The study of embryology in the last century has helped us to understand the development of the embryo, it is only in the last few years that has led us to understand the significance of homeobox genes in the proper development of the head and facial region of the vertebrate. Nowadays it is possible to study how these particular homeobox genes act on their natural environment, how they are expressed phenotypically and what will be their side effects if their expression is blocked. Without proper knowledge of gene activity and their relevant cellular signal transduction pathways, elucidating the mechanism that controls development would be impossible. With the advancements in research, now it is possible to explain the cause of craniofacial defects and also the magnitude of defect if a homeobox gene is missing. Therefore, homeobox genes are of utmost important for the clinician to have a superior understanding regarding these abnormal developments.

HOMEOBOX GENES

In 1983, Walter J Gehring along Amy Weiner and Mathew Scott discovered Homeobox genes at the University of Basel, Switzerland and Indiana University Bloomington respectively. They have 180 base pairs long DNA sequence which are found within genes that are involved in the morphogenesis in plants, animals and fungi. A homeobox gene encodes a 60-amino acid helix loop which binds a DNA helix within an encoded transcription factor. It has a homeodomain protein which act as transcription factors that activate or inhibit the transcription of other genes.

The homeobox genes were found initially in the Drosophila melanogaster and it was clustered in two segments namely Antennapedia and bithorax on the chromosome no 3 and hence was known as HOM-C complex. These complexes gave the molecular representation of the anterior and posterior embryonic axis of the developing fly. A search began to find these similar genes in vertebrates. The first time that any vertebrate homeobox ever cloned was done in frog Xenopus Levis and was soon followed by cloning of the same in mouse. These vertebrate genes are called HOX genes which involves in the proper development of the head and facial region of the vertebrate. Nowadays it is possible to study how these particular homeobox genes act on their natural environment, how they are expressed phenotypically and what will be their side effects if their expression is blocked.

**TABLE 1: HOMEBOX GENE CLUSTER IN HUMAN**

<table>
<thead>
<tr>
<th>Homeobox</th>
<th>Number Of genes</th>
<th>Chromosome Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOX A</td>
<td>11 (1-7, 9-11, 13)</td>
<td>7P</td>
</tr>
<tr>
<td>HOX B</td>
<td>10 (1-9, 13)</td>
<td>17q</td>
</tr>
<tr>
<td>HOX C</td>
<td>9 (4-6, 8-13)</td>
<td>12q</td>
</tr>
<tr>
<td>HOX D</td>
<td>9 (1,3,4,8-13)</td>
<td>2q</td>
</tr>
</tbody>
</table>

The expression of these HOX genes can be seen along the dorsal axis within the central nervous system from the anterior region of hindbrain through the length of spinal cord. When the neural crest cells migrate from the rhombomeres into specific branchial arches, it retains a specific Hox code, which details shape and outline of different derived regions of head and neck. It is engrossing to note that neural crest cells destined for first branchial arch do not express Hox genes related to homeotic homeobox but relies on its subfamilies.

The homeobox genes have been classified in different ways into subfamilies, superfamilies, classes, subclasses, or groups but there has been inconsistency the terms. The subfamilies of Homeobox genes are, - muscle segment (Msx), distal less (Dlx), orthodenticle (Otx), goosecoid (Gsc), Bar class (Bazx), paired-related (Pxr, SHOT) & LIM class. All this homeobox genes plays important role in craniofacial patterning and morphogenesis.

**MUSCLE SEGMENT (MSX)**

Vertebrate Msx genes are homologous to Drosophila muscle segment homeobox gene. These genes are expressed at multiple sites during embryonic development of vertebrates. Msx genes are essential for normal development of craniofacial skeleton, limb and ectodermal organ. Improper expression of it can lead to morphological abnormalities in human and mice.

Mammalian Msx gene family consists of 3 physically unlinked members named as Msx1, Msx2 and Msx3. Among these Msx1 and Msx2 plays an important role in development of craniofacial complex, including development of teeth. Msx1 and Msx2 are expressed in suture mesenchyme and duramater in development of skull. The expression of Msx1 extends into the postnatal stages of skull morphogenesis while Msx2 expression is decreased after birth.

Msx1 expression is found in cap stages of dental papilla and follicle during tooth morphogenesis. Msx1 has also been expressed in developing palate and anterior portion palate shelves. Failure of expression of Msx1 leads to growth impairment of anterior palate and region forming cleft palate. In the development of face, combined expression of Msx1 and Barx1 in the mandibular mesenchyme specifies patterning events including tooth formation.

The expression of Msx2 is detectable at 7.5 weeks of human embryonic development. Expression of Msx2 gene is essential in initiating development of the orofacial skeleton such as the mandibular and maxillary bones, Meckle's cartilage, and tooth germs. Msx2 expression is found can be detected in both the epithelial and mesenchymal tissues of the developing tooth germs. The earliest marker of asymmetric expression is in the buccal distribution within the invaginating dental lamina of molar tooth germs. In the cap stage, Msx2 expression is prominently seen in the components of the enamel organ as well as in the inner enamel epithelium. With the commencement of the bell stage, Msx2 expression is lost from the inner enamel epithelium because they differentiate into the ameloblasts. A strong expression of Msx2 is detected in the odontoblasts and subodontoblastic regions of the dental papilla. Therefore, a spatial and temporal expression of Msx1 and Msx2 genes appear to correlate with crucial aspects of craniofacial morphogenesis.
Missense mutation of Msx1 gene causesagenesis of second premolars and third molars. Non-sense mutation of Msx1 causes Wiskit syndrome, which causes tooth genesis and dysgenesis.\(^\text{13}\) The deletion of Msx1 locus on chromosome 4 results in congenital human syndrome named as Wolf-Hirschhorn syndrome (WHH). The manifestations seen are midline fusion defects, ear defects, supernumery teeth and microcephaly. Other manifestations may include tooth agenesis, nail dysgenesis, mental retardation, cardiac defects and a variety of skeletal deformities.\(^\text{12}\) Boston type craniosynostosis was caused by a mutation in the MSX2 gene. It is characterized by premature fusion of skull bones together with certain orofacial bone abnormalities.\(^\text{15}\)

**DISTALESS GENE (DLX)**

As the name suggests, Distal-less is required for distal limb development. Six Dlx genes are known, each in mice and humans. The Dlx genes are expressed within the branchial arches in nested patterns along the proximal-distal axis of maxillary and mandibular primordia, apart from the facial midline and also in a few cells of the medial nasal process. The mutation of Lhx6 and Lhx7 do not lack absence of nasal structures and do not develop permanent clefts in the frontalonasal region. Reduced maxillar growth is characterised by morphological abnormalities of the frontonasal region, where upper incisors develop.\(^\text{20}\)

**PAIRED RELATED HOMEBOX GENE**

Prx1 and Prx2 are closely related members of paired related (Prx) family of homeobox genes. Mice experiments have shown that Prx1 is expressed in first and second brachial arch, mainly in frontonasal process, mesenchyme of maxillary and mandibular process.\(^\text{19}\) Mutated Prx1 and Prx2 genes caused defects in the external, middle and inner ear, reduced or loss of skull bones, a decreased or sometimes cleft mandibular and limb abnormalities.\(^\text{21}\)

Another paired related homebox gene Pax9 has been associated with selected tooth agenesis.\(^\text{5}\)

**GOOSECEOID (GSC)**

A homeobox-containing gene named Gsc was originally isolated in *Xenopus* from a dorsal blastopore lip.\(^\text{17}\) Experiments in mice have found that Gsc transcripts are detected in mandible and tongue, eustachian tube and the base of auditory meatus that form the nasal chambers, and proximal limb buds and ventrolateral body wall that form the proximal limb structures and ventral ribs. Thus, Gsc may also be required in mouse during later embryogenesis for craniofacial, limb and thoracic development.\(^\text{18}\) In wild type mice, at later stages of development in the osteogenic mesenchyme of mandible and tympanic ringbone, Gsc transcripts have been detected. Gsc when mutated resulted in formation of hypoplastic mandible with lack of coronoid and angular process along with several defects on other bones such as maxilla, palatine bone and pterygoid plates.\(^\text{19}\)

**BAR CLASS GENE (BARX)**

Barx genes (Human ANTP class NK1 subclass) forms the transcription factor that exhibits regional expression within the mesenchyme of the first branchial arch. Barx1 appeared in the mesenchyme of the mandibular and maxillary processes, whereas Barx1 and Barx2 showed complementary patterns of expression. The expression of Barx1 is found intense throughout lateral and caudal region of head, particularly in diencephalon region. Barx2 transcripts were restricted to the head region particularly in telencephalon, frontonasal region and mesencphalon. Both Barx1 and Barx2 showed diffused expression in the limb mesenchyme, spinal cord and dorsal root ganglion.\(^\text{20}\) Mutation of Barx1 and Barx2 may result in development of cleft lip and palate.\(^\text{21}\)

**LIM CLASS GENE (LHX)**

LIM/homeodomain genes are characterized by the association of two LIM domains with a homeodomain which encode transcription factors. These two homeodomains are Lhx6 and Lhx7 and they are also termed Lhx6 and Lhx7. In the first brachial arch, the expression domains of Lhx6 and Lhx7 were highly overlapping which were present in the palatal shelves, the developing tongue and in vibrissae follicles. Higher levels of expression for Lhx6 and Lhx7 were found in mesenchymal cells condensed around the molar tooth buds. The mRNA of Lhx6 was localized in the proximal parts of the maxillary and mandibular processes. Lhx7 transcripts were detected mostly in the proximal-distal axis of maxillary and mandibular primordia, apart from the facial midline and also in a few cells of the medial nasal mesenchyme.\(^\text{22}\)