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HOMEOBOX GENES AND OROFACIAL DEVELOPMENT -A REVIEW

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ABSTRACT

Body organization requires cell differentiation and morphogenesis which are controlled by gene expression. Gene expression is defined as an activation of a gene that results in production of polypeptide/protein that can activate/deactivate other genes with the influence of transcription factors (growth factors). Every organism has a unique body pattern because of the influence of Homeobox genes. Hox genes are critical regulators of embryonic development, being involved in formation of the Skeleton and Limbs, Craniofacial morphogenesis, and in development of the Central Nervous System, Gastrointestinal and Urogenital tracts. Aberrant expression of Hox genes have been described in developmental abnormalities and solid tumors as well as in hematologic malignancies. The purpose of this review article is to explore the growth and formation of the head and neck from embryological development through puberty in order to understand how this knowledge is necessary for the development.

KEYWORDS:

INTRODUCTION

A gene is a sequence of DNA nucleotides: Adenine, Cytosine, Thymine, or Guanine. These nucleotides encode for specific traits and features. Distinct sections of nucleotides are read and turned into a chain of RNA, ribonucleic acid, through a process known as transcription.[1]

Genes code for every Genetic difference between species and individuals, from blue eyes to skin color in humans to the color and number of petals in flowers.[2]

Thomas Hunt Morgan, who received the Nobel Prize in Physiology or Medicine in 1933 for discovering the role of genes in heredity, first theorized that there were genes that regulate body formation.[2]

Human genes called the Hox genes have the same pattern of organization, follow the same order of gene arrangement, their expressions and functions are also in sequences as observed in Drosophila. The genes HOX A, HOX B, HOX C and HOX D are arranged on four different chromosomes 7, 17, 12 and 2. Homeobox genes are characterized by a conserved 180-bp DNA sequence coding for a 60-aminoacid DNA-binding domain called the ‘‘**homeodomain.**’’[3]

The role of Homeobox genes were discovered independently by Walter J Gehring in 1983 working at the University of Basel, Switzerland and Matthew Scott and Amy Weiner who were working at Indiana University Bloomington.[4]

Edward Lewis was the first person to identify the homeotic genes in the fly Drosophila melanogaster, which help in controlling the developmental response of groups of cells along the body's antero-posterior axis.[5]

In the fly, the homeotic genes are predominantly clustered in two regions-Antennapedia and Bithorax-on chromosome 3 which together make up a single HOM – C complex.[4,6]

The first vertebrate homeobox was cloned in frog Xenopus Levis and was soon followed by cloning in mouse. The vertebrate genes are called HOX genes and consist of 39 genes both in human and mouse.[7]

The neural crest cells destined for first branchial arch does not express Hox genes related to homeotic homeobox butrelies on its subfamilies.[8]

The subfamilies of Hox genes, which are of particular interest in Craniofacial patterning and morphogenesis include - muscle segment *(Msx)*, distal less *(Dlx)*, orthodenticle *(Otx)*, goosecoid *(Gsc)*, Bar class*{Barx)*, paired-related *{Prx*, SHOT) & LIM homeobox.[3, 4, 9]

The expressions of these genes are mediated through two main groups of regulatory proteins - Growth factor

family and steroid/thyroid/retinoic acid super family. The vehicles through which Hox gene information is expressed for the regulation of the growth process include fibroblast growth factor(FGF), Transforming growth factor a and b (TGF a and TGF b) and bone Morphogenetic protein 4 (BMP 4). Mutations of fly homeobox genes can lead to bizarre homeotic transformations, where one segment can even assume the phenotype of other. Thus, the most complex part of CNC migration is the understanding of how the combinations of Hox genes are expressed to specify the fate of the cells.[10]

Tooth development is complex phenomenon between epithelium and ectomesenchyme, which is being governed by the set of these complex genes.[11]

There are two classes of homeobox genes:[11]
Class1 genes called Hox genes share a high degree of identity in their homeodomain.

Class2 genes share a low degree of identity their homeodomain.

This paper updates a review on the importance of cell condensations in skeletal development that we published in 1992.

Human Hox Gene Abnormalities[12]
A mutation in the *HOX A13* gene causes Synpolydactyly (SPD). In this disorder digits on the hands and feet can be fused together or additional digits may be present. This rare condition was first described in 1916 in an Australian family who faced extreme prejudice and stigma from their community, despite the fact that patients do not suffer from any other physical or mental impairment.

Hox Genes and Oncogenesis[13]
Hox genes has significant role in Oncogenesis, the formation of cancer. The protein products of *Hox* genes act as transcriptional factors that promote carcinogenesis, the initiation of cancer formation, by being unregulated or down regulated in cancer cells.

Muscle Segment Box (*Msx1, Msx2*)
The *Msx* homeobox gene (Human ANTP class NKL subclass) family plays a crucial role in the development of craniofacial development.[5]

Three subtypes are present *Msx 1, Msx 2* and *Msx 3*; in which *Msx 1* and *Msx 2* are expressed in craniofacial development including the brachial arches especially in the region of epithelial mesenchymal organogenesis including the developing teeth. Both the *Msx 1* and *Msx 2* are expressed in the sutural mesenchyme and duramater but while the expression of *Msx 1* continues at a higher level in the postnatal stages of skull morphogenesis as well the level of *Msx 2* expression declines.[14]

During the tooth development *Msx 1* is expressed in the bud stage and in the morphogenetic cap stage. *Msx 1* becomes localized in the mesenchymal cells of the Dental follicle and the papilla and *Msx 2* becomes more expressed in the enamel organ besides expressing in dental papilla and the follicles.[15]

Msx 2 plays role in the expression in the formation of the extracellular matrix and ameloblast differentiation .[16]

In the late stage of morphogenesis, *Msx 1* expression is absent in root sheath epithelium indicating that *Msx* does not plays a role in root morphogenesis.[17]

Msx1 also plays an important role in the development of the palate specially the anterior portion of the palatal shelves.[18]

Msx1 is co- expressed with *Msx2* at the site of epithelial - mesenchymal interactions. Its expression is increased in cap stage in enamel knot, inner enamel epithelium and Dental papilla whereas *Msx2* expressed in odontoblasts, cuspal formation, and root initiation.[11]

Wolf-Hirschhorn syndrome (WHS) is a congenital human syndrome resulting from a deletion of *Msx1*locus on chromosome 4. It manifests as midline fusion defects, ear defects, supernumery teeth and microcephaly. It may also cause tooth agenesis, nail dysgenesis, mental retardation, cardiac defects and variety of skeletal deformities.[19]

***Msx-Dlx* interaction**[20]
Msx and Dlx genes are those that are expressed early enough in the CNC cells to specify its differential fate as they populate the branchial arches and subsequently shape the skull and its associated sensory structures.

Msx expression is restricted to cells that are proliferating or dying whereas *Dlx* expression is found in regions undergoing differentiation or are capable of doing so.

Accordingly *Msx* and *Dlx* proteins appear to have opposing transcriptional properties – *Msx* proteins function as transcriptional repressors whereas *Dlx* proteins act as activators.

The various biological and cellular activities of both *Msx and Dlx* genes are mediated through the homeoproteins they encode, which can bind to specific DNA sequences.

The preferred binding site for *Msx1*, *Msx2*, *Dlx3* and *Dlx5* are essentially the same –the T- A- A- T sequence. However the competition for DNA binding site does not appear to represent primary mode of regulation of neural crest cells as *Msx* proteins repress transcription through protein - protein interaction mediated by the homeodomain. Although this process may occur, *Msx1* and *Msx2* each can form a protein complex with *Dlx 2* and *Dlx 5* and this heterodimer formation has a

neutralizing effect on transcriptional activities of both the *Msx* and *Dlx* proteins.

Role of *Msx* - *Dlx* in Tooth Development

Msx and *Dlx* genes participate in tooth development by reciprocal epithelial mesenchymal signaling. As the epithelium of the prospective oral cavity thickens to form the Dental lamina, the expression of *Msx2* localizes.[20]

Activation of *Msx1, Msx2, Dlx1* and *Dlx2* in Dental mesenchyme occurs in response to BMP 4 and FGF signals from the overlying epithelium. The BMP 4 mediated induction of *Msx1*expression and subsequent *Msx* dependent activation and maintenance of BMP4 expression in the Dental mesenchyme are the key steps in conferring odontogenic potential to this tissues.[20,21]

In humans, a point mutation in*Msx1* homeobox results in agenesis of second premolars and third molars in affected individuals.[22]

Goosecoid (*Gsc*)

Goosecoid (Human PRD class) encodes a protein that acts as a transcription factor and was previously isolated from Xenopus.[5]

Mutants exhibited a hypoplastic mandible with lack of coronoid and angular process along with several defects on other bones like maxilla, palatine bone and pterygoid plates.[6]

Distal-less (*Dlx*)

Distal-less genes (Human ANTP class NKL subclass) as the name suggest requires for the development of the limbs. There are at least six *Dlx* genes in humans and named as *Dlx 1* to *Dlx 6*. Similar to *Msx, Dlx* genes are primarily expressed in regions that give rise to highly derived or vertebrate specific structures.[5]

Dlx genes are mainly expressed in branchial arches incomplete spatio – temporal patterns. *Dlx1* and *Dlx2* are expressed throughout the first and second arches whereas expression of *Dlx 3*, *Dlx 5* and *Dlx 6* are restricted to a more distal location. In contrast to the *Msx* genes, the expression of *Dlx 1* and *Dlx 2* in the maxillary and mandibular arch mesenchyme is restricted to the region where the future molar teeth will develop specially for the ectodermal and mesenchymal compartments of the developing tooth.[6,22]

Barx genes

Barx genes (Human ANTP class NKL subclass) consist of transcription factor that exhibits regionalized expression within the ectomesenchyme of the first branchial arch.[5]

As tooth development proceeds, Bar expression becomes more localized exclusively to the mesenchymal regions around the developing molars to produce specific folding pattern of the dental epithelium that produce molar cusps.[5,20]

Barx genes also play a role in the development of central nervous system and are expressed in the telencephalon, diencephalon, mesenchephalon, spinal cord and in the cranial and dorsal root ganglion. *Barx 1* and *Barx 2* show complementary patterns in their expression. *Barx1* appears in the mesenchyme of the maxillary and the mandibular process where as Expression of *Barx 2* is most prominent in mantle layer, where post- mitotic neurons are located, the palatal floor and dorsal root ganglia, mutations of which can produce cleft of secondary palate hence, the association of *Barx 1* with *Barx 2* in the possible etiology of cleft lip and palate.[23]

LIM HOMEOBOX DOMAIN (*Lhx*)

Lim genes (Human LIM class) have been found to play an important role in the cell type specification and differentiation during embryogenesis.[5]

These are found to be related with the expression of the ectomesenchyme of the maxillary and the mandibular process and also suggested to control patterning of the first brachial arch.[14]

Experiments have shown that homeodomain proteins of Lim genes are important for craniofacial development and patterning of mammalian dentition.[24]

Lh x6, Lhx 7 are the earliest mesenchymal markers of tooth development.[11]

***Prx* genes (Pair related gene)**

Prx1 and *Prx2* are closely related members of *Prx* family of homeobox genes. At 9.5 days post coitum, *Prx1* is expressed in central nervous system derived mesenchyme of Fronto nasal process, first and second branchial arches and group of cells that form maxillary process. Its expression decreases once differentiation is initiated. *Prx1* in combination with *Prx2* is essential to stabilize and maintain cell fates in craniofacial mesenchyme.[5]

Another paired related homeobox gene -*SHOT*- has been described recently. *SHOT was* mapped to human chromosome 3q25-q26 and OG -12 with a syntenic region on chromosome 3. This chromosomal region is involved in development of Cornelia-de- hange syndrome characterized by mental retardation and microcephaly, cleft palate, abnormally situated eyelids, nose and ear deformities as well as heart and limb defects.[25]

Pax a family of 9 genes. Regulators of organogenesis, maintains pleuripotency of stem cell. It is the earliest mesenchymal gene which localizes site of tooth bud.[11]

Sonic Hedgehog (*Shh*)
In craniofacial development, *Shh* is first expressed in axial mesendoderm, mutations of which lead to abnormal patterning of neural plate resulting in holoprosencephaly and cyclopia.[26]

Later in facial development, *Shh* is expressed in the ectoderm of frontonasal process (FNP) and maxillary process (MXP). Transient loss of these signals can produce collapse of the facial midline and hypotelorism. Disrupting *Shh* signaling in FNP and MXP leads to interruption in their outgrowth, resulting in clefting between the primordia; cleft lip/palate.[3, 27-28]

It has been shown that pharmacological doses of retinoids and cholesterol analogues induce facial dysmorphogenesis in part through their misregulation of *Shh* signaling.[28]

Humans with cholesterol metabolism disorders - Smith - Lemli-Optiz syndrome exhibit holoprosencephaly and micro-cephalic characteristics, which may result from an inability of target cells to respond appropriately to *Shh*.[29]

It has also been shown that *Shh* and proteins in *Shh* signaling pathways such as *Gli1,BMP2* and *Ptc* play key roles in regulating patterned outgrowth of the *FNP* and *MXP* and specfying the mediolateral axis of the face.[27]

Shh expressed in Bud stage, cap stage (enamel knot) and in hertwigs epithelial root sheath for root formation.[11]

Endothelin, dHAND and eHAND[29]
The endothelin family of signaling peptides has been implicated in development and migration of neural crest cells. Appearance of marked craniofacial and cardiac abnormalities similar to those of CATCH -22 syndrome (Cardiac defects, abnormal facial features, Thymic hypoplasia, Cleft palate, Hypocalcaemia) which is associated with chromosome-22 deletion.

The other two novel b HLH (basic helix-loop- helix) proteins – *d HAND* and *e HAND* are co-expressed with endothelin-1 in developing branchial arches, aortic arch arteries and cardiac mesoderm.

In endothelin null embryos, both these proteins are down regulated resulting in hypoplasia of first and second branchial arches. *Msx1*, which is implicated in growth of branchial arches, was also found to be undetectable in the mesenchyme of *d HAND* null branchial arches, thus suggesting the regulatory role played by endothelin 1 in stimulating mesenchymal expression of *d HAND* thus regulating *Msx1* expression in growing distal branchial arch.[29]

Lymphoid Enhancing Factors (*Lef*-1)
Lef-1 gene is involved in Wnt signaling pathway and it may function in hair cell differentiation and follicle morphogenesis. Expressed in condensing mesenchyme in bud stage &adjacent basal cells of epithelium. It is Essential in initiation and cytodifferentiation.[11]

Fate of Neural Crest Cells[30]
The individual neuroectodermal cells are multipotent which are imparted positional identity by the action of homeobox gene and other transcription factors. Later this regulative capacity is lost leading to the cessation of migration of neural crest cells mainly through:· Adhesion changes - Down regulation of certain integrins and re-expression of NCAM, N - cadherin and E-cadherin.

Decrease in intercellular space through decline in levels of hyaluronic acid. Decrease in extracellular material molecules such as fibronectin, reducing the availability of migratory substrate. With time, the precursors become progressively restricted to form *NCC* derivatives and eventually to individual phenotypes.

CONCLUSION
Research into Hox genes has opened the door to greater human understanding of genomics, evolution, craniofacial, limb, nervous system development and cancer.

Many human syndromes and genetic abnormalities have now been attributed to defects in individual genes, which lose its transcriptional ability, thus its control over neural crest cell migration.

With the advancements in understanding the role of genes, it is now possible to explain the cause of craniofacial defects and there magnitude if a particular geneis missing.

It is therefore of utmost importance for a clinician to have an understanding of the underlying genetic mechanism to facilitate proper diagnosis and therapeutic intervention.

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