JOURNAL OF ORAL MEDICINE SURGERY PATHOLOGY BIOLOGY

Cleft lip and cleft palate: A short communication

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Keywords: Cleft lip; Cleft palate; genetics; environmental; medial epithelial edge (MEE); medial epithelial seam (MES)

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How to cite this article: Shibu P, Anjana Arora, Aravind Meena Shanmugam and Ajmal Mohammed. Cleft lip and cleft palate: A short communication. Journal of Oral Medicine Surgery Pathology Biology. Dec-Jul 2016; 1(2): 80-91

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Running Title: Cleft Lip and Cleft Palate

Clinical Significance: The knowledge, of the factors responsible for clefts of the orofacial region, enables a physician to prevent its occurrence and treat it, before it manifests itself.

ABSTRACT

The facial clefts usually occur due to the disruption in the interrelationship of several cells, and the co-operation of the signaling pathways responsible for the formation of the head and neck. To understand the reason for the clefts it is necessary to know the background of the normal development of the head and neck region.

Aims and Objectives: The clefts of the lip and/or palate (CLP) are common birth defects having a very complex etiology. The cleft of the palate may occur in isolation or may be a part of a wide spectrum of teratogenic, chromosomal or Mendelian syndromes. Genetic and environmental factors responsible for the syndromic CLP (10-15%) have been identified, but the etiology of the more common (40-50%) non-syndromic (isolated) form remains poorly understood.

To identify the etiology, genomics and phenotype of clefts in animal modelsis the objective of this article.

Conclusion: Extensive etiopathologic identification of the orofacial clefts by genetic means is fundamental for treatment; as the ideal preventive measures could be implemented to rectify the mutations in the genes that have resulted in the orofacial clefts.

INTRODUCTION

One of the common congenital anomalies especially from the Asian subcontinent (about 1:400) and American Indians, is the cleft lip with or without cleft palate. The Europeans or the European descendants show less prevalence of the cleft, although, in the USA, there have been reports of nearly 1/ 750 live births due to the condition. Africans and African Americans have less prevalence of clefts; with a ratio of about 1:1500. [1]

Fogh-Andersen identified two basic types of orofacial clefts while performing his ground-breaking 1942 genetic study, namely, CL with or without CP (CL/P) and CP alone. [2]The cleft lip (CL), the cleft lip and palate (CLP), the cleft palate (CP) alone, the transversal, median, and the oblique facial clefts are the clefts affecting the orofacial apparatus. [3]The treatment for all types of clefts is multidisciplinary, due to which America spends nearly US$101,000 towards its treatment [4]

Social integration, speaking, hearing, and feeding may be some of the problems caused by CL/P. [1]The cleft lip and palate may also cause difficulty in hearing, respiration, ventilation in the middle ear, etc. due to the absence of functional structures involved. There may also be some disturbing, psycho-social, and educational difficulties. [1]

The cleft lip and palate is said to have both genetic and environmental etiologies. The use of the animal models for gene targeting technology and basic conventional techniques has helped in identifying the basic source of this malady.

The recent data obtained from the American Medical set-up indicated that in recent times most of the oro-facial clefts were: syndromic [(van der Woude syndrome), [2] and 22q11 deletion syndrome (sometimes known as DiGeorge or velocardiofacial syndrome)] [5]rather than non-syndromic; as compared to the observation of the earlier days.

Environmental factors for Cleft Lip and Palate

Environmental factors playing a role in occurrence of clefts may be

- ✓ Alcohol consumption,
- ✓ tobacco,
- ✓ Anti-convulsants [2]

- ✓ Teratogen exposure such as ethanol, thalidomide, phenytoin
- ✓ Amniotic banding, maternal diabetes and maternal folate deficiency [6] may exaggerate the risk of CL/P.

Folic acid consumption is said to have a protective effect on CL/P and neural tube defects. The National Birth Defect Prevention Network's recent data has indicated a reduction in the defects arising from the neural tube; from 5/10,000 to less than 2/10,000, after inclusion of folic acid in the diet. The finding suggests the relevance of this vitamin, the metabolism of this vitamin- including its uptake of proteins;thus making it a candidate gene in craniofacial development.

DISCUSSION

A multitude of complicated and genetically programmed events,cause the embryological development of the upper lip, palate, and nose. At about the 3rd and the 8th weeks of gestation, there is a fusion of 5 important facial processes: resulting inthe development of the lip between the 3rd and 7th weeks, and the development of the palate, between the 5th and 12th weeks.[7]

Development of the palate

The anterior 1/3rd of the palate develops from the posterior extension of the mesial, lateral and frontal processes forming a triangle that terminates at the sulcus terminalis; thereby separating the anterior and the posterior palates. The anterior 1/3rd is also called as the primitive palate or the primary palate. The posterior 2/3rd of the palate or the secondary palate arises from the maxillary process; which is derived from the 1st branchial arch. They arise in the form of the palatal shelves that initially grow vertically along the sides of the tongue- (E13.5-m; p.c.7wk-h), in the case of mice, or at about the 6-7th week of the embryo, in humans. These shelves then rise against force, above the tongue; and become horizontal, after the latter drops in the floor of the mouth following the forward and downward growth of the mandible- E14.0-m. These shelves then undergo continued growth, and appose at the midline at E14.5-m and eventually fuse- E15.5-m. These actions have been controlled by genes; that may be similar in mice and humans. [2]

While studying the molecular aspect of the fusion between the palatal shelves it has been said that the epithelium from both the process thins out, and forms a seam. The seam is called the midline epithelial seam (MES). The cause of this action is said to be either apoptosis, in which case the cells just die out, or an EMT (epithelial mesenchymal transition) whereby the epithelial cells become mesenchymal. Some authors are of the opinion that both these actions take place during the fusion.

Development of the upper and lower lip

The upper lip forms primarily by the fusion of the medial nasal process and the maxillary process. [1] This is called as the lambdoidal junction. The frontal process forms the nose and together with the medial nasal and lateral nasal process, it forms the nasal cavity and the nasal bridge. A fusion similar to that occurring in the palate takes place here. Instead of the 'midline epithelial seam (MES)' a 'nasal fin' forms. This nasal fin undergoes an epithelial mesenchymal transition (EMT) and becomes completely mesenchymal; unlike the palate where it remains lined by the epithelium.

Development of cleft lip-unilateral

Either the arterial supply is defectively resulting in the lack of fusion between the maxillary and medial nasal process

Or

The Orbicularis Oris (OO) inserts abnormally into the piriform aperture extending along the cleft.

Or

The muscle inserts into the nasal base on the cleft side, and the non-cleft OO fibers insert abnormally into the nasal spine and septum. This makes the base of the nose to spread laterally when the infant smiles. [7]

Development of cleft lip-bilateral, with or without palate

In bilateral cleft lip with or without cleft palate, the arterial network and musculature of the lateral elements parallel that of the lateral segment of the unilateral deformity. The abnormal insertion of the cleft lip musculature traverses along the cleft margin, up to the piriform aperture. [7]

Reasons for the occurrence of the cleft palate with or without cleft lip

Genes' that is defective in pathogenesis:

Include the growth factors and the signaling molecules mainly

- Sonic hedgehog (Shh)
- The transforming growth factor β (TGfβ) super family members such as the
    - Bmps or the bone morphogenetic proteins and
    - Tgfβs,
- Fgfs or Fibroblast growth factors and their receptors, namely the FgfR,
- Effectors and
- Targets.

In the case of the fibroblast growth factor; Fgf10 (Fibroblast growth factor 10) and FgfR2b (Fibroblast growth factor receptor 2b) are mutated and affect the initial formation of the palatal shelves.

Factors that hinder the elevation of palatal shelves

Jag2-Notch genes, by signaling,control the epithelial differentiation and do not allow the inappropriate adhesion of the palatal shelves to other oral epithelia. Hence when mutated, the anterior palate may fuse with the tongue and the posterior and middle palate to the mandible, thereby hindering the elevation of the palatal shelves.

An important factor in the palatal shelf elevation is the role of the extra-cellular matrix (ECM) of palatal processes as stated in some studies, and these findings are currently being accepted as important, in the palatal shelf elevation. The primary reason for this belief is the presence of the glycosaminoglycan, the hyluran, in the ECM. The glycosaminoglycan that is highly charged absorbs water, transforming into a hydrated gel that leads to the stretching of the ECM which; thereby results in the cleft palate.

mesenchymal derived Fgf10

↓

signals via its receptor FgfR2

↓

supports epithelial proliferation

↓

Induces expression of Shh in epithelium

↓

signals to the mesenchyme and

↓

stimulates cell proliferation.

Signaling activities are subject to tight spatiotemporal control

Cleft palate occurring due to failure of fusion of the palatal shelves

Primarily the **mutations in the** ***Msx1*** **and** ***Lhx8*** **genes**and the conditional **inactivation** **of** ***Tgfbr2*** **in CNC** (cranial neural crest) cells **or** ***Shh*** in the epithelium are all responsible for the retarded palatal shelf development as mentioned earlier, during embryogenesis. **The** ***Hand 2*** **gene**plays a role in the development of structures from the pharyngeal arches and if mutated may result in the lack of descent of the mandible downward and forward. This affects the descent of the tongue and the lack of fusion of the palatal shelves. [1]

The fusion of the palatal shelves results in the formation of the medial edge epithelial (MEE) seam and therefore it is a crucial step in palatal development. Initial adherence of the opposing shelves is by the adherens junction, and later the desmosomes play a role. The MEE seam is replaced by mesenchyme due to epithelial mesenchymal transition (EMT). If there is a failure in the EMT, a cleft palate arises.

The CP has a multifactorial etiology. The **TGFβ primarily the** **TGF β 3** plays an important role in regulating the palatal development. Its absence results in cleft palate.**TGFbeta1 and** **TGFbeta2** are also involved in growth. Therefore, it is essential to understand the TGFβ family signaling, to develop therapeutic strategies.

Mutations in TBX22 (T-box genes encode the various transcription factors included in the regulation of developmental processes) have been reported in families with X-linked cleft palate and ankyloglossia

Mutation of activin-βA causes a severe facial primordial development defect,

↓

which may result in the retardation of palatal shelf development and

↓

Complete cleft palate.

Msx1, Lhx8, Shox2, and *Osr2*, assume important roles in the palatal shelf growth.

Neurotransmitters (γ-aminobutyric acid (GABA)

|

Nervous stimulation

|

Absent

|

Cleft palate

Recent data has showed **Smad2 and 4** has been used by the TGF β 3 during palatal EMT, rather than using beta-catenin, to activate LEF1 (lymphoid enhancer binding factor), which was earlier considered to play a role in EMT. [9]For the fusion of the 2 palatal shelves it was found that the periderm of the two-layered embryonic epithelium begins to bog down slightly before these primordia fuse, thereby causing the basal epithelial cells to closely contact each other. Transmission Electron Microscope also showed that the basal epithelial cells were found to contact each other forming a midline seam that showed numerous desmosomes meeting each other. This was followed by the basement membrane disappearance and extension of filopodia from the basal surfaces of epithelial cells. This caused the space between them to enlarge, breaking the seam apart and causing the exposure of the underlying mesenchymal cells and hence a cleft. The absence of this entire action may result in a cleft lip and palate [10, 11]

After the completion of the fusion, the ossification of the palatal processes start. The cartilage development is controlled by the ***Sox9*** **gene** which also blocks the expression of a transcription factor, ***Runx2*****;** that is essential for osteoblast differentiation and bone formation. This condition is associated with cleidocranial dysplasia where the *Sox9* is mutant, and the Runx2 expression is not repressed thereby causing ossification to begin prematurely.

Premature ossification does not allow the palatal shelves to grow towards the midline and fuse with each other. The fusion of the secondary palate to result in the mid-palatine suture occurs before the formation of the mandibular condyle. **Genes such as Bmps, Fgfs, core binding proteins (Cbf), and hedgehog (Hh) proteins** interact with multiple signaling pathways to regulate the arrangement of the undifferentiated mesenchyme. [1]

The suture formedis found to be similar to that occurring in sagittal suture but not similar to that of the frontonasal suture. [12]Bone spicules from the suture margins along with the poorly calcified tissues form a scallop, at the sutural margin. The intertwining of the suture increases, as the age advances, and it extends in a posterior to anterior direction. The bone changes from cortical to cancellous. [13]This information is made use of in cases of V-shaped palate and cases of a cross-bite. The differentiation of the osteoblasts and chondroblasts is controlled by **genes Nell-1 and Bmp-7,** at the suture site. [1]

Pierre Robin sequence shows a deformational cleft palate, where the micrognathia or small mandible reduces the space for the tongue, and hence a prominent tongue (glossoptosis) may interfere with the palatal fusion, leading to the classic triad of glossoptosis, micrognathia, and an isolated cleft palate. [7]

Muscles and Occurrence of Cleft lip

It has been inferred that the **Orbicularis Oris muscle** has a role to play in a conversion of the mesenchyme in the nasal fin, an epithelial structure that arises due to the fusion of the (OO) medial nasal and maxillary process. The OO muscle fibers start formation by 12 weeks and reach completion by 16 weeks. Any defect in this formation will delay the replacement by the mesenchymal cells and result in a mild form of the cleft lip. This is represented by a slight scar along the philtrum or the mid portion of the upper lip. A high-resolution ultrasonography (USG) is used while identifying these changes. **Bmp 4** is found to control this event.

Cleft Uvula:

The mildest form of the cleft palate is the cleft in the uvula. The majority of the soft palate muscles is derived from occipital myotome cells, which enter the apparatus of the tongue and then shifts to the palate by carrying the nerve supply of the vagus with them.

Experiments on rats have shown that not all causes for the Cleft lip and Cleft palate are genetic. Environmental factors have been attributed to them too [1]

Among the environmental factors, diet plays an important role. **Vitamin E deficiency, overload of Vitamin A, folic acid, Vitamin B12, Biotin (Vitamin H), Vitamin U, choline, minerals such as Zinc** and maintenance of a **good BMI** during pregnancy, **smoking, and alcohol** are all factors that may cause the occurrence of the CL and CP. [3]

Table 1. Genes that have been implicated in cleft palate in the mice	
Genetic loss-of-function	**The proposed cause of cleft palate**
Activin-A	TEL

Activin receptor type II	TEL
αv integrins	Palatal shelves elevate but do not make contact
Bmpr1a (Alk3), nestin-Cre-mediated ablation)	Cell proliferation defects and a transformed anterior-posterior modeling
Bmp type I receptor (Alk2)Wnt1-Cre-mediated ablation	TEL
Different compound mutants of Alx4and Cart1	TEL
Dlx1	TEL
Dlx2	TEL
Dlx5	TEL
Foxc2 (previously Mfh1)	Craniofacial defects similar to those in Gli2mutants TEL
Foxe1(previously Titf2)	Palatal shelves elevate but fail to fuse with each other
Foxf2	TEL?
Gli2	TEL
Gli3 xtJ	TEL
Hic1	TEL
Hoxa2	TEL
Myf5; MyoD	Absence of fusion of primary and secondary palate
Lhx8	Elevation of palate which fails to make contact

Ryk	TEL
γRAR	TEL
Shh (K14-Cre-mediated ablation)	Proliferation is altered, and there is an increase in the apoptosis within the palatal shelves
Tgfb2	TEL
Tgfb3	Absence of fusion of the palatal shelves
Tgfbr2 (Wnt1-Cre-mediated removal)	Proliferative defects of palatal mesenchyme
Tgfbr2 (K14-Cre-mediated removal)	Impaired or a partial palatal fusion which may be due to lack of apoptosis and there may also be a persistent proliferation of the MES/MEE
Tgfbr1 (Alk5)K14-Cre-mediated removal	Impaired or partial palatal adhesion and fusion occur probably due to a decreased MEE filopodia and a lack of apoptosis of the MES
Tgfbr1 (Alk5)Wnt1-Cre-mediated removal	Increase in the apoptosis and cell proliferation within the palatal shelves. Anomalies in other craniofacial structures may also give rise to CP.
Msx1	Proliferation in palatal shelves is altered
Osr2	Impairment in the mediolateral patterning and proliferation in the palatal shelves
p63	Alteration in the epithelial–mesenchymal interactions and there may be palatal shelf epithelial differentiation defects
Pax9	TEL
Pitx1	TEL

Pitx2	Elevation of palatal shelves takes place, but they may be hypoplastic
Prx1 (previously Mhox)	TEL
Prx1; Prx2	TEL
Rae28	TEL
Satb2	Defects in the patterning of the developing palate. Anomalies of other craniofacial structures may also give rise to the CP.
Sall3	Soft palate and epiglottis are hypoplastic
Shox2	The anterior portion of the secondary palate is cleft due to atypical proliferation and apoptosis.
Sim2	Hypocellular palatal shelves that exhibit an increased extracellular glycosaminoglycans
Sox9haploinsufficiency	TEL
Tbx1	TEL
Apaf1	Fusion of palatal shelves fails due to failure of apoptosis
Gad1	Delayed elevation of palatal shelves
3b-hydroxysterol-D7-reductase	Palatal shelves are hypoplastic
IKK1	Cleft palate
p57kip2	TEL
Viaat	TEL
Extracellular matrix components	
Col2a1	TEL
Perlecan	TEL

Insertional mutations	
CASK (loss-of-function)	TEL
Dlg (loss-of-function)	TEL
Tbx10 (gain-of-function). Dancer mutation	Ectopic expression of Tbx10 causes cleft lip and cleft palate
p23-Tbx10 in transgenic mice	Similar cleft lip and cleft palate to that of Dancer mice
TEL Indicates cleft palate secondary to other cranio- maxillofacial bone defects and by the caused by obstruction of the tongue.	

Prenatal Diagnosis and Prevention:

A prenatal diagnosis is crucial for prevention of clefts as

- The entire data is available during a prenatal diagnosis to allow individuals and couples to make decisions about childbearing.
- For this, the ultrasound machine used should be of high standard. The ultrasonographer must be experienced. The ideal time for the examination should be around 18-20 weeks of pregnancy. The position of the baby is also important during the procedure.
- The American College of Medical Genetics and the Centers for Disease Control and Prevention have recommended a vitamin supplement of 400mg of folic acid, daily, for all pregnant women, to minimize the risk of neural tube defects, spina bifida, and the probable cleft lip and palate in the baby.

Cleft Prevention can be achieved by:

1. Fetal surgery:
   This procedure is done by the feto-endoscopic approach. The procedure has the following advantages:
   - scar-less fetal wound healing and bone healing without callus formation, which would also allow a better/normal maxillary growth, and
   - a significant decrease in fetal and maternal morbidity.
2. Genetic counselling

If a family has a risk for the cleft, then a genetic screening is mandatory.

Genetic Evaluation may include-

- The screening to observe that the cleft is non-syndromic.
- Enquire whether other relatives have similar defects and how closely they are related to the patient,
- The type and severity of the cleft.

- Testing in a laboratory for Chromosome tests (karyotypes) and molecular testing. [14]

The **treatment strategy** for cleft lip and palate include:

- Surgery – surgery for correction of cleft lip is usually carried out at 3-6 months for repairing a cleft palate it is usually at 6-12 months. [5]

  There has been no consensus regarding the perfect timing of lip repair. Surgery in the early neonatal period has been suggested by some authors citing the benefits such as minimum scar appearances and nasal cartilage adaptability. So that anesthetic risks to the baby are reduced it has been suggested to follow the rule of 10s:

  - ✓ When the child's hemoglobin level is 10 g,
  - ✓ the weight of 10 pounds and
  - ✓ is aged 10 weeks.

  To minimize the anesthetic risks, the lip reconstruction is deferred to about 2-4 months of age, so that the patient can tolerate the stress of surgery; and lip structure is largeenough to allow a careful reconstruction. [15]

- Provide feeding support
- Monitor, the baby, 's hearing
- Speech and language therapy
- Good maintenance of dental hygiene and orthodontic treatment

NOTE:

1. The occurrence of a second child with a cleft lip or palate occurs only with a probability of around 2-8%.
2. The chance of a child born with a cleft lip or palate parent with a similar cleft would be around 2-8%
3. The chances are much higher in cases of syndromic rather than a non-syndromic variant.
   For example, there is a 50% chance of CL/P in a child born to parents with 22q11 deletion syndrome (Di George syndrome). [5]

Footnotes:

Conflict of interest: No conflict among the authors about the article
Source of funds*:* No funding received

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