

Review Article

The Role of Genetics in Craniofacial Biology

Sperber GH^{1*†} and Sperber SM^{2†}¹Faculty of Medicine & Dentistry, University of Alberta, Canada²Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA

*Corresponding author: GH Sperber, Faculty of Medicine & Dentistry, University of Alberta, Canada

†These authors contributed equally to this work.

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Introduction

Of all the billions of extant human faces, no two are exactly alike, even between monozygotic identical twins. This astonishing diversity is to be traced to the three billion nucleotides contained in the human genome and the epigenomic variations imposed during development, maturation and ageing [1]. Understanding the relationship between genotype and phenotype is one of the key challenges of developmental biology. The increasing integration of genetics with embryology has led to the coining of “embryogenetics” as a new discipline of developmental biology [2].

With the transformative advent of genetic identification of morphogenesis, an added new discipline of “molecular morphology” is emerging that is applicable to the study of craniofacial development. The revelations of form, shape, size, formation and malformation revealed by genetic perspectives on development, open up new avenues of research, of which stem cell recognition is one aspect [3,4]. The synthesis of genetics and embryology into the new embryogenesis conjugation is the purpose of this review. The rapidly progressing field of biogenesis encompassing genetics and genomics is being advanced by the new technologies of optogenetics, optical projection tomography, 3D imaging, Magnetic Resonance Imaging (MRI), visualsonics, morphometrics and six dimensional X-ray tomography [5]. Nano-particle tracking stem cells will reveal the dispersion of these embryonic cells. New genes and genetic pathways have been expanded upon as rare diseases are increasingly scrutinized by whole exome sequencing [6]. The role of hormones in influencing facial shape is undergoing increasing scrutiny [7]. The explosion of the new targeted genome Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR/Cas9) technology that allows specific genetic engineering- deletion, insertion or replacement of genes, heralds an exciting period of developmental and evolutionary genetics [8].

Craniofacial Embryogenesis

The craniofacial complex encompasses the most diverse conglomeration of tissues of any part of the body [9]. The intricate assembly of specialized organs and tissues comprising many of the primary sense organs, the central and peripheral nervous systems and the skeletomuscular constituents of the head and face make the embryology particularly challenging. The bones of the skeletal system arise from two separate evolutionary sources, the exoskeleton

Abstract

A review of recent advances in genetic identification of the embryonic components of the craniofacial complex. The revelations by the technical achievements of optical projection tomography, 3D imaging, Magnetic Resonance Imaging (MRI) and geometric morphometrics provide insights into gene expression patterns occurring in developmental morphogenesis. Revelations of facial evolution are revealed.

Keywords: Genetics; Craniofacial; Morphogenesis; Embryogenetics; Evolution

(desmocranium) forming intramembranously and the endoskeleton developing endochondrally. Further complicating craniofacial embryogenesis is the intermingling of the foundational germ layers of ectoderm, neural crest, endoderm and mesoderm, creating the viscerocranium and the chondrocranium. This conglomeration invokes a great multitude of genes in facial fabrication, of which only a select few are schematically portrayed for their ontogenetically ephemeral expression patterns in regions of the face of a seven-week-old human embryo (Figure 1).

PAX6: Paired Box 6. Encodes DNA transcription factor for eye development; affected individuals display eye aplasia irides or aniridia and is expressed in the nasolacrimal duct region in the Figure [10].

WNT: Wingless type. Nineteen genes for differentiation and proliferation; Ubiquitous in craniofacial tissues; responsive to stem/progenitor cells [11]. They are expressed in the upper facial region in the Figure.

DLX: Distal-less. Homeobox gene family involved in craniofacial morphogenesis [12]. Expressed in the cheek and mandible in the Figure.

BARX: Homeobox gene plays a role in tooth development and craniofacial mesenchyme of neural crest origin [13,14]. It is expressed in the cheek region in the Figure.

GSC: Goosecoid homeobox: Gene located at 14q32.1. A protein-coding, transcription factor in craniofacial development [15].

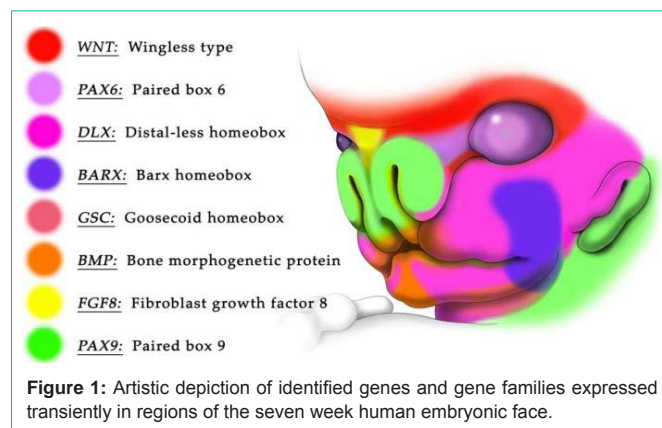


Figure 1: Artistic depiction of identified genes and gene families expressed transiently in regions of the seven week human embryonic face.

BMP: Bone Morphogenetic Protein gene family: BMP signaling regulates facial skeletal morphogenesis by controlling a balance between self-renewing progenitors, osteoblast differentiation and differentiating lineage-restricted cells, and is a major determinant of the dentate development [16,17]. It is expressed in the mandible in the Figure.

FGF: Fibroblast Growth Factor: 23 members of the FGF family of signaling molecules that control a wide range of cellular proliferation, migration, differentiation and regulate bone growth [18]. Expressed in the mid-frontal region in the Figure.

PAX9: Paired box 9. Gene on chromosome 14q13.3 is a member of the pair box family of transcription factors. Mutations are associated with tooth agenesis [19]. It is expressed in the medial and lateral nasal and second pharyngeal arch regions in the Figure 1.

Skull Development

The regions of the skull that are developmentally discrete are the calvaria, the basicranium, the mid-face and the mandible, each of which exhibit different genetic expression patterns during embryogenesis.

The Calvaria: The embryonic neural crest and mesodermal origins of various portions of the calvaria are determined by different genetic expression patterns. The calvaria composed of the frontal and parietal components separated by a frontal suture demarcating the differing embryonic origins exhibit alternative gene expression patterns [20-22]. The *MSX2* gene is associated with craniosynostosis by a single amino acid substitution leading to a transient increase of sutural osteogenesis, resulting in fusion [23]. Progression of bone development requires *FOXC1* regulation of *MSX2* and *ALX4* [24]. *MN1* is a key gene and transcriptional coactivator that evolutionary analysis reveals arose at the base of the bony vertebrates (Eutelostomi) when head ossification first appeared [25]. The coronal suture develops from a group of Sonic Hedgehog (*SHH*) responsive cells in the mesoderm [26].

The Basicranium: The chondrogenic origin of the skull base is determined by genes involved in cartilage development [27, 28]. The location of the basicranium interposed between the brain and the face is subject to increasing encephalization and decreasing facial size in hominin evolution (Figure 2) [29].

Midface: The mesodermal origin of the bones comprising the midface, viz. zygomatic, maxilla, nasal and turbinate bones incur a cluster of genes and gene families: *BMP*, *FGF*, *MSX*, *DLX*, *BARX*, *LHX*, *GSC*, *PAX9*, *PDGF* that create a network of signaling pathways to form the oronasal facial complex [30,31].

Mandible: The embryonic mandible is foreshadowed by the prior development of Meckel's cartilage that, in turn, is dependent upon cranial neural crest migration into the first pharyngeal arch. *BMP2* and *BMP4* ligands are required for the development of cartilage and bone that creates the mandible [16]. *TGF-β* signaling further regulates chondrogenesis and osteogenesis during mandibular development [32].

Evolutionary Considerations

The transition of the chimpanzee genome that is 99.5% similar to

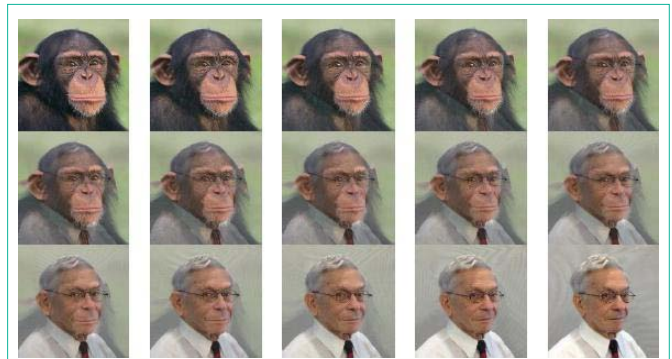


Figure 2: Facial translation of Pan troglodytes to Homo sapiens.

the human genome required genomic rearrangements to transform the craniofacies of Pan Troglodytes to Homo sapiens (Figure 2). Head-to-head fusion of two ancestral chromosomes that occurred in the great apes resulted in reduction of the 48 chromosomes in nonhuman primates to 46 in humans [33-35]. Comparative primate genomic analyses reveal massive genomic rearrangements during evolution. [36,37].

The comparative sequences of the human and chimpanzee genomes have led to the discovery of the regulatory machinery of genes involved in human development and accounting for the differing phenotypic portrayals of the two genera [38]. Exploration of recent facial enhancer cis-regulatory divergence in cranial neural crest cells reveals the genetic basis of the evolution of the human face. Advancements in embryogenetic explorations allow for cellular levels of enquiry into "cellular anthropology" to account for the morphological variations underlying phenotypic evolution and the ontogenetic development of the human face [39]. Regulatory changes in the genomes of chimpanzees and humans account for the rapid phenotypic divergence of these two closely related genera [40].

Evidence for the evolution of mankind has hitherto relied upon paleoanthropological discoveries of fossilized skeletal remains. Evolution takes place in two areas- in the embryo and in the environment. Changes in the embryo have to function cooperatively to form an organism that survives in the environment [41]. Interspecies epigenetic development accounts for evolutionary phylogenetic diversity [42]. A new era of embryogenetic evidence of evolution incurred by gene mutations and chromosome copy number variations is heralding new insights into the molecular mechanisms that successively evolved Pliopithecus, Dryopithecus, Pithecanthropus and Australopithecus into Homo over geologic eons [33]. The molecular basis underlying phenotypic diversity is now being explored in the human methylome as a key regulator of genomic function [43].

Molecular genetic analysis demarcates a dramatic transformation in tracing the evolutionary lineage of humankind in future investigations. The enlargement of the hominin brain in evolution has impacted the shaping and morphogenesis of the human face (Figure 2) [44,45]. New revelations of palaeogenetics and ontogenetics await discovery in the new field of evolutionary developmental anthropology [46]. The recent development of 3D Facial Norms Database (3DFN) that correlates facial phenotypic measurements

with genotype data creates a powerful resource of homology and relevancy of high-resolution genetic markers with anthropometric phenotypes. The fields of orthodontics, forensics and evolutionary physical anthropology are on the verge of an extraordinary exploration of evo-devo and embryogenetics [47]. The future holds much promise.

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