limited and are not evenly distributed across the country. Paucity of committed cleft surgeons, poverty, illiteracy, superstitions, poor connectivity in some remote areas; severely limit the chances of an average cleft lip Indian child from receiving rational and effective treatment for his malady. The Oscar winning documentary film "Smile Pinki" highlighted the plight of cleft patients in our country. The film stood testimony to the passion of dedicated doctors, social workers and paramedical staffs toiling in the Indian heartland as they treated unfortunate cleft children with the support of the "Smile Train Project".

**KEY WORDS:** Orofacial clefting, Syndromic & Non Syndromic forms, Gene mutations, candidate genes, Sequencing, Statistical geneticists.

**Address for Correspondence:** Dr. Subhra Mandal, M.B.B.S. , M.D. (Anatomy), Associate Professor, Dept. of Anatomy, Medical College Kolkata, West Bengal, India. Mobile. No: +919477458100  
**E-Mail:** [drsuvramandal@gmail.com](mailto:drsuvramandal@gmail.com)

### Access this Article online

#### Quick Response code



DOI: 10.16965/ijar.2015.164

**Web site:** International Journal of Anatomy and Research  
ISSN 2321-4287  
[www.ijmhr.org/ijar.htm](http://www.ijmhr.org/ijar.htm)

Received: 23 Apr 2015

Accepted: 12 May 2015

Peer Review: 23 Apr 2015

Published (O): 31 May 2015

Revised: None

Published (P): 30 June 2015

### INTRODUCTION

Today, in India, about 35,000 children are born yearly with cleft lip or palate. Most of these chil-

dren are from very poor illiterate families who believe such defects were curse of God caused due to an eclipse that occurred while the baby

was inside mother's womb. Tragedy is, most of these children never receive any treatment for what is an easily correctible orofacial defect occurring sometimes between the 4<sup>th</sup> to 12<sup>th</sup> week of IUL. American film maker Megan Mylan directed a 39 minute documentary film "**SMILE PINKI**" (made in Hindi & Bhojpuri language) which won the 81<sup>st</sup> Academy Awards (2008) [1]. The documentary followed the remarkable journey of two children from a socially isolated shameful life to that of a normal happy childhood. Thankfully, Pinki Sonkar (cleft lip), 8 years and Ghutaru Chauhan (cleft palate), 11 years –were spotted by social workers from G S Memorial Plastic Surgery Hospital, Varanasi. Both of them received free cleft surgery under the US based global cleft charity programme – "**SMILE TRAIN** " [2]. This charitable organization sponsors surgical treatment and follow up of cleft surgeries to underprivileged children worldwide. Since 2000, Smile train has sponsored over 4, 25,000 surgeries across India. But there are still an estimated one million kids waiting in the wings for a simple surgery. Also ignorance about the condition, no access to treatment, poor antenatal care, anemia etc will continue to hinder efforts by medical and social workers in providing help to the children who actually need it. Smile Train partner Hospitals are now homogeneously spread over almost all parts of India. Varanasi, the birth place of *Sushruta*, has the distinction of the highest rate of cleft surgeries in the world in its four centers'. This is a tribute to the father of plastic surgery.

Orofacial clefts are common congenital structural anomalies of the lip and/or palate, that affect approximately 1/1000 live births. Their frequent occurrence as well as their extensive psychological, surgical, speech and dental involvement require complex multidisciplinary treatment and have lifelong implications for affected individuals [3]. The etiology of both cleft lip (CL) with or without cleft palate (CLP) and isolated cleft palate (CP) is complex and multifactorial with both genetic and environmental factors playing crucial roles. In recent years, significant breakthrough has occurred with respect to the characterization of the underlying gene defects associated with clefting syndromes. These include the identifica-

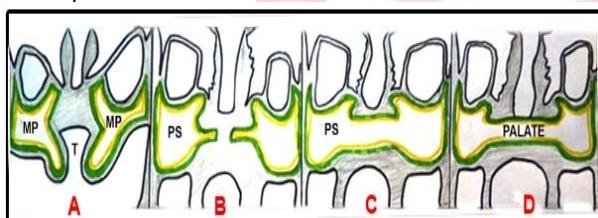
-tion of mutations in the interferon regulatory factor-6 (IRF6) gene as the cause of Van der Woude syndrome (VWS) and Polio virus receptor related-1 (PVRL-1) gene as the culprit for an autosomal recessive ectodermal dysplasia syndrome associated with clefting [4]. While no specific disease causing gene mutations have been identified in non syndromic clefting, a number of candidate genes have been isolated through both linkage and association studies. Nevertheless it's clear that environmental factors also interfere with lip and/or palate formation, when present during first trimester of pregnancy; whereas ethanol, retinoid, or folate antagonists are clearly teratogenic, inclusion of more common exposures like caffeine is merely tentative. Orthodontists are intimately involved in the therapeutic management of CL/P patients. So it's essential that they are having current updated knowledge of the etiology behind these conditions. As the CL/P patient continues to grow, defect in tooth development and malocclusion require dental and sometimes surgical treatment. At the stage of speech development, speech therapy is often needed to rectify problems resulting from muscular defects of the clefts. The prolonged series of treatment from birth to adulthood is a heavy burden for the patient, family and society. The Dentist can assess from a good family history, the possible extent to which genetic factors are involved in the etiology of CL/P in a given proband and provide genetic counseling [4]. Various efforts have been made to understand the etiology of CLP so as to predict its occurrence and to prevent it from occurring in the future. In this article, we have targeted to summarize some of the significant advances in the genetics of CLP and discuss the different modes of inheritance and genetic loci underlying this common, complex orofacial malformation. We have reviewed the recent data on aetiology of CLP, taking relevant information's from MEDLINE literature search [5].

This review will concentrate on genetic contributions to facial clefts with or without cleft palate. We will begin with an overview of early palatal development, concentrate on identification of genes associated with SCLP and NSCLP, recent molecular signaling pathways in

palatogenesis and incorporate the effects of environmental insults and known genetic mutations that affect human palatal development.

**EMBRYONIC PALATE DEVELOPMENT [4]:** The palatal structures are made up of-1) Cranial neural crest (CNC) derived mesenchyme & 2) Pharyngeal ectoderm. Epithelia covering the palatal shelves are regionally divided into—Oral, Nasal & Medial edge epithelia (MEE).The nasal & oral epithelia differentiate into pseudo stratified & squamous epithelia, where as MEE is removed from the fusion line.

**Fig. 1:** Schematic drawing showing coronal view of a normal palate shelf and key stages of normal palatal development.



(Courtesy-Dr Lynne Opperman , councilor of Dallas chapter of the American Association of Dental Research).

The Secondary Palate originates as an outgrowth of the Maxillary Prominences (MP) at approximately embryonic day 11.5 in the mouse (E11.5-m) and post coital six weeks in humans (p.c.6wks-h).

**A) Vertical growth**-Initially the palate shelves grow vertically along the sides of the tongue (E13.5-m; p.c7wks-h)

**B) Elevation**-Then they rise above the tongue as the latter drops in the oral cavity due to the forward and downward growth of the mandible (E14.0-m; p.c8wks-h).

**C) Adhesion**-With continued growth, the shelves appose at the midline (E14.5-m; p.c10 wks-h) and

**D) Eventually fusion** occurs (E15.5-m; p.c13wks-h).

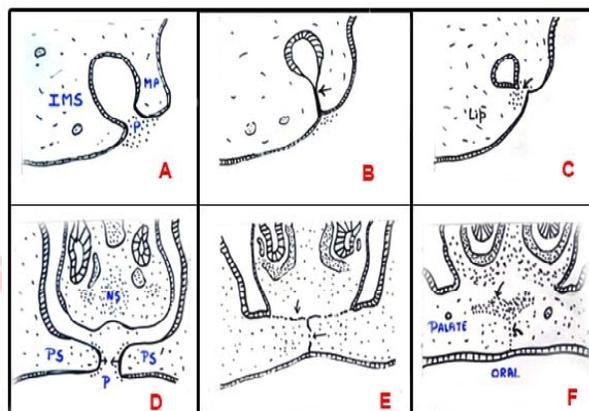
Numerous genes similar in mice and humans are expressed during palatal development (Table-1).

During fusion, the epithelium covering the tip of the opposing palatal shelves (MEE cells), adheres, intercalates, and thins into a single layer midline epithelial seam (MES). The disintegration of this seam results in the confl-

-uence of the palatal mesenchyme. Probable cellular mechanisms underlying MES degradation are:

- 1). Epithelial- Mesenchymal transition (EMT).
- 2). Apoptosis-All MEE cells die during fusion.
- 3). MES cells disappear by migrating from the midline towards the nasal and oral epithelia [4].

**Fig.2:** Morphogenesis of palate.



(Courtesy- Dr Lynne Opperman).

After the bilateral maxillary processes (MP) fuse externally, with the intermaxillary segment (IMS), the resultant epithelial seam (arrow, B), forms the Mesenchyme (arrowhead, C), to create a confluent lip. Later, the palatal shelves (PS) arising internally from the maxillary processes fuse with each other (arrows, D) and with the nasal septum (NS) lying above them, forming an epithelial seam that transforms to mesenchyme (arrowheads, E). P- Sloughed periderm cells.

**Table 1:** Syndromic genes associated with cleft lip and palate.

SYNDROME	CLINICAL FEATURES	GENES
1. Apert Syndrome(AS)	AD; high arched palate, bifid uvula and cleft palate.	FGFR2
2. Down Syndrome(DS)	Macroglossia, microstomia, atlantoaxial subluxation	Duplication of portion of chromosome 21.
3. Ectrodactyly-ectodermal dysplasia-cleft syndrome(EEC)	AD; Triad of ectrodactyly, ectodermal dysplasia and facial clefting	P63
4. Fetal alcohol syndrome(FAS)	Minor facial anomalies, prenatal and postnatal growth retardation	Alcohol dehydrogenase 1B(ADH1B)
5. Margarita Island ectodermal dysplasia(ED4)	AR; Unusual facies, dental anomalies, syndactyly and cleft lip/cleft palate	PVRL1(nectin-1) mutation.
6. Stickler syndrome(SS)	AD; mid face hypoplasia, micrognathia, Pierre Robin sequence, retinal detachment and early cataracts, deafness, hypermobility of joints	COL11A1,COL11A2,COL2A1
7.Treacher Collins(TC)	AD; Downward slanting eyes, micrognathia, conductive hearing loss, underdeveloped zygoma	Mutations inTCOF1 gene at chromosome5q32-q33.1.
8. Van der Woude syndrome(VDWS)	AD; Cleft lip/palate, distinctive pits of the lower lips or both	Interferon regulatory factor 6(IRF6) mutations.
9. Velocardiofacial syndrome(VCFs)	AD; abnormal facial structure, cleft palate, heart defects, learning problems	Chromosome22q11 microdeletion

**Table 2:** Non -Syndromic genes: Interaction effects of genes and environmental risk factors on oral clefts.

Genes	Functional Role	Risk Factor
1) Glutathione Transferase Gene family(GST)	Enzymes that catalyse the conjugation of reduced glutathione with electrophilic groups of a wide variety of environmental agents.	GSTM1-Negative gene-smoking interaction effect GSTT1 & GSTP1-Positive gene-smoking interaction effect
2) Hypoxia induced factor 1(HIF1)	Maternal smoking may affect development due to the production of carbon monoxide which interferes with oxygen transfer to placenta, or, nicotine, which constricts the uterine wall leading to hypoxia.	Positive gene-smoking interaction effect
3) Methylene tetrahydrofolate reductase(MTHFR) MTHFR C677T	Folate metabolism by reducing methylenetetrahydrofolate, primary donor for folate synthesis. Variant of MTHFR reductase	Positive gene-smoking interaction effect Negative gene smoking interaction effect
4) Transforming growth factor A(TGF $\alpha$ )	Transmembrane protein expressed at the MEE of fusing palatal shelves	Positive gene-smoking interaction effect (smoking, alcohol drinking, vitamins)
5) Transforming growth factor $\beta$ 3(TGF $\beta$ 3)	Regulator of many biological processes like proliferation, differentiation, EMT, and apoptosis.	Positive gene-smoking interaction effect (smoking, alcohol drinking, vitamins)
6) Muscle segment Homeobox 1 MSX1, MSX2	Transcriptional repressor- important in craniofacial, nervous system & limb development. It is a rare cause of isolated cleft lip with or without cleft palate.	Positive gene-smoking interaction effect (smoking, drinking alcohol, vitamins)

### Palatal shelf development defects and probable gene mutations leading to Cleft lip/ Cleft palate are the following [4]:

- 1). Failure of palatal shelf formation [Gene mutations- Sonic hedgehog (Shh), Transforming growth factor $\beta$  (TGF $\beta$ ), Fibroblast growth factors (Fgf) etc.]
- 2). Inhibition of fusion of palatal shelf with tongue or mandible [Gene mutations- Jagged 2(Jag2), T-box transcription factor22 (TBX22) etc.]
- 3). Failure of palatal elevation [Gene mutations- Pax9, Pitx1 or Osr2; GABA regulation]
- 4). **Failure of palatal shelves (MEE) to meet after elevation- Commonest cleft palate defect documented in animal studies.** [Gene mutations - Msx1, Tgfbr2 or Shh etc.]
- 5). Persistence of middle edge epithelium- [Gene mutations of CDH1/E Cadherin, Tgf  $\beta$  3 or Egfr etc]
- 6). Defective ossification of palate-[Gene mutations-Sox9, Runx2, TGF $\beta$ , Cbf etc.]
- 7). Defective development of oral and or palatal musculature-[Gene mutations in Bmp4]

**Syndromic versus Nonsyndromic cleft:** Clefts (CL, CP or Both) represent a complex phenotype and reflect a breakdown in the normal mechanisms involved during early embryological development of the face. The incidence of these

defects varies according to geographical location, ethnicity and socioeconomic status, but in Caucasian population it is reasonably uniform, with 1:800 to 1:1000 (CLP) and approximately 1:1000 (CP) live births affected (Fraser, 1970). The clinical manifestations of these defects are diverse, ranging from isolated clefts of lip to complete bilateral clefts of lip, alveolus and palate. Clinically, when CLP appears with other (usually two or more) malformations in recognizable patterns, it is classified as syndromic CLP (SCLP-30%). It is nonsyndromic CLP (NSCLP- 70%) [6], if it appears as an isolated defect or if there are multiple anomalies resulting from a single initiating event/primary malformation or if multiple anomalies are limited to a single developmental field. The number of SCLP's is large (>300 syndromes) and still growing. Syndrome identification is extremely important because of the need for accurate counseling. In families with SCLP, some affected members may present with only CLP, because of variable expression of the syndrome. On the other hand, more than 20% of patients with NSCLP were found to have associated congenital malformations in one study [6]. Thus some cases of SCLP and NSCLP might share a common etiology.

### RECENTLY DISCOVERED CANDIDATE GENES CAUSING OROFACIAL CLEFT SYNDROMES (SCLP)

**1). T-box transcription factor-22:** X-linked cleft palate (CPX) is a rare semi dominant X-linked disorder characterized by isolated cleft palate (CP) and ankyloglossia (tongue tie). Clinical expression of CPX is highly variable. High arched palate, bifid uvula, or ankyloglossia could be the only presenting sign in affected males. Female carriers could be asymptomatic or they could express full features of CPX. By using genetic linkage analysis, the causative gene was originally localized to chromosome Xq21 (Moore et al, 1987) [7]; but very recently Braybrook et al (2001) [8] succeeded in pinpointing a variety of mutations in the TBX22 gene (which encodes a number of the Tbox family of transcription factors) in individuals from a number of separate families, as being responsible for CPX. These mutations, including missense, nonsense splice site and frameshift,

were all predicted to result in a complete loss of function of TBX22. Animal experiments showed that expression of TBX22 was highly restricted to the palatal shelves just before their elevation to adopt a horizontal position, and at the base of the tongue (frenulum); both these expression patterns closely matched the clinical presentation of CPX. Involvement of TBX22 in NSCLP also has recently been indicated from a genome-wide sibling-pair analysis in which the chromosome Xcen-q region, where TBX-22 is located, showed promising multipoint logarithm of odds (LOD) scores [9]. Mutation analysis of TBX22 in these patients could reveal whether the gene is involved in NSCLP as well [9].

**TBX22 is the first gene to be identified for a major CP syndrome:** Another special feature is that targeted disruption of TBx1 in the mouse results in a wide range of developmental anomalies which encompass almost all of the common features of the DiGeorge or velocardiofacial syndromes (deletion in chromosome 22q11) including cleft palate also.

**2). Poliovirus receptor like -1:** Using positional cloning, Suzuki et al (2000) [10]. identified a homozygous nonsense mutation in the poliovirus receptor related-1 (PVRL-1) gene (called W185X) as being responsible for an autosomal recessive CLP-ectodermal dysplasia syndrome (CLPED-1), found in families from Margarita Island (north Venezuela), Israel and Brazil. CLPED is characterized by cleft lip with or without cleft palate, hidrotic ectodermal dysplasia, syndactyly, and occasionally mental retardation. Two other syndromes—Zlotogora-Ogur syndrome and Margarita island ectodermal dysplasia are also stamped as CLPED.

The protein product of PVRL-1 was initially identified as poliovirus receptor-related protein (PRR). Takahashi et al [11] confirmed the function of PRR as a cell adhesion molecule and renamed it nectin-1. All three PVRL-1 mutations found in families with CLPED resulted in truncations in nectin-1, thereby destroying the nectin-afadinsonin (NAP)—dependent cell adhesion system. In mouse embryo, PVRL-1 was expressed at the medial edge epithelium of the palatal shelves and the skin surface epithelium—locations that corresponded to the clinical phenotypes of CLPED. Above mentioned findings suggest that

normal PVRL-1 function is important in mediating fusion of the palatal shelves during the later stages of palatogenesis.

**3). Interferon regulatory factor-6:** One of the most common human autosomal dominant disorders associated with CLP is Van der Woude syndrome (VWS) which accounts for 2% of all syndromic CLP cases (Van der Woude, 1954) [12]. This condition is associated with—cleft lip with or without cleft palate/isolated cleft palate, highly characteristic pitting of the lower lip mucosa and hypodontia.

The genetic locus for VWS has previously been localized to chromosome 1 (1990). Through linkage and chromosomal analysis, the critical area for VWS was gradually narrowed to 1q32-q41. Recently, a unique approach has exploited the discovery of monozygotic twins who demonstrated VWS in one member of the pair, but not in the other twin or parents ((Kondo et al, 2002) [13]. The VWS in the affected twin was thought to arise from somatic mutation. Sequence analysis revealed a nonsense point mutation in the interferon regulatory factor-6 (IRF6) gene in the affected twin. IRF6 encodes a transcription factor belonging to a nine member family involved in regulating the expression of Interferon  $\alpha$  and  $\beta$  following viral infections.

However, the exact role of IRF6 during palatogenesis is unknown. In the developing mouse embryo, IRF6 demonstrates high levels of expression in a variety of craniofacial structures, including the medial edges of the fusing palatal processes, tooth buds, hair follicles and skin. This expression pattern and the haploinsufficiency of IRF6 causing VWS, suggests an important role during craniofacial development, with some indication that it mediates interactions between members of the transforming growth factor- $\beta$  (TGF  $\beta$ ) super family of signaling peptides (Kondo et al, 2002) [13]. Indeed, 45 additional unrelated families affected by VWS have also been demonstrated to carry mutations in the IRF6 gene (Kondo et al, 2002) [13]. The IRF6 mutations, however, were missense mutations that affected the DNA binding domain and caused a dominant-negative effect, which resulted in severe phenotypes. A partial or modifying role of IRF6

in NSCLP has been demonstrated in a study applying the transmission disequilibrium test, in which specific parental alleles at the VWS locus were preferentially transmitted to the individuals with NSCLP.

### **CANDIDATE GENES OR LOCI FOR NON SYNDROMIC CLEFT LIP AND PALATE (NSCLP)**

Non syndromic orofacial clefting arises as a complex multifactorial trait, being a mosaic of mendelian patterns, exhibiting varying levels of penetrance, sex differences, various ethnic backgrounds and environmental overlays; with the result that gene identification is difficult (Murray J, 2002) [14]. A more recent genome wide linkage study in families with multiple cases of non syndromic CLP concluded that no single major CLP locus exists; instead a multifactorial model was widely accepted cause. But it is not unreasonable to declare that both together and individually, the candidate genes might have a modifying role in causing a non syndromic CLP (Prescott et al, 2001) [9].

**1). Transforming growth factor $\alpha$ (TGF $\alpha$ ):** Ardinger et al. (1989) [15], in a case control study (association studies using candidate gene approach), concluded that—TGF  $\alpha$  was associated with NSCLP. Transforming growth factor  $\alpha$  is member of a large group of developmentally important intercellular signaling molecules which have been localized in the epithelium of the palatal shelves prior to fusion (in mouse). However, while targeted disruption of TGF $\alpha$  produces defects in hair follicles and eyes of the mouse, it does not produce CLP, but probably acts as a modifier (Murray, 2002; Prescott et al, 2001) [14,9]. The combined effect of TGF $\alpha$  mutations and environmental influence in NSCLP has been analyzed by a group of researchers. The rare TGF  $\alpha$  variant (TaqlC2 allele) and maternal smoking could increase the risk of cleft palate by 6 to 8 times and of cleft lip with or without cleft palate by 2 times. If multivitamins were not consumed during first trimester of pregnancy and the baby is carrying the TGF $\alpha$ TaqlC2 allele, the relative risk for cleft lip with or without cleft palate increased by 3 to 8 times (Shaw GM et al, 1996) [16].

**2). Drosophila msh homeo box homolog-1:**

Msx1 (formerly Hox7), is a member of a distinct subfamily of homeobox genes related to the Drosophila msh (muscle segment homeobox) gene; Msx1 encodes a transcription factor which demonstrates a regionally restricted expression pattern in the developing murine craniofacial complex (MacKenzie et al, 1991a) [17]. Mice lacking functional Drosophila msh homeobox homolog -1 (Msx1) exhibit a cleft of the secondary palate and tooth agenesis. Heterozygous Msx1 nonsense mutation has recently been identified in a three generation dutch family exhibiting various combinations of CLP, CP and selective tooth agenesis (Van den Boogaard et al, 2000) [18]. Recently, a large scale sequence analysis of Msx1 performed on 917 CLP patients identified mutations in 16 patients with cleft lip with or without cleft palate, or cleft palate alone, providing evidence that this gene could be involved in both forms of cleft (Jezewski PA et al, 2003) [19]. The authors estimated that Msx1 mutations contributed to 2% of all NSCLP cases. A recent study showed that the combined genetic background of rare variants of TGF $\alpha$  and Msx1 could increase the risk of cleft palate by up to 9.7 times, demonstrating the significance of gene—gene interaction in the etiology of NSCLP (Jezewski et al 2003) [19].

**3). Transforming growth factor $\beta$ 3 (TGF $\beta$ 3):** Another locus that has been identified in association with non syndromic CLP (NSCLP) encodes the TGF $\beta$  3 gene on chromosome 14q24 (Lidral et al 1998) [20]. Mice lacking functional gene encoding (TGF $\beta$  3) displayed cleft palate because of defective adhesion of opposing palatal shelves.

**4). 5, 10 Methylene tetrahydrofolate reductase (5,10-MTHFR):** The association between folic acid deficiency and neural tube defects has been well established. 5, 10-MTHFR is the enzyme responsible for catalyzing the conversion of 5, 10 Methylene tetrahydrofolate into 5 Methyl tetrahydrofolate in the folate metabolism pathway. The MTHFR C677T single nucleotide polymorphism (SNP) is thermally labile and considered a risk factor for neural tube defects. In NSCLP, the MTHFR C677T genotype in the mother conferred a risk of CLP in the offspring that was increased by 4.6 times.

In periconceptional folic acid deficiency, the MTHFR thermally labile variant could lead to a risk of CLP that was increased by 10 times (Prescott et al, 2002) [21].

**5). Other genes and loci:** a) Cleft lip and palate associated transmembrane protein-1 (CLPTM-1) Yoshiura et al [22] reported on a family with CLP in three generations; all affected members had a balanced translocation at chromosome 19q13. Breakpoint cloning revealed a novel gene called CLPTM1—eight rare variants of CLPTM-1 were found in 74 patients with NSCLP, but none was significantly associated with cleft lip or palate. The authors concluded that though CLPTM1 was not a major contributor to CLP, yet this gene could still be associated with NSCLP.

b) Chromosome 6p 23, By linkage studies, chromosome 6p23 has been indicated in some patients with NSCLP as well as SCLP.

### **THERE ARE TWO NEW ENTITIES IN THE ARENA OF GENETICS IN BOTH SYNDROMIC AND NONSYNDROMIC CLEFTING**

I). SUMO modification of signaling pathways in palatogenesis [23]

Recent researchers elucidated small ubiquitin related modifiers, belonging to the ubiquitin related protein family, These SUMO proteins are ubiquitously expressed throughout the eukaryotic kingdom. A significant role in orofacial development has been revealed for protein modification by the SUMO, which might hint at a possible interaction with environmental factors [23].

\*SUMO1 shows strong expression in the medial edge epithelia (MEE) of the secondary palate.

\*A translocation breakpoint interrupting SUMO1 was found in a patient with CLP. The causative nature of the same translocation defect has been confirmed in SUMO1 deficient mice having a distinct CP phenotype. \*Other SUMO targets (Genes which may get SUMOYLATED) [24] are—TBX22, MSX1, SATB2, P63, PAX9, FGF signaling etc. \* Destabilizing the normal balance of expression and activity of the above mentioned genes, during early pregnancy most likely provides a high risk environment for the occurrence of CLP.

Establishing the relationship between environmental factors, the SUMO pathway and

the complex of craniofacial genes, influenced by this post transcriptional modification is crucial to our understanding of the idiopathic forms of orofacial clefting.

II). A-P Gradient of molecular signaling in Palatal Development [23].

- Important research currently revealed the confirmation of not only the genetic but also mesenchymal heterogeneity along the anterior–posterior as well as medial–lateral Axes of the developing palate.

- Genes responsible for restricted expression patterns in the anterior region of the palate are-Msx1, Bmp4, Bmp2, Fgf10 and Shax2. The specific gene expression patterns in the posterior region of the palatal mesenchyme are less understood.

- The odd, skipped related genes Osr1 and Osr2 are expressed in the medial lateral gradient in the palatal shelf and retards palatal shelf elevation. The expression of Fgfr2 is also found to be focused on the development and elevation of medial aspect of palatal shelf.

- This heterogeneity may provide a differential regulatory mechanism for the fusion of the anterior vs. posterior region of the palate. MEE cells undergo apoptosis at different times during palatal fusion. Apoptosis of MEE cells is triggered by palatal shelf contact in the anterior region, whereas no such initiating factor is required in the posterior region. This difference may be the result of dissimilar molecular signals in the palatal mesenchyme along the anterior-posterior axis that instruct different fates to the palatal epithelium [4].

- Recent studies have revealed that constant and reciprocal interactions between palatal epithelium and CNC derived mesenchyme are responsible for setting up this heterogeneity along the AP axis and are essential for normal palatal development and fusion.

- The specific gene expression patterns in the posterior region of the palatal mesenchyme are less understood. Fgfr2 is expressed in the epithelium, and the CNC derived mesenchyme is found in the middle and posterior palate. Fgf8 signaling selectively induces the expression of Pax9 in the posterior region of the palatal mesenchyme [4]. The loss of Pax9 results in a

defect in palatal shelf development and ultimately forming a cleft palate.

**Environmental Influences:** A number of researches also suggest a significant environmental contribution in the etiology of CLP/CP; the lack of total concordance in monozygotic twins, the relatively rare findings of non syndromic cases being present throughout large family groups and the varying social, geographical and ethnic incidence of these malformations (Spritz RA, 2001) [25]. The majority of CLP cases are, therefore, multifactorial and a variety of environmental factors have been implicated (Wyszynski & Beaty, 1996) [26]. It is logical to state that the true etiology relevant to these conditions cannot be treated in isolation, but it should be remembered that intrauterine environmental factors will influence fetal development in combination with the individual genetic background of the embryo (Prescott et al, 2002) [21].

**Smoking:** Maternal cigarette smoking- leading to embryonic hypoxia has been associated with an increased incidence of non syndromic CLP. A relatively recent meta analysis of relevant studies produced over 20 years, prior to 1996- suggested a small, but statistically significant association between maternal cigarette smoking during 1<sup>st</sup> trimester of gestation and an increased risk of having a child with CLP or CP (Wyszynski et al, 1997b) [27]. When maternal smoking was considered together with certain genetic background the synergistic effect was more significant. Furthermore, Van Rooj et. Al [28] found that maternal glutathione S-transferase Q-1 (GSTT-1) genotype, when combined with smoking, could significantly increase the risk of CLP (Odd ratio=4.9). Beaty et al [27] reported that maternal smoking and infant MSX1 genotypes contributed to an elevated risk for CLP by 7.16 times.

**Altitude Hypoxia:** During pregnancy, altitude hypoxia might also be associated with an increased incidence of several birth defects, including CLP (Castilla et al. 1999) [29].

**Maternal Alcohol (Ethanol) Ingestion:** Maternal Alcohol abuse, during pregnancy, apart from causing foetal alcohol syndrome (FAS),

increases the risk of CLP (Romitti et al, 1999) [30]. Some study found evidence for gene environment interactions in nonsyndromic CLP aetiology with a greater incidence of CLP in children carrying allelic variants at the MSX1 site. Shaw and Lammer, 1999 [31] showed that the heavier the consumption, the more likely a CLP/CP phenotype will form a component of the craniofacial defect- Low level alcohol consumption, however did not seem to increase the risk of orofacial clefts. The definite link between alcohol consumption and genotypes on the risk of CLP has yet to be shown.

**Folic Acid Supplementation:** Some studies have paid attention to the nutritional status of pregnant mothers (especially role of folic acid supplementation) with respect to incidences of clefting phenotypes in their offspring's. Certainly, there is conclusive evidence for maternal folic acid / folate supplementation in the prevention of neural tube defects (Medical Research Council, 1991) [32] and some epidemiological investigation have suggested that deficient maternal folic acid intake may predispose to orofacial clefting. Shaw et al [33] reported that if vitamin supplements were not taken during early pregnancy, the risk for CLP could be tripled. Folic acid deficiency with the background of the TGFA Taq1C<sub>2</sub> genotype was also found to increase the risk of CLP [34]. In addition, defective maternal vitamin dependent homocystine metabolism is a risk factor for CLP in offspring. From recent studies, it can be concluded that low dose folic acid supplementation by fortifying cereal grain products could not protect against CLP. Only a very high dose of supplementary folic acid (10mg/d) could reduce the risk of CLP significantly (65%) [35].

## CONCLUSION

Abovementioned review of literature clearly elucidates that etiology of CL/P is multifactorial, including both genes and environment. With recent draft sequencing of both the human and mouse genomes and the introduction of gene microarray technology, further identification of candidate genes and newer genetic pathways involved in syndromic clefting can be analyzed. Advanced complex and

widespread multifactorial genetic analysis are likely to be required to know further the etiology of non syndromic CLP and , in particular the emergence of studies linking environmental influences with the genetic background of susceptible embryos. Ultimately, all of these advances will allow more accurate methods of genetic screening (followed by genetic counseling), the identification of high risk individuals or family clusters and improved pre natal diagnosis. In turn, we may witness the introduction of both preventive measures (like dietary supplementation or life style modifications) as well as in vivo fetal therapy for these orofacial clefting conditions.

**Conflicts of Interests: None**

## REFERENCES

- [1]. Megan Mylan: "Smile Pinki" documentary.org (2008)
- [2]. The official Smile Train website (2008) www.smiletrainexpress.org
- [3]. Puneet Batra et al. Genetics of cleft lip and palate revisited J Clin Pediatr Dent. 2003;27(4):311-320.
- [4]. Wenli Yu et al. Cleft lip and palate genetics and application in early embryological development. Indian J Plast Surg. 2009;42 Suppl S1:35-50.
- [5]. MEDLINE DATABASE—MEDLINE literature search (1986-2003) study.
- [6]. Wong FK et al.2004 An update on the etiology of orofacial clefts. Hong Kong Medical Journal 20014;10(5):331-336.
- [7]. Moore G E et al. Linkage of an X-chromosome cleft palate gene. Nature 1987;326:91-92.
- [8]. Braybrook C et al. T-box transcription factor gene TBX22 is mutated in X-linked cleft palate and ankyloglossia. Nature Genetics 2001;29:179-183.
- [9]. Prescott NJ et al. Identification of susceptibility loci for nonsyndromic cleft lip with or without cleft palate in a two stage genome scan of affected sib-pairs. Hum Genet 2000; 106:345-50.
- [10]. Suzuki K et al. Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpes virus receptor, in cleft lip/palate-ectodermal dysplasia. Nature Genetics 2000;25:427-430.
- [11]. Takahasi K et al. Nectin/PRR: an immunoglobulin like cell adhesion molecule recruited to cadherin based adherens junctions through interaction with Afadin, a PDZ domain containing protein. J Cell Biol 1999;145:539-49.
- [12]. Van der Woude A. Fistula labii inferioris congenita and its association with cleft lip and palate.American Journal of Human Genetics 19546:244-256.
- [13]. Kondo S et al. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nature Genetics 2002;32:285-289.
- [14]. Murray J. Gene/environment causes of cleft lip and/or palate.Clinical Genetics 2002;61:248-256.
- [15]. Ardinger HH et al. Association of genetic variation of the Transforming growth factor alpha gene with cleft lip and palate. American Journal of Human Genetics. 1989;45:348-353.
- [16]. Shaw G M et al. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. Am J Hum Genet 1996;58:551-61.
- [17]. MacKenzie et al. Hox 7 expression during murine craniofacial development. Development 1991a;113:601-611.
- [18]. Van den Boogaard MJ et al. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. Nature Genetics 2000;24:342-343.
- [19]. Jezewski PA et al. Complete sequencing shows a role for MSX1 in non syndromic cleft lip and palate Med Genet 2003;40:399-407.
- [20]. Lidral A C et al. Association of MSX1 and TGFB3 with non syndromic clefting in humans. Am J Hum Genet 1998;63:557-568.
- [21]. Prescott NJ et al. Maternal MTHFR genotype contributes to the risk of non syndromic cleft lip and palate.J Med Genet 2002;39:368-369.
- [22]. Yoshiura K et al. Characterisation of a novel gene disrupted by a balanced chromosomal translocation t (2; 19)(q11.2;q13.3) in a family with cleft lip and palate.Genomics 1998;54:231-40.
- [23]. Pauws E, Stanier P. FGF signaling and SUMO modification : new players in the etiology of cleft lip and/or palate. Trends Genet Dec 2007;23(12):631-640.
- [24]. Andreou AM et al. TBX22 missense mutations found in patients with Xlinked cleft palate affect DNA binding, sumoylation, and transcriptional repression.Am J Hum Genet 2007;81(4):700-712.
- [25]. Spritz R A. The genetics and epigenetics of orofacial clefts. Current opinion in pediatrics 2001;13:556-560.
- [26]. Wyszynski DF,Beaty TH. Review of the role of potential teratogens in the origin of human non syndromic oral clefts. Teratology 1996;53:309-317.
- [27]. Wyszynski DF et al. Maternal cigarette smoking and oral clefts: a meta analysis.Cleft Palate Craniofacial Journal. 1997b;34:206-210.
- [28]. Van Rooij IA et al. Smoking, genetic polymorphisms in biotransformation enzymes, and non syndromic oral clefting:a gene-environment interaction. Epidemiology 2001;12:502-7
- [29]. Castilla EE et al. Altitude as a risk factor for congenital anomalies. Am J Med Genet 1999;86:9-14.
- [30]. Romitti PA et al. Candidate genes for non syndromic cleft lip and palate and maternal cigarette smoking and alcohol consumption: evaluation of genotype-environment interactions from a population based case- control study of oro-facial clefts.Teratology 1999;59:39-50.

- [31]. Shaw DM, Lammer EJ. Maternal periconceptional alcohol consumption and risks for orofacial clefts. *J Ped.* 1999;134:298-303.
- [32]. Medical Research Council 1991 Prevention of neural tube defects: results of the Medical Research council vitamin study. *Lancet* 338:131-137.
- [33]. Shaw GM et al. Maternal periconceptional vitamins: Interactions with selected factors and congenital anomalies. *Epidemiology* 2002;13:625-630.
- [34]. Jugessur A, Lie RT, Wilcox AJ, et al. Cleft palate, Transforming growth factor alpha gene variants, and maternal exposures: assessing gene-environment interactions in case-parent triads. *Genet Epidemiol* 2003;25:367-374.
- [35]. Tolarova M, Harris J. Reduced recurrence of orofacial clefts after periconceptional supplementation with high dose folic acid and Multi vitamins. *Teratology* 1995;51:71-78.

**How to cite this article:**

Subhra Mandal, Prabir Mandal, Swapan Bhattacharjee. REMINISCENCING THE GENETICS OF OROFACIAL CLEFTING: A REVIEW. *Int J Anat Res* 2015;3(2):1063-1072.

**DOI:** 10.16965/ijar.2015.164

