

# **THE INFLUENCE OF FGFR1 VARIANTS ON NORMAL HUMAN CRANIOFACIAL SHAPE**

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The factors influencing the morphology of the human face are of interest to researchers in a variety of different fields. Craniofacial morphology is modified by both genetic and epigenetic events, and factors influencing craniofacial morphology include, but are not limited to, age, sex, mechanical function, soft-tissue matrices, hormones, and genetics. Mutations discovered within FGFR1 offer insight into the importance of this particular gene in controlling craniofacial skeletal development, and the evidence thus far connecting FGFR1 variants to quantitative craniofacial traits in the general population is inconclusive. The purpose of this study was to investigate the association between FGFR1 variants and several measures of cranial and facial shape in a sample of healthy human subjects and to serve as a replication sample for prior genotype-phenotype studies with positive findings for FGFR1. This study comprised 1375 subjects (544 Male, 795 Female, 36 unknown sex) recruited as part of the 3D Facial Norms Project. 3D facial surface images were captured using digital stereophotogrammetry and eight craniofacial measurements were analyzed: maximum cranial width, maximum cranial length, morphological face height, upper face height, nasal protrusion, cephalic index, facial index and upper facial index. Two SNP's of FGFR1 were genotyped: rs6983315 (intronic variant) and rs13317 (3' UTR variant). Genotype-phenotype associations were tested with linear regression, using an additive model and a full dominant model, where age and sex were included as

covariates in all analyses. Results were considered significant if  $p \leq 0.0015$ . No significant associations were observed between either of the two SNPs and any of the eight craniofacial measurements, and the association results of previous studies could not be replicated.

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## **1.0 BACKGROUND**

### **1.1 GENERAL FACTORS INFLUENCING FACIAL FORM**

The factors influencing the morphology of the human face are of interest to researchers in a variety of different fields, including but not limited to maxillofacial surgery, plastic surgery, orthodontics, anthropology/evolutionary biology, developmental anatomy, genetics, and psychology. Both favorable and harmful events occurring within the womb, and even continuing into early childhood, may significantly impact craniofacial growth and development (Toma et al., 2011). Craniofacial morphology develops from numerous, detailed and interactive processes, modified by both genetic and epigenetic events (Cheverud, 1988; Enlow, 1990; Atchley and Hall, 1991; Wagner, 1996; Wagner and Altenberg, 1996; Hallgrimsson et al., 2002; Lieberman et al., 2002; Klingenberg et al., 2003; Hallgrimsson et al., 2004; Gonzalez-Jose et al., 2004; Paschetta et al., 2010). Factors influencing craniofacial morphology include, but are not limited to, age, sex, mechanical function, soft-tissue matrices, hormones, and genetics (Huggare and Ronning, 1995; Kiliaridis, 1995; Kjaer, 1995; Ronning, 1995; Persson, 1995; Thilander, 1995; Oliveira-Neto et al., 2011).

Over the lifespan, the human face is constantly changing in size and shape. After birth, the growth of the human craniofacial skeleton, which consists of several individual elements such as the neurocranium and facial skeleton, varies both in rate and direction. This is

exemplified by the early and quick growth of the neurocranium compared to the more gradual growth of the facial skeleton (Enlow, 1990; Schumacher, 1997; Sperber, 2001; Ross, 2010). After the age of four, the patterns of growth transition into relatively quicker mandibular growth, intermediate facial skeleton growth, and slower neurocranium growth (Sperber, 2001; Ross, 2010). By the age of six, craniofacial growth, specifically the cranium, is approximately 94 per cent complete (Farkas, 1981; Arboleda et al., 2011). The patterns of craniofacial growth are comparable to both weight and height growth patterns, which present with the average pattern of declining growth rates during childhood and elevated growth rates during adolescence (Tanner, 1962; Malina et al., 2004; Veldhuis et al., 2005; Arboleda et al., 2011). Although growth largely ceases by early adulthood, the craniofacial complex continues to change, albeit to a much lesser degree. In a study by Ross et al. (2010) the shape and size of the cranium in subjects age 25 years and older differed significantly from those of other, younger age groups. In addition, a study by Sarna's and Solow (1980) discovered nasion and sella alterations from age 21 to 26 years of age, indicating bone turnover in adulthood, and Akgul and Toygar (2002) also found significant changes, primarily in the mandible, after the age of twenty (Ross and Williams, 2010). More dramatic are the facial soft-tissue changes that take place well into old age (Farkas, 1981).

The biological sex of an individual also influences their facial morphology. Sexual dimorphism of the skull and/or facial soft-tissues has been well documented in humans (Rosas and Bastir, 2002; Pretorius et al., 2006; Kimmerle et al., 2008; Toma et al., 2008). Variations in hormonal balance, especially during puberty, provide the foundation for many of the sexual differences in craniofacial form. Alternatively, the sex chromosomes may contain genes that directly influence facial form, possibly by influencing overall somatic growth. Research

conducted by Kimmerle et al. (2008) found that sex, but not size, considerably impacts craniofacial morphology in both American Whites and Blacks. Thus, whether an individual is small or large, according to this study, the shape of the individual is similar within the same gender category. Correspondingly, Rosas and Bastir (2002) conducted a study on craniofacial morphology and sexual dimorphism in a Portuguese population; results concluded that the craniofacial region is significantly influenced by both size and sex and that males present with a larger nasopharyngeal space with more prominent muscle attachments, while females present with nares reduced in size (Pretorius et al., 2006; Kimmerle et al., 2008). Another study by Pretorius et al. (2006) found that the orbital shape is more gender diverse than the mandibular ramus. Furthermore, according to a study by Toma et al. (2008), females display more pronounced eyes and cheeks, versus males display larger noses and mouths.

In addition to age and sex, function can influence craniofacial form. Melvin Moss's "functional matrix theory" of growth claims that control of growth in the craniofacial skeleton depends upon the neighboring soft tissues (Moss and Young, 1960). For example, the cranial vault grows in direct response to the growth of the brain, which exerts pressure leading to separation of the cranial bones at the sutures. With Moss's theory, the expansion of the nasal and oral cavities, which grow in response to functional needs, is the main factor in growth of the maxilla and mandible. Based on this theory, it can be assumed that the lack of normal function would also produce an array of consequences (Proffit et al., 2013).

Lack of function, as seen with ankylosis of the condyle, may certainly influence growth of the face and exhibits an excellent example of the functional matrix theory. For instance, 20% to 25% of children have a growth discrepancy after condylar fracture, most likely due to a hindrance of function (Proffit et al., 2013). Reduction of mandibular growth occurs from

ankylosis at the temporomandibular joint, and soft tissue wounds may also lead to scar tissue, which interferes with motion during the healing process (Proffit et al., 2013). Temporomandibular joint ankylosis occurs when the mobility of the jaws becomes inhibited, and variations occur from slight inability to open the mouth to a complete restriction of opening (Ko et al., 2005). Variations in the manifestation of unilateral TMJ ankylosis include asymmetry of the chin, deviating toward the affected side, and elongation and flattening of the face on the non-affected side with roundness presenting on the involved side (Nwoku, 1979; Ko et al., 2005).

Mastication also exemplifies the functional matrix theory and is a very significant environmental factor in generating craniofacial variation. Evidence from prior research indicates that the different physical consistencies of food impacts craniofacial growth and morphology in differing ways dependent upon the diet (Yamamoto, 1996). Food consistencies depend upon factors such as particle size, hardness, and toughness and the chewing of foods alters bone loading in both areas of muscle insertion (Engstrom et al., 1986) and in areas without direct muscle insertion (Bouvier and Hylander, 1981; Kiliaridis, 1989; Yamada and Kimmel, 1991; Bresin et al., 1994; Yamamoto, 1996). Based upon former research which concludes that mechanical stress of mastication induces bone growth, the consensus remains that unprocessed diets with harder foods ultimately cause larger and more robust skulls (Larsen, 1995, 1997), relatively larger faces compared to the skull (Carlson and Van Gerven, 1977), larger temporal muscle areas (Carlson, 1976; Carlson and Van Gerven, 1977; Gonzalez-Jose et al., 2005; Sardi et al., 2006), larger temporo-mandibular joint size (Hinton and Carlson, 1979; Corruccini and Handler, 1980) and increased thickness of the cranial vault (Hylander, 1986; Lieberman, 1996; Paschetta et al., 2010). Mastication of tougher foods induces greater lower face strain and more growth, and often leads to a more brachycephalic facial form. Alternatively, softer diets

containing more processed foods may cause relatively less craniofacial growth in the mandible, lower maxilla, and other areas that normally undergo mechanical loading with mastication (Paschetta et al., 2010). With softer diets, a lack of mechanical stress on craniofacial bones and skeletal muscles will cause decreased growth (Paschetta et al., 2010), and cause for example, longer faces or dolicocephalic facial forms.

Craniofacial morphology is also influenced by a host of hormones. Thyroid hormone, growth hormone, adrenocortical and sex steroids all have essential roles (Pirinen, 1995; Funatsu, 2006; Oliveira-Neto et al., 2011). In 1934, the first studies on growth hormone deficiency and the relationship to facial morphology emerged with evidence revealing an “immature” facial manifestation in these patients. The length and depth of the face were incongruously small for age, which created a “child- like convexity” (Markus, 1942; Cohen, 1948; Bevis, 1977; Edler, 1979; Pirinen, 1994; Kjellberg, 2000; Segal, 2004; Funatsu, 2006). One study by Oliviera-Neto et al. (2011) was conducted on a group of adult patients with untreated lifetime isolated growth hormone deficiency (IGHD), resulting from the same genetic abnormality. Their data revealed that these patients manifest decreases in all linear measurements, predominantly total maxillary length. Moreover, other studies have shown greater reductions in anterior cranial base length (Cantu, 1997; Salvatori, 1999), mandibular corpus length (VanErum, 1998), and total mandibular length (de Faria, 2009; Oliveira-Neto et al., 2011). In hypopituitary children, posterior facial height appears to be the most reduced linear dimension (Spiegel, 1971; Pirinen, 1994). Funatsu et al. (2006) also studied idiopathic GHD patients and found that the most affected craniofacial areas included anterior cranial base, anterior facial height, and maxillary length (Oliveira-Neto et al., 2011).

In addition to studies on growth hormone, thyroxine and its impact on bone growth and remodeling has also been a subject of previous research. Evidence has shown that untreated hyperthyroidism has led to increased bone resorption (Adams et al., 1967; Persson et al., 1989). This is exemplified in rats, for example, where high doses of thyroxine display this increased bone resorption (Frost et al., 1962; Adams et al., 1967; Adams and Jowsey, 1967). Other research on thyroxine and craniofacial growth has shown premature closure of cranial sutures in children given therapeutic thyroxine (Penfold and Simpson, 1975; Johnsonbaugh et al., 1978; Persson et al., 1989). A study by Persson et al. (1989) concluded that the craniofacial growth pattern of growing rats is altered by low doses of thyroxine administration, leading to an increased curvature of the superior portion of the skull and ventral rotation of the viscerocranium in comparison to control animals. Reduction of linear dimensions following the administration of thyroxine, indicates a retardation of the normal forward growth in the viscerocranium, and altered angular values reveal induction of rat skulls with a klinorhyncial versus the normal orthocranialization present in rat skulls (Moss, 1958; Ronning, 1971; Pucciarelli, 1978; Vilmann and Moss, 1979; Persson et al., 1989). Under normal circumstances, the rat skull straightens during adolescence, but with increased thyroxine, a pronounced flexure between the viscerocranium and neurocranium was present (Persson et al., 1989). Based on this initial research, it can be concluded that cortical bone dimensions during growth are undeniably influenced by thyroxine.

In summary, there are numerous intrinsic and extrinsic factors capable of influencing human craniofacial morphology over the lifespan. The major factor yet to be discussed, however, is genetics.



## 1.2 EVIDENCE FOR THE ROLE OF GENETICS

One of the goals in the field of genetics is to decipher the role of specific genes in producing the final phenotype of organisms, including the craniofacial complex (Lee et al., 2008; Gómez-Valdés et al., 2013). The face and craniofacial complex help to make us who we are as individuals, and the interrelationship of genes, along with their redundancy and polymorphisms, contributes to our unique make up (Kim et al., 1998; Coussens and Daal, 2005).

Twin, family, and animal studies have consistently shown that genes impact craniofacial morphology (Hunter, 1970; Nakata, 1973; Kohn, 1991; Johannsdottir, 2005; Paternoster, et al., 2012). Increasing knowledge in molecular genetics continues to provide new information about the control of craniofacial growth. Families of growth factors and their cognate receptors must function correctly to regulate embryonic processes. This process of cell interaction between different tissues within the craniofacial complex creates another level of regulation of growth and development. The process of facial growth and development is regulated by numerous signaling pathways, including the BMP, WNT, SHH, and FGF pathways (Nanni et al., 1999; Cai et al., 2005; Chai et al., 2006; Brugmann et al., 2007; Feng et al., 2009; Kang et al., 2009; Buchtová et al., 2010).

Understanding the genetic basis of facial form is also vital to medical fields like orthodontics, where the goal is to therapeutically manipulate facial growth. The projected outcome of orthodontic and dentofacial orthopedic treatment on patients depends upon the ability of “environmental” treatment to alter the existing malocclusion (Naini et al., 2004). Ideally the orthodontist can diagnose the genetic and the environmental role in a particular malocclusion, understanding that orthodontic treatment will have a poorer prognosis should the genetic contribution be large (Mossey, 1999; Baydas et al., 2007). Identifying etiology of malocclusion

and the challenges presented during orthodontic treatment may be easier to accomplish when genetic and environmental influences on craniofacial form are better understood (Baydas et al., 2007). Thus, studies focused on specific facial traits, and their association with corresponding genes, are extremely beneficial for the specialty of orthodontics.

There are several converging lines of evidence, from both animal models and humans, supporting the link between genetic variation and specific craniofacial phenotypes. It is well known, for example, that selective breeding can alter facial traits in domesticated animals (e.g. snout length in dogs) (Stockard, 1941). This tells us that certain craniofacial features are at least heritable. The study of animal mutants can provide an additional window into how specific genes work in isolation or in networks to influence craniofacial morphology (Juriloff and Harris, 2008). Spontaneous or experimentally induced knockouts/knockdowns can reveal the effects of reduced expression/function of specific genes on craniofacial morphology (Ingraham et al., 2006). Likewise, gain-of-function mutations can also be modeled.

A variety of craniofacial-focused gene mapping and trait association studies have been carried out using animal models (Nishimura et al., 2003). A study by Haworth et al. (2001) explored genetic differences in DNA from dog breeds presenting with varying craniofacial phenotypes, identifying an association between *Tcof1* variants and overall head/face shape. In humans, mutations in *TCOF1* lead to Treacher Collins syndrome. In another study, Dohmoto et al. (2002) identified quantitative trait loci (QTLs) on chromosomes 10 and 11 associated with anteroposterior length of the mandible in mice. The relevance of a study such as this pertains to the possibility of predicting mandibular size prior to growth cessation of the maxillofacial bones by seeking chromosome region polymorphisms (Dohmoto et al., 2002). Similarly, Oh et al.

(2007) used congenic mice to successfully map QTLs for snout length to chromosome 12. Similar QTL mapping studies have been carried out on baboons (Sherwood et al., 2008).

In humans, there is a great deal known about patterns of craniofacial disturbance that occur when specific genes are mutated or chromosomal regions are deleted (Gorlin et al., 2001; Jones et al., 2006). Mendelian syndromes caused by either genetic mutations or chromosomal anomalies often have very specific craniofacial presentations; examples include Van der Woude syndrome, Apert syndrome, 22q deletion syndrome and Treacher Collins syndrome. By linking phenotypic outcomes to the specific gene/region affected, information can be gleaned about possible gene function. For example, well over 400 syndromes are associated with orofacial clefting. Many of the genes involved in these clefting syndromes are involved in the process of normal lip and/or palate development.

Family heritability studies are one of the strongest lines of evidence supporting the role of genetics in determining facial morphology in humans. Both twin concordance and parent-offspring resemblance studies have shown repeatedly that facial morphology and the variance in phenotypes observed are partially attributable to genetics (Weinberg et al., 2013). In fact, it has been estimated by twin studies that genetics contributes as much as 80% of variation in some facial features (Johannsdottir et al., 2005; Liu et al., 2012; Peng et al., 2013). Utilization of twin and parent-offspring studies provides valuable information regarding which particular features of the human craniofacial complex are strongly regulated by genetics. For example, evidence from numerous generalized studies indicate that tooth anatomy and eruption patterns as well as vertical facial dimensions in comparison to sagittal dimensions, are more genetically influenced and thus more difficult to modify via treatment (Hunter, 1965; Manfredi et al., 1997; Lundström, 1987; Savoye, 1998; Carels et al., 2001; Naini et al., 2004; Weinberg et al., 2013). A

study by Carels et al. (2001) concluded this as well and found that angular measurements depend the least upon genetic contribution. Because genetics appear to influence vertical measurements more than sagittal and angular, it is expected that orthodontic therapy would have a larger impact on both angular and sagittal dimensions than vertical (Carels et al., 2001). A twin study by Weinberg et al. (2013) found evidence that variation in the length and breadth of the central midfacial structures of nose, interorbital region, and upper lip are under considerable genetic control. These studies are important because they point to traits most likely to be associated with genes.

While it is clear that many aspects of human facial morphology are heritable and the link between genes and certain craniofacial abnormalities is apparent, relatively little is known about the role of specific genes in producing the range of normal craniofacial phenotypes evident in humans. Only a handful of studies, to date, attempted to identify genes/regions associated with quantitative facial traits, like facial length or width (Coussens and van Daal, 2005; Boeringer et al., 2011; Paternoster et al., 2012; Liu et al., 2012; Gómez-Valdés et al., 2013; Peng et al, 2013; Claes et al., 2014). Some of the genes identified make sense biologically. For example, in the first ever genome-wide association (GWA) study of normal human facial shape, Paternoster et al. (2012) reported a significant association between the shape of the nasal root (position of nasion) and SNPs in the PAX3 gene in a large sample of European Caucasians 15 year olds. This finding is of considerable interest because mutations in PAX3 have been implicated in Waardenburg Syndrome, which is characterized by phenotypic abnormalities involving the nasal root region.

Thus far, however, only a small number of findings have been independently replicated. PAX3 was only partially replicated by Liu et al (2012) in a second European Caucasian GWA

study. The gene with the most robust evidence to date seems to be the fibroblast growth factor receptor 1 (FGFR1), which has been identified by several groups independently (Coussens and van Daal, 2005; Gómez-Valdés et al, 2013; Claes et al, 2014).

### **1.3 FGFR1 AND HUMAN CRANIOFACIAL VARIATION**

Fibroblast growth factors (FGFs) are comprised of a family of polypeptides, displaying mitogenic activity in cells of mesenchymal, neuronal and epithelial origin (Williams, 1994). FGFs also promote survival and/or neurite outgrowth from hippocampal neurons, cerebral cortical neurons and spinal motor neurons (Walicke, 1989; Morrison et al., 1986; Hughes et al., 1993; Williams, 1994). FGFs are important in cellular differentiation and because they are present during embryogenesis, it is believed that they may play other important roles during development (Williams, 1994).

Four FGF receptors exist, all of which are proteins with a role in important cellular functions. Examples include embryonic development, cell division, wound healing, regulation of cell growth and maturation, and formation of blood vessels. Distinct genes encode for the four receptors in this gene family (Jaye et al., 1992; Williams, 1994). FGFRs are located simultaneously inside and outside of cells, permitting FGF proteins outside of the cell to transmit extracellular signals to within the cell. This attachment of FGF proteins with their receptors leads toward a chemical reaction and ultimately an environmental response within the cell. When FGF attaches to its receptor, dimerization of the receptor follows, leading toward autophosphorylation of defined tyrosine residues, ultimately forming binding sites for the SH2 domains of multiple transduction molecules such as src and PLCg. Independent signaling cascades are then triggered,

causing survival, mitogenesis or differentiation (Schlessinger and Ullrich, 1992; Williams, 1994). Some fibroblast growth factor receptors are high affinity tyrosine kinase receptors, which are thought to regulate FGF cellular responses, and some are low affinity receptors, characterized by the presence of heparan sulfate (Johnson and Williams, 1993; Wilkie et al., 1995; Stachowiak et al., 2003).

The cytogenic location of FGFR1 is 8p11.23-p11.2, the short arm of chromosome 8 between positions 11.23 and 11.22. FGFR1 is a cell surface receptor guiding signals between two major transduction pathways: Ras/mitogen-activated protein kinase and PLC $\gamma$  pathways (Groth and Lardelli, 2002; Hünemeier, 2014). Possible roles of FGFR1 include development of the nervous system (Wanaka et al., 1990; Asai et al., 1993) and regulation of long bone growth and development (<http://ghr.nlm.nih.gov/gene/FGFR1>).

Mutations discovered within FGFs and FGFRs offer insight into the importance of these particular genes in controlling craniofacial skeletal development (Ornitz and Itoh, 2001; Dorey and Amaya, 2010; Hünemeier, 2014). Specifically, mutations within genes of the FGF/FGFR signaling pathway have been connected to congenital craniosynostosis and related syndromes such as Jackson-Weiss, Beare-Stevenson, Muenke, Crouzon, Apert, and Pfeiffer syndromes, leading to the conclusion that these specific genes are critical to skull development, particularly suture and synchondrosis regulation. (Robin et al, 1998; Nie et al, 2006; Hajihosseini et al., 2009; Hünemeier, 2014). Pfeiffer syndrome, which is a common syndrome presenting with craniosynostosis, midface hypoplasia, and partial syndactyly of the digits is one example specific to FGFR1 and FGFR2 mutations (Chokdeemboon, 2013). Another rare syndrome known as Hartsfield syndrome presents in patients with holoprosencephaly and ectrodactyly occurring together. Other possible phenotypes of this syndrome include multiple congenital anomalies such

as cleft lip and palate, malformed ears, and hypo- or hypertelorism. A study by Simonis et al. (2013) concluded Hartsfield syndrome represents many developmental anomalies caused by FGFR1 loss-of-function mutations. FGF signaling pathways play an important role in palate development as well (Simonis, 2013). Mutations in FGFR1, FGFR2, and loss of function mutations of FGFR1 often cause cleft palate and associated syndromes including Kallmann, Pfeiffer, Apert, and Crouzon syndromes (Alappat, 2005; Pitteloud, 2006; Riley, 2007; Wang, 2013). A study by Wang et al. concluded that specific FGFR1 signaling and balance must be present for proper CNC-derived mesenchymal cell proliferation and palate shelf development to occur and that both excess and a deficit of FGFR1 signaling may lead to defects in palatogenesis (Wang, 2013).

Because FGFR1 clearly plays an important role in normal craniofacial morphogenesis, any change that alters its function may affect the resulting craniofacial phenotype. As described above, mutations in the gene can have serious phenotypic consequences in humans. However, while mutations in FGFR1 can result in syndromes with quite severe presentations, there is still a wide range of phenotypic expression (Passos-Bueno et al., 1999; 2008). Normal variants of FGFR1 may also affect the gene's expression and function, albeit to a lesser extent than a functional mutation. In healthy individuals, alternative FGFR1 variants may differentially influence the shape of the cranial vault or components of the midface, the craniofacial regions most likely to be affected when mutations in this particular gene result in syndromic phenotypes. Coussens and van Daal (2005) were the first to evaluate the association between FGFR1 SNPs and craniofacial shape in a sample of healthy subjects. Using traditional anthropometry and anthroposcopy, they reported significant associations between the tagging SNP rs4647905 and both cranial index and facial shape; individuals with at least one C allele at this SNP tended to

have a reduced cephalic index (more dolicocephalic cranial vault shape) and longer and narrower face (more leptoprosopic facial shape). The results were most pronounced in females and those of Asian ancestry.

Gómez-Valdés et al. (2013) looked at the association between five FGFR1 SNPs (rs4647905, rs2304000, rs2293971, rs3213849, rs930828) and a variety of anthropometric craniofacial measurements and indices in several Mexican populations. Although most results were marginal, they reported significant genetic associations for several traits including cephalic index, cranial vault length and height, and total facial height. The particular SNP identified by Coussens and van Daal (2005) was associated only with cranial vault length in this study. Most recently, Claes et al. (2014) investigated 50 candidate genes, including FGFR1, for their effects on facial shape using 3D facial imaging and surface-based morphometry. FGFR1 SNP rs13267109 was associated with variation in several facial features including the breadth of midfacial structures (nose, philtrum and mouth) and the projection of the orbits, brow ridges and nose.

However, not all studies have reported positive associations between quantitative facial traits and FGFR1 variants in healthy samples. In an assessment of 10 SNPs in four candidate genes, Peng et al. (2013) failed to find any association between FGFR1 SNP variants (rs4647905, rs3213849) and a wide variety of facial shape measures in their sample of healthy Han Chinese individuals. Furthermore, neither of the two GWA studies investigating facial shape (Paternoster et al., 2012; Liu et al., 2012) identified SNPs in or near FGFR1. The three studies mentioned above all used 3D facial imaging in concert with landmark-based morphometry and focused exclusively on the face. None measured the cranial vault.



The evidence thus far connecting FGFR1 variants to quantitative craniofacial traits in the general population is inconclusive. While a few studies have reported associations with certain craniofacial measurements, others have not. Further, the positive results reported to date have been inconsistent with regard to the specific SNPs or traits involved.

## **2.0 PURPOSE OF THE PRESENT STUDY**

The present study will investigate the association between FGFR1 variants and several measures of cranial and facial shape in a sample of healthy human subjects. Understanding how normal variation in FGFR1 result in typical craniofacial variation may provide insight into how functional mutations in this same gene results in specific craniofacial anomalies and syndromes. This study will also serve as a replication sample for prior genotype-phenotype studies with positive findings for FGFR1.

### **3.0 MATERIALS AND METHODS**

#### **3.1 SAMPLE DESCRIPTION**

The sample for this study was comprised of 1375 subjects (544 Male, 795 Female, 36 unknown sex) recruited as part of the 3D Facial Norms Project – an NIDCR funded component of the FaceBase Consortium (Hochheiser et al., 2011). The overall goal of the 3D Norms Project was to generate age, sex and ethnicity-specific norms for craniofacial measurements based on 3D facial surface imaging technology. A further aim was to test for genetic associations with quantitative craniofacial traits.

##### **3.1.1 Inclusion/Exclusion Criteria**

To be enrolled, subjects had to be of recent European-Caucasian ancestry (as far back as their maternal and paternal grandparents) and between 3-40 years of age. Ancestry, age and sex were self-reported. Subjects were excluded if they reported any of the following: a personal history of facial trauma or disfigurement, facial reconstructive or plastic surgery, orthognathic/jaw surgery or jaw advancement, any palsy, stroke or neurological condition affecting the face, or any facial prosthetics or implants; a personal or family history of any facial anomaly, birth defect, or any syndrome or congenital condition with a facial manifestation;, and the presence of conspicuous facial hair or any facial piercings other than small studs.

### **3.1.2 Recruitment Strategy**

Subjects were recruited at three US sites: Pittsburgh, Seattle and Houston. As a means of enrolling subjects into the database, multiple methods of advertising were utilized such as direct mail printouts, public setting kiosks, word-of-mouth, cooperation with general dental and medical clinics, and via registries linked to universities and hospitals.

## **3.2 QUANTITATIVE PHENOTYPING**

### **3.2.1 Demographic Interview**

Following informed consent, subjects were given a brief demographic interview to record age, biological sex, height, weight, ancestry (race) and ethnicity (Hispanic). All items were self-reported. For young children, parents completed the interview on their behalf.

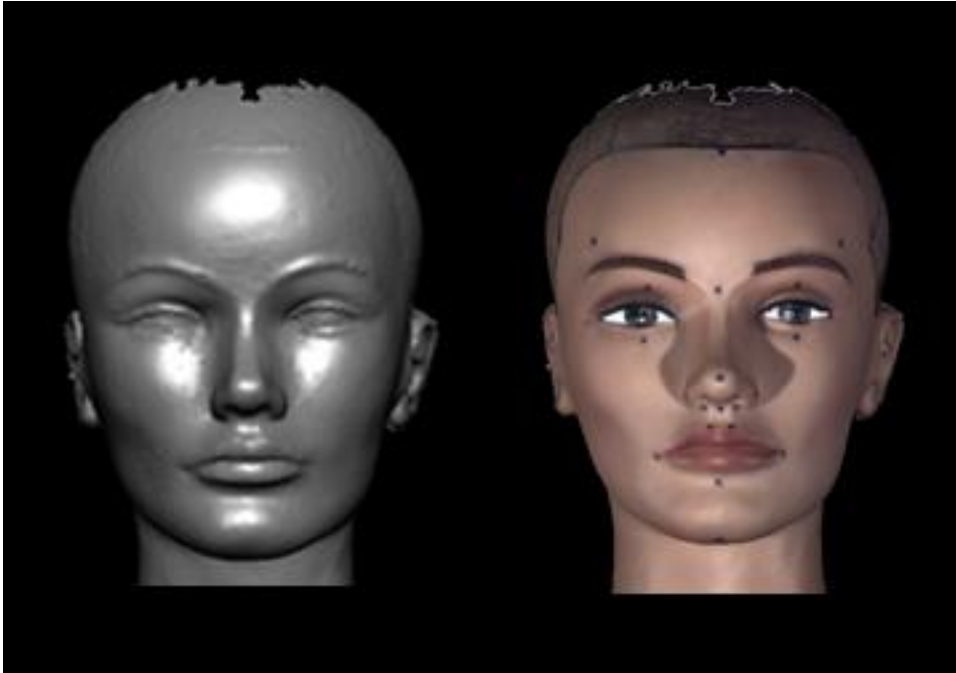
### **3.2.2 Facial Image Acquisition**

3D facial surface images were captured using digital stereophotogrammetry (3dMDface system). The 3dMD system is comprised of a number of machine vision CCD cameras situated in positions with fixed angles to allow overlapping views of the face and head. The system can capture a 180-degree facial surface in approximately 2 milliseconds, including 3D geometry and mapped skin color and texture (Lane and Harrell 2008) (Figure 1). Previous studies have

investigated the precision and accuracy of 3dMD systems (Aldridge et al., 2005; Losken et al., 2005; Weinberg et al., 2006; Wong et al., 2008; Heike et al., 2009).

Several steps were conducted prior to 3D image capture, including the removal of loose hair from blocking the forehead and ears and labeling facial landmarks (pronasale, gnathion, left/right trigion) via a liquid eyeliner pencil directly onto the skin. Both steps assisted in the later identification of points on the 3D facial model.

The 3D image capture process involved placing the subject's head directly between the imaging pods in an upward position (15 degrees above Frankfort horizontal) and centered in front of the camera. With a relaxed and neutral facial expression, with eyes open and pupils facing in a slight upward direction, the image was taken. Once processed, the resulting 3D model was evaluated for non-neutral facial expressions, mesh defects, calibration errors, missing surface data, and motion artifacts. If the 3D images had any of the above problems, they were retaken.



**Figure 1: Example of a 3D facial surface image captured with the 3dMDface system. The left image shows the underlying surface geometry. The right image shows the surface with color and texture mapping applied.**

### **3.2.3 Image Landmarking and Measurement**

The 3D images were loaded into the 3dMDpatient software program, cleaned by removing extraneous hair and portions of the neck, and oriented in a position that would provide a clear frontal view of the face. No hole-filling, mesh-repair or smoothing routines were used on the 3D surfaces. The re-oriented 3D facial images were then loaded into the 3dMDvultus software program, where a series of 24 standard facial landmarks were collected by hand on each 3D surface and their associated x,y,z coordinates saved to a simple text file. Using simple Euclidean geometry, a pre-defined set of 29 standard facial anthropometric linear distances was calculated from the 3D coordinates. Details about the full set of landmarks and derived measurements can be found here: [https://www.facebase.org/facial\\_norms/notes](https://www.facebase.org/facial_norms/notes).

### **3.2.4 Traditional Anthropometry**

In addition to facial measurements obtained via 3D imaging, five additional anthropometric measurements were obtained directly on subjects' head with spreading calipers: maximum head width, minimum frontal breadth, maximum face width, mandibular width and maximum head length. These are standard anthropometric measurements and were collected in order to get a more complete picture of the craniofacial complex. These particular measurements are not possible to obtain via 3D surface imaging.

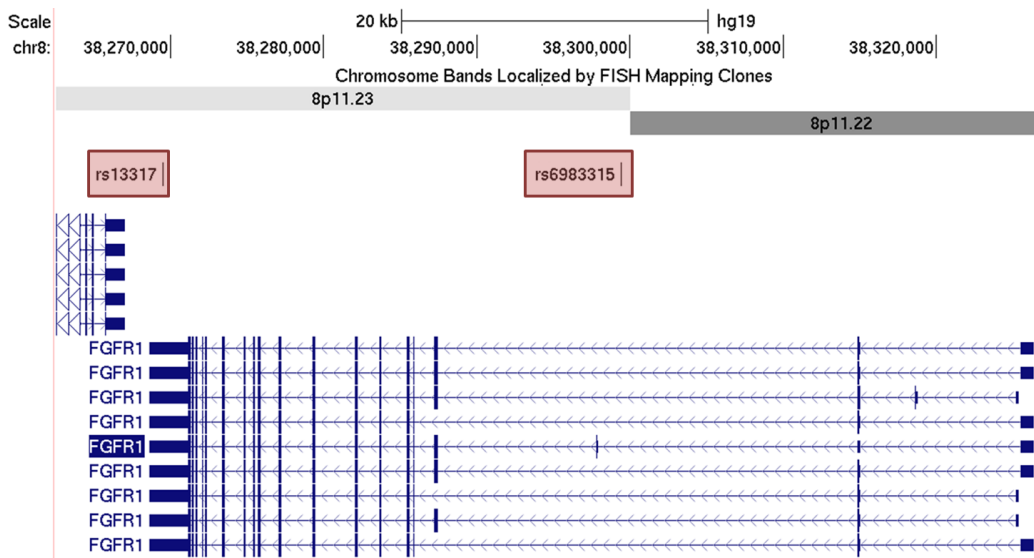
## **3.3 SALIVA COLLECTION AND SAMPLE PROCESSING**

Subjects provided saliva samples using Oragene collection kits. For children too young to spit, collection swabs were used. Children under 6 years of age provided two samples. DNA was extracted using standard methods.

## **3.4 SNP SELECTION AND GENOTYPING**

Multiple groups have previously reported associations with SNPs in FGFR1 (primarily rs4647905) and quantitative craniofacial traits. The genotyping assay for rs4647905 was unavailable so we selected two nearby SNPs to serve as surrogates: rs6983315 (intronic variant) and rs13317 (3' UTR variant) (Figure 2). Two statistics used to determine pairwise linkage disequilibrium (LD) between the SNP's were  $r^2$  and  $D'$ . A pair of SNPs is in perfect LD if both

$r^2$  and  $D'$  = 1.0. Pairwise LD was calculated using genotype data from European and European-American samples selected from the 1000 Genomes Project, a public database with genome-wide data from multiple populations. SNPs rs13317 and rs6983315 were in high LD with rs4647905, but were not in complete LD with each other (Table 1). Therefore, these SNPs can be regarded as surrogates for rs4647905. Genotyping was performed with Taqman SNP assays following standard protocols.



**Figure 2: Schematic representation of the FGFR1 gene showing the location of the two SNPs tested in this study**



**Table 1: LD Relationship between SNPs**

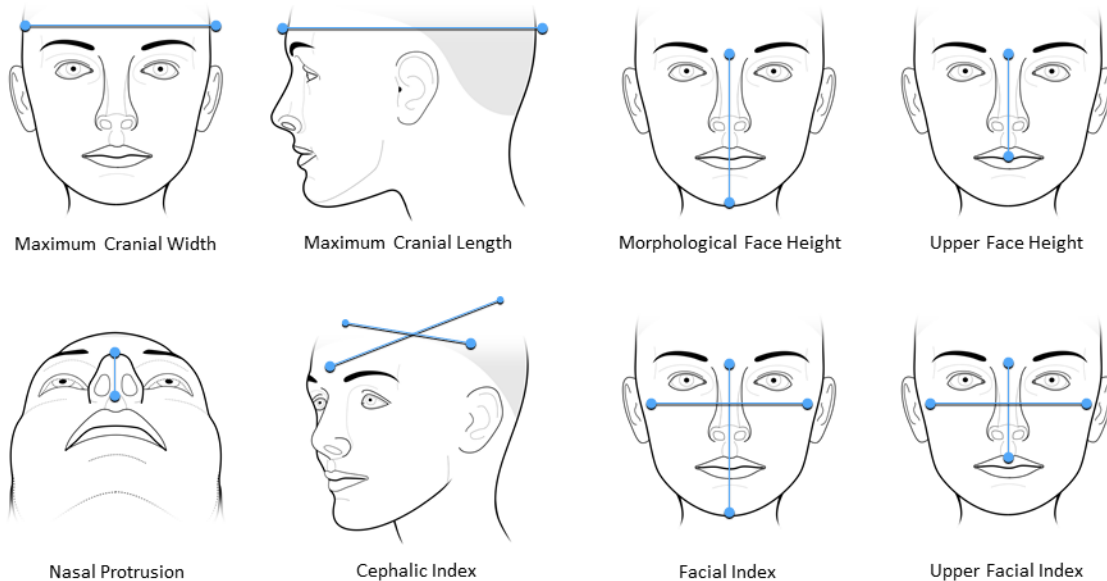
SNP's	r <sup>2</sup>	D'
rs4647905 and rs13317	0.932	1.0
rs4647905 and rs693315	0.314	0.958
rs13317 and rs693315	0.279	0.873

\* RS4647905 NOT USED IN THE PRESENT STUDY, BUT WAS IDENTIFIED IN PREVIOUS REPORTS

### **3.5 FINAL SELECTION OF MEASUREMENTS FOR ANALYSIS**

From the complete set of 3D-based and caliper-based linear distance measures, a subset of five distances were selected for analysis; these distances were each tested separately, but also used to construct three indices (Figure 3). The choice of these eight distances and indices was based on the results of prior studies investigating the relationship between facial characteristics and FGFR1 SNP variants (Coussens and van Daal, 2005; Gómez-Valdés et al., 2013; Claes et al., 2014). The eight quantitative variables included maximum cranial width, maximum cranial length, morphological face height, upper face height, nasal protrusion, cephalic index, facial index and upper facial index. Maximum cranial width and length are standard measures of cranial vault size. Morphological face height is the distance between nasion and gnathion and is a conventional facial measurement for the complete vertical length/height of the face or viscerocranium. Upper face height measures the distance from nasion to stomion and measures

the vertical height of the upper portion of the face or viscerocranium, not including the mandible. Nasal protrusion, or nasal depth, refers to the distance from subnasale to pronasion and measures the nose from the nasal floor to the nasal tip. The cephalic index is calculated as maximum cranial width / maximum cranial length x 100 and provides a basic measure of cranial vault shape; higher numbers indicate a more brachycephalic head shape. The facial index is calculated as morphological face height / maximum face width x 100 and is basic measure of face shape; higher numbers indicate a face that is relatively long for its width. Upper face index is calculated as upper face height / maximum face width x 100 and is similar to the facial index except that it deals only with the portion of the face above the labial fissure; interpretation is similar to the facial index.



**Figure 3: The measurements and indices used in the current study**

### 3.6 GENOTYPE-PHENOTYPE ANALYSIS

Genotype-phenotype associations were tested with linear regression, using an additive model and a full dominant model to code the genotypes. In the additive model, the direction of the regression coefficient (Beta) is represented by the effect each extra minor allele contributes to the mean of the phenotype. For example, if there is a positive correlation between the dependent and independent variables, the mean of the presenting phenotype should increase as extra minor alleles are added, which is presented as a positive regression coefficient. The assumption is that by adding alleles, the mean of the craniofacial measurements will increase. With the full dominant model, individuals that are heterozygous or homozygous for the minor allele are combined into a single group. In this scenario, having one or two copies of the minor allele would result in the same effect on the phenotype. Age and sex were included as covariates in all analyses. Thirty two individual association tests were conducted: two SNPs x eight traits x two models. Results were considered significant if  $p \leq 0.0015$ , corrected for multiple testing (0.05/32).

## **4.0 RESULTS**

### **4.1 PROFILES OF THE TWO FGFR1 SNPS**

For SNP rs6983315, the minor allele was A and the major allele G. The frequency of the minor allele was found to be 0.4345 (43% A allele, 57% G allele). The distribution of genotypes was as follows: frequency of AA was 253 (18.4%), frequency of AG was 689 (50.1%) and frequency of GG was 433 (31.5%). The genotypes were observed to be in Hardy Weinberg equilibrium ( $p = 0.476$ ).

For SNP rs13317, the minor allele was A and the major allele was C. The frequency of the minor allele was found to be 0.224 (22% A allele, 78% C allele). The distribution of genotypes was as follows: frequency of AA was 71 (5.2%), frequency of AC was 474 (34.5%), and frequency of CC was 830 (60.3%). The genotypes were observed to be in Hardy Weinberg equilibrium ( $p = 0.756$ ). Summary statistics for the two SNPs are shown in Table 2.

**Table 2: Summary statistics for the two FGFR1 SNPs included in the present study**

SNP	BP	A1	A2	MAF	GENO DIST	HWE P	NCHROBS
rs6983315	38299419	A	G	0.4345	253/689/433	0.4762	2750
rs13317	38269514	A	C	0.224	71/474/830	0.7564	2750

A1= minor allele; A2= major allele; MAF=minor allele frequency; GENO DIST= genotype distribution (e.g., AA, AG, GG); HWE P=P-value for Hardy Weinberg Equilibrium; NCHROBS = number of chromosomes.

## 4.2 ASSOCIATION TESTS

No significant associations were observed between either of the two SNPs and any of the eight craniofacial measurements. This was the case under both dominant and additive models. The two covariates included in the regression model (sex and age) had a significant ( $p < 0.001$ ) effect on all measurements, except for sex on upper facial index. The detailed results for each SNP are shown in Table 3 and Table 4.

**Table 3: Association Results for SNP rs6983315**

TRAIT	N	MODEL	BETA	STAT	P
Maximum Cranial Width	1330	DOM	-0.084	-0.252	0.801
		ADD	-0.185	-0.831	0.406
Maximum Cranial Length	1333	DOM	-0.870	-1.916	0.056
		ADD	-0.356	-1.174	0.241
Morphological Face Height	1316	DOM	-0.368	-0.790	0.429
		ADD	-0.117	-0.3774	0.706
Upper Face Height	1334	DOM	-0.036	-0.115	0.909
		ADD	-0.076	0.362	0.718
Nasal Protrusion	1337	DOM	-0.110	-0.927	0.354
		ADD	-0.044	-0.554	0.580
Cephalic Index	1328	DOM	0.312	1.507	0.132
		ADD	0.049	0.353	0.725
Facial Index	1313	DOM	-0.099	-0.289	0.773
		ADD	-.007	-0.029	0.977
Upper Facial Index	1330	DOM	0.032	0.132	0.895
		ADD	0.086	0.538	0.591

**Table 4: Association Results for SNP rs13317**

TRAIT	N	MODEL	BETA	STAT	P
Maximum Cranial Width	1330	DOM	-0.047	-0.148	0.882
		ADD	-0.114	-0.435	0.663
Maximum Cranial Length	1333	DOM	-0.540	-1.258	0.209
		ADD	-0.536	-1.505	0.133
Morphological Face Height	1316	DOM	-0.064	-0.145	0.885
		ADD	-0.096	-0.263	0.793
Upper Face Height	1334	DOM	0.029	0.099	0.921
		ADD	0.028	0.114	0.910
Nasal Protrusion	1337	DOM	0.012	0.108	0.914
		ADD	-0.024	-0.253	0.800
Cephalic Index	1328	DOM	0.223	1.144	0.253
		ADD	0.177	1.090	0.276
Facial Index	1313	DOM	0.105	0.324	0.746
		ADD	0.027	0.101	0.919
Upper Facial Index	1330	DOM	0.090	0.397	0.692
		ADD	0.065	0.349	0.727

## 5.0 DISCUSSION

Our results showed no evidence of an association between SNPs in *FGFR1* and several measures of the head and face. Thus, we failed to replicate the results of prior studies. There are several possible reasons for our failure to replicate. In our study, the sample population consisted of only US Caucasians, whereas in the study by Coussens and Daal (2005), the sample consisted of a mix of Caucasians, Asians, Australian Aborigines, and African Americans. The investigation by Gomez-Valdes et al. (2013) comprised Mexican Native and mestizo Mexican populations. Claes et al. (2014) utilized subjects from European and West African ancestry, but all were from three different countries. Ancestry could certainly impact association results, as allele frequencies may be significantly different among various populations. Further, craniofacial traits may be influenced by environmental factors, such as diet or climate, which can vary greatly by region and potentially affect the relationship between genotype and phenotype.

Prior studies also focused primarily on adult samples, whereas the present study included a large number of subadults. While we did attempt to account for age as a covariate in our statistical analysis, the simple linear regression approach used here approach may not have been adequate to account for the effects of age on the traits.

There were additional important methodological differences. Coussens and Daal (2005) did not quantitatively assess facial shape, but rather visually categorized subjects by eye. Gomez-Valdes et al. (2013) used traditional caliper-based anthropometry for their facial



measurements, whereas the present study primarily used indirect 3D stereophotogrammetry. Claes et al. (2014) also used 3D stereophotogrammetry, but they used a totally different method for quantifying facial shape based on geometric morphometrics. The traits in the present study were limited to simple linear distances and indices.

A limitation in our study (and all prior studies) is the fact that there was no attempt to account for environmental factors that can affect craniofacial variation. Nutrition, mouth breathing and related facial forms (e.g. dolicocephalic), diet (coarse versus soft), and hormones all impact facial form and may be confounding factors. Even if the particular SNPs studied impact facial form, there is no evidence precluding the idea that the environment may obscure the genetic contribution. Future studies may be conducted to account for such environmental influences.

Our study was also limited by the choice of SNPs. Because assays for some of the SNPs we wanted were not available the chosen SNP's in our report were surrogates, but they were not identical and could therefore have produced different results. There were numerous SNPs in the *FGFR1* gene that we did not assess, including several that had shown associations in prior studies.

Some of the craniofacial measurement methods also present limitations. Our cranial vault shape measurements, like Coussens and Daal (2005) and Gomez-Valdes et al. (2013), were obtained with direct anthropometry. This method can be very prone to measurement error unless individuals are well trained and collection is done in a very uniform manner. For our study, since recruitment was performed at multiple sites, several different individuals carried out the measurements. Although attempts were made to calibrate data collection staff at the sites, this type of collection effort inevitably affects the quality of the data.

One strength of our study was its sample size, which is larger than any of the previous three studies that have looked at this question. The lack of observed association was not likely due to a lack of power. It is a distinct possibility that the previously observed associations were in fact false positives.

Results from this study are significant because they propose the need for further research on FGFR1, by looking at other SNP's within this gene and their connections to the craniofacial complex. Accounting for different variables, adjusting sample selection, and altering methods of material collection are all possible changes in future studies that may lead to more conclusive findings. The selected SNPs are just two of many and the FGFR1 gene is just one of a large number of genes accounting for the complex facial phenotypes that exist in populations all across the globe. More focused studies such as this and those previously described are absolutely necessary to continue the path toward understanding the biological basis of craniofacial variation in humans.

## **6.0 CONCLUSIONS**

Findings of this study revealed no significant association between two SNPs in the FGFR1 gene and dimensions of the head and face. The association results of previous studies could not be replicated.

## BIBLIOGRAPHY

- Adams P, Jowsey J. Bone and mineral metabolism in hyperthyroidism: An experimental study. *Endocrinology*. 1967; 81: 735-740.
- Adams PH, Jowsey J, Kelly PJ, Riggs BL, Kinney VR, Jones JJ. Effects of hyperthyroidism on bone and mineral metabolism in man. *Quarterly Journal of Medicine*. 1967; 14:1-15.
- Akgül AA, Toygar TU. Natural craniofacial changes in the third decade of life: a longitudinal study. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2002;122:512-522.
- Alappat SR, Zhang Z, Suzuki K, Zhang X, Liu H, Jiang R, Yamada G, Chen Y. The cellular and molecular etiology of the cleft secondary palate in Fgf10 mutant mice. *Developmental Biology*. 2005; 277, 102–113.
- Aldridge K, Boyadjiev SA, Capone GT, DeLeon VB, Richtsmeier JT. 2005. Precision and error of three-dimensional phenotypic measures acquired from 3dMD photogrammetric images. *Am J Med Genet Part A* 138A:247-253.
- Arboleda C, Buschang PH, Camacho JA, Botero P and Roldan S. A mixed longitudinal anthropometric study of craniofacial growth of Colombian mestizos 6–17 years of age. *European Journal of Orthodontics*. 2011; 33(4):441-9.
- Asai T, Wanaka A, Kato H, Masana Y, Seo M and Tohyama M. Differential expression of two members of FGF receptor gene family, FGFR-1 and FGFR-2 mRNA, in the adult rat central nervous system. *Brain Research. Molecular Brain Research*. 1993; 17, 174-178.
- Atchley WR, Hall BK. A model for development and evolution of complex morphological structures. *Biological Reviews of the Cambridge Philosophical Society*. 1991; 66 (2), 101–157.
- Baydaş B, Erdem A, Yavuz I, Ceylan I. Heritability of facial proportions and soft-tissue profile characteristics in Turkish Anatolian siblings. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2007 Apr; 131(4):504-9.
- Boehringer S, van der Lijn F, Liu F, Günther M, Sinigerova S, Nowak S, Ludwig KU, Herberz

- R, Bohnsack BL, Kasprick DS, Kish PE, Goldman D, Kahana A. A zebrafish model of axenfeld-rieger syndrome reveals that *pitx2* regulation by retinoic acid is essential for ocular and craniofacial development. *Investigative Ophthalmology and Visual Science*. 2012 Jan 3; 53 (1):7-22.
- Bevis RR, Hayles AB, Isaacson RJ, Sather AH. Facial growth response to human growth hormone in hypopituitary dwarfs. *Angle Orthodontist*. 1977; 47:193–205.
- Bouvier M, Hylander WL. Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). *Journal of Morphology*. 1981. 167: 1-12.
- Bresin A, Johansson C B, Kiliaridis S. Effects of occlusal strain on the development of the dentoalveolar process in the growing rat: A morphometric study. *European Journal of Experimental Musculoskeletal Research*. 1994; 3(2-3): 112-122.
- Brugmann Sa, Goodnough LH, Gregorieff A, Leucht P, ten Berge D, et al. Wnt signaling mediates regional specification in the vertebrate face. *Development*. 2007; 134 (18): 3283–3295.
- Buchtová M, Kuo WP, Nimmagadda S, Benson SL, Geetha-Loganathan P, et al. Whole genome microarray analysis of chicken embryo facial prominences. *Developmental dynamics: an official publication of the American Association of Anatomists*. 2010; 239: 574–591.
- Cai J, Ash D, Kotch LE, Jabs EW, Attie-Bitach T, et al. Gene expression in pharyngeal arch 1 during human embryonic development. *Human molecular genetics*. 2005; 14: 903–912.
- Cantu G, Buschang PH, Gonzalez JL. Differential growth and maturation in idiopathic growth hormone-deficient children. *European Journal of Orthodontics*. 1997;19:131–139.
- Carels C, Van Cauwenberghe N, Savoye I, Loos R, Derom C, Willems G, Vlietinck R: A quantitative genetic study of cephalometric. *Clinical Orthodontic and Research*. 2001; 4(3)130–140.
- Carlson DS. Temporal variation in prehistoric Nubian crania. *American Journal of Physiology and Anthropology*. 1976; 45:467–484.
- Carlson DS, Van Gerven DP. Masticatory function and post-Pleistocene evolution in Nubia. *American Journal of Physiology and Anthropology*. 1977; 46:495–506.
- Chai Y, Maxson RE. Recent advances in craniofacial morphogenesis. *Developmental dynamics: an official publication of the American Association of Anatomists*. 2006; 235: 2353–2375.
- Cheverud JM. A comparison of genetic and phenotypic correlations. *Evolution*. 1988; 42:958–968.
- Chokdeemboon C, Mahatumarat C, Rojvachiranonda N, Tongkobpetch S, Suphapeetiporn K,

- Shotelersuk V. FGFR1 and FGFR2 mutations in Pfeiffer syndrome. *Journal of Craniofacial Surgery*. 2013; Jan; 24(1):150-2.
- Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, Pearson LN, McEvoy B, Bauchet M, Zaidi AA, Yao W, Tang H, Barsh GS, Absher DM, Puts DA, Rocha J, Beleza S, Pereira RW, Baynam G, Suetens P, Vandermeulen D, Wagner JK, Boster JS, Shriver MD. Modeling 3D facial shape from DNA. *PLoS Genetics*. 2014; 10(3):e1004224.
- Cohen MM, Wagner R. Dental development in pituitary dwarfism. *Journal of Dental Research*. 1948; 27:445–458.
- Corruccini RS, Handler JS. Temporomandibular joint size decrease in American Blacks: evidence from Barbados. *Journal of Dental Research*. 1980; 59:1528.
- Coussens AK, van Daal A. Linkage disequilibrium analysis identifies an FGFR1 haplotype-tag SNP associated with normal variation in craniofacial shape. *Genomics*. 2005; 85:563-573.
- de Faria MEJ, Carvalho LR, Rossetto SM, Amaral TS, Berger K, Arnhold IJP, Mendonca BB. Analysis of craniofacial and extremity growth in patients with growth hormone deficiency during growth hormone therapy. *Hormone Research*. 2009; 71:173–177.
- Dohmoto A, Shimizu K, Asada Y, Maeda T. Quantitative trait loci on chromosomes 10 and 11 influencing mandible size of SMXA RI mouse strains. *Journal of Dental Research*. 2002; 81(7):501-504.
- Dorey K, Amaya E. FGF signaling: Diverse roles during early vertebrate embryogenesis. *Development*. 2010; 137: 3731-3742.
- Edler RJ. Cephalometric parameters in hypopituitary patients. *British Journal of Orthodontics*. 1979; 6:19–22.
- Engstrom C, Kiliaridis S, Thilander B. The relationship between masticatory function and cranial morphology II. A histological study in the growing rat fed a soft diet. *European Journal of Orthodontics*. 1986; 8: 271-279.
- Enlow D. 1990. Facial growth. Philadelphia: Saunders.
- Farkas LG. Anthropometry of the head and face in medicine. New York: Elsevier; 1981.
- Feng W, Simoes-de-Souza F, Finger TE, Restrepo D, Williams T. Disorganized olfactory bulb lamination in mice deficient for transcription factor AP-2epsilon. *Molecular and cellular neurosciences*. 1990; 42: 161–171.
- Frost HM, Villanueva AR, Stanisavljevic S, Roth H. Effect of thyroxine on chondro-osseous complex resorption in the rat. *Henry Ford Hospital Medical Bulletin*. 1962; 10(2):199-207.

- Funatsu M, Sato K, Mitani H. Effects of growth hormone on craniofacial growth. *Angle Orthodontist*. 2006; 76:970–977.
- Gómez-Valdés JA1, Hünemeier T, Contini V, Acuña-Alonzo V, Macin G, Ballesteros-Romero M, Corral P, Ruiz-Linares A, Sánchez-Mejorada G, Canizales-Quinteros S, Martínez-Abadías N, Salzano FM, González-José R, Bortolini MC. Fibroblast Growth Factor Receptor 1 (FGFR1) Variants and Craniofacial Variation in Amerindians and Related Populations. *American Journal of Human Biology*. 2013 Jan-Feb;25(1):12-9. Epub 2012 Oct 16.
- González-José R, Van der Molen MS, González-Pérez E, Hernandez M. Patterns of phenotypic covariation and correlation in modern humans as viewed from morphological integration. *American Journal of Physiology and Anthropology*. 2004; 123:69–77.
- González-José R, Ramirez-Rozzi F, Sardi M, Martínez-Abadías N, Hernández M, Pucciarelli HM. Functional-cranial approach to the influence of economic strategy on skull morphology. *American Journal of Physiology and Anthropology*. 2005; 128:757–771.
- Gorlin RJ, Cohen MM, Hennekam RCM. *Syndromes of the Head and Neck*. Oxford: Oxford University Press. 2001.
- Groth C, Lardelli M. 2002. The structure and function of vertebrate fibroblast growth factor receptor 1. *Int J Dev Biol* 46:393-400. Hallgrímsson B, Willmore K, Hall BK. Canalization, developmental stability, and morphological integration in primate limbs. *American Journal of Physiology and Anthropology*. 2001; 35:131–158.
- Hajihosseini MK, Duarte R, Pegrum J, Donjacour A, Lana-Elola E, Rice DP, Sharpe J, Dickson C. Evidence that Fgf10 contributes to the skeletal and visceral defects of an Apert syndrome mouse model. *Developmental Dynamics*. 2009; 238:376-385.
- Hallgrímsson B, Willmore K, Hall BK. 2002. Canalization, developmental stability, and morphological integration in primate limbs. *American Journal of Physiology and Anthropology*; 35: 131-158.
- Hallgrímsson B, Willmore K, Dorval C, Cooper DM. Craniofacial variability and modularity in macaques and mice. *Journal of Experimental Zoology. Part B Molecular and Developmental Evolution*. 2004; 302:207–225.
- Haworth KE, Islam I, Breen M, Putt W, Makrinou E, Binns M, Hopkinson D, Edwards Y. Canine TCOF1; cloning, chromosome assignment and genetic analysis in dogs with different head types. *Mammalian Genome*. 2001; 12(8):622-629.
- Heike CL, Cunningham ML, Hing AV, Stuhau E, Starr JR. 2009. Picture perfect? Reliability of craniofacial anthropometry using three-dimensional digital stereophotogrammetry. *Plast Reconstr Surg* 124(4):1261-1272.
- Hinton RJ, Carlson DS. Temporal changes in human temporomandibular joint size and shape.

- American Journal of Physiology and Anthropology. 1979; 50:325–333.
- Hochheiser H, Aronow BJ, Artinger K, Beaty TH, Brinkley JF, Chai Y, Clouthier D, Cunningham ML, Dixon M, Donahue LR, Fraser SE, Hallgrímsson B, Iwata J, Klein O, Marazita ML, Murray JC, Murray S, de Villena FP, Postlethwait J, Potter S, Shapiro L, Spritz R, Visel A, Weinberg SM, Trainor PA. 2011. The FaceBase Consortium: A comprehensive program to facilitate craniofacial research. *Dev Biol* 355(2):175-182.
- Huggare JA, Rønning OV. Growth of the cranial vault: influence of intracranial and extracranial pressures. *Acta Odontologica Scandinavica*. 1995; 53:192–195.
- Hughes, RA, Sendtner, M, Goldfarb, M, Lindholm, D and Thoenen, H. Evidence that fibroblast growth factor is a major muscle derived survival factor for cultured spinal motoneurons. *Neuron*. 1993; 10, 369-377.
- Hünemeier T, Gómez-Valdés J, De Azevedo S, Quinto-Sánchez M, Passaglia L, Salzano FM, Sánchez-Mejorada G, Alonzo VA, Martínez-Abadías N, Bortolini MC, González-José R. FGFR1 signaling is associated with the magnitude of morphological integration in human head shape. *American Journal of Human Biology*. 2014; 26(2):164-75.
- Hunter, WS, Balbach, DR, and Lamphiear, DE. The heritability of attained growth in the human face. *American Journal of Orthodontics*. 1970; 58: 128–134.
- Hylander WL. In vivo bone strain as an indicator of masticatory force in *Macaca fascicularis*. *Archives of Oral Biology*. 1986; 31:149–157.
- Ingraham CR, Kinoshita A, Kondo S, Yang B, Sajan S, Trout KJ, Malik MI, Dunnwald M, Goudy SL, Lovett M, Murray JC, Schutte BC. Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (*Irf6*). *Nature Genetics*. 2006; 38(11):1335-1340.
- Jaye, M, Schlessinger, J and Dionne, CA. Fibroblast growth factor receptor tyrosine kinases: molecular analysis and signal transduction. *Biochimica Biophysica Acta*. 1992; 1135, 185-199.
- Johannsdóttir B, Thorarínsson F, Thordarson A, Magnusson TE. Heritability of craniofacial characteristics between parents and offspring estimated from lateral cephalograms. *American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics*. 2005; 127: 200–7.
- Johnson DE, Williams LT. Structural and functional diversity in the FGF receptor multigene family. *Advanced Cancer Research*. 1993; 60:1-41.
- Johnsonbaugh RE, Bryan RN, Hierlwimmer UR, Georges LP. Premature craniosynostosis: A common complication of juvenile thyrotoxicosis. *The Journal of Pediatrics*. 1978; 93: 188-191.



- Jones KL. *Smith's Recognizable Patterns of Human Malformation*. Philadelphia: Elsevier Saunders. 2006; 954 p.
- Juriloff DM, Harris MJ. Mouse genetic models of cleft lip with or without cleft palate. *Birth Defects Research Part A Clinical and Molecular Teratology*. 2008; 82(2):63-77.
- Kang EH, Yamaguchi T, Tajima A, Nakajima T, Tomoyasu Y, et al. Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population. *Archives of oral biology*. 2009; 54: 556–562.
- Kiliaridis S. Muscle function as a determinant of mandibular growth in normal and hypocalcaemic rat. *European Journal of Orthodontics*. 1989; 11: 298-308.
- Kiliaridis S. Masticatory muscle influence on craniofacial growth. *Acta Odontologica Scandinavica*. 1995;53:196–202.
- Kim HJ, Rice DP, Kettunen PJ, Thesleff, I. FGF-, BMP- and Shh-mediated signalling pathways in the regulation of cranial suture morphogenesis and calvarial bone development. *Development*. 1995; 125, pp. 1241–1251.
- Kimmerle, EH, Ross, A and Slice, D. Sexual Dimorphism in America: Geometric Morphometric Analysis of the Craniofacial Region. *Journal of Forensic Sciences*. 2008; 53: 54–57.
- Kjaer I. Human prenatal craniofacial development related to brain development under normal and pathologic conditions. *Acta Odontologica Scandinavica*. 1995; 53:135–143.
- Kjellberg H, Beiring M, Albertsson WK. Craniofacial morphology, dental occlusion, tooth eruption, and dental maturity in boys of short stature with or without growth hormone deficiency. *European Journal of Oral Sciences*. 2000; 108:359–367.
- Klingenberg CP, Mebus K, Auffray JC. Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? *Evolution Development*. 2003; 5:522–531.
- Ko EW, Huang CS, Chen YR, Figueroa AA. Cephalometric craniofacial characteristics in patients with temporomandibular joint ankyloses. *Chang Gung Medical Journal*. 2005; Jul; 28(7):456-66.
- Kohn, LAP. The role of genetics in craniofacial morphology and growth. *Annual Review of Anthropology*. 1991; 20: 261–278.
- Lane C, Harrell W. 2008. Completing the 3-dimensional picture. *Am J Orthod Dentofacial Orthop* 133(4):612-620.
- Larsen C. Biological changes in human populations with agriculture. *Annual Review of Anthropology*. 1995; 24:185–213.
- Larsen C. *Bioarchaeology: interpreting behavior from the human skeleton*. 1997; Cambridge:

Cambridge University Press.

- Lee I, Lehner B, Crombie C, Wong W, Fraser AG, Marcotte EM. A single gene network accurately predicts phenotypic effects of gene perturbation in *Caenorhabditis elegans*. *Nature Genetics*. 2008; 2:181–188.
- Lieberman DE. How and why humans grow thin skulls: experimental evidence for systemic cortical robusticity. *American Journal of Physiology and Anthropology*. 1996; 101:217–236.
- Lieberman DE, McBratney BM, Krovitz G. The evolution and development of cranial form in *Homo sapiens*. *Proceedings of the National Academy of Sciences of the USA* 99. 2002;1134–1139.
- Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, Wollstein A, Lao O, de Bruijne M, Ikram MA, van der Lugt A, Rivadeneira F, Uitterlinden AG, Hofman A, Niessen WJ, Homuth G, de Zubicaray G, McMahon KL, Thompson PM, Daboul A, Puls R, Hegenscheid K, Bevan L, Pausova Z, Medland SE, Montgomery GW, Wright MJ, Wicking C, Boehringer S, Spector TD, Paus T, Martin NG, Biffar R, Kayser M. A genome-wide association study identifies five loci influencing facial morphology in Europeans. *PLoS Genetics*. 2002; 8(9):e1002932.
- Losken A, Fishman I, Denson DD, Moyer HR, Carlson GW. 2005. An objective evaluation of breast symmetry and shape differences using 3-Dimensional images. *Ann Plast Surg* 55(6):571-575.
- Lundström A, JJ. McWilliam. A comparison of vertical and horizontal cephalometric variables with regard to heritability. *European Journal of Orthodontics*. 1987; pp. 104–108.
- Lundstrom A. The importance of genetic and non-genetic factors in the facial skeleton studied in one hundred pairs of twins. *European Orthodontic Society Report of Congress, 30 (1954)*, pp. 92–107.
- Malina RM, Bouchard C, Bar-or O. Growth, maturation, and physical activity. Champaign: Human kinetics; 2004.
- Manfredi C, Martina R, Grossi GB, Giuliani M. Heritability of 39 orthodontic cephalometric parameters on MZ, DZ twins and MN-paired singletons. *American Journal of Orthodontics and Dentofacial Orthopedics*. 1997 Jan;111(1):44-51.
- Markus MB, Goosman SD, Einhorn NH, Lerner J. Facial development in hypopituitary dwarfism. *American Journal of Orthodontics and Oral Surgery*. 1942; 23:334–350.
- Morrison, RS, Sharma, A, DeVellis, J and Bradshaw, RA. Basic fibroblast growth factor supports the survival of cerebral cortical neurons in primary culture. *Proceeding of the National Academy of Sciences of the USA*. 1986; 83, 7537-7541.
- Moss ML. Rotations of the cranial components in the growing rat and their experimental

- alteration. *Acta Anatomica*. 1958; 32: 65-86.
- Moss ML, Young RW. A functional approach to craniology. *American Journal of Physiology and Anthropology*. 1960; 18(4):281-292.
- Mossey PA. The heritability of malocclusion: part 2: The influence of genetics in malocclusion. *British Journal of Orthodontics*. 26 (1999), pp. 195–203.
- Naini FB, Moss JP. Three-dimensional assessment of the relative contribution of genetics and environment to various facial parameters with the twin method. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2004; Dec; 126(6):655-65.
- Nakata, N, Yu, PI, Davis, B, and Nance, WE. The use of genetic data in the prediction of craniofacial dimensions. *American Journal of Orthodontics*. 1973; 63: 471–480.
- Nanni L, Ming JE, Bocian M, Steinhaus K, Bianchi DW, et al. The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly. *Human molecular genetics*. 1999; 8: 2479–2488.
- Nie X, Luukko K, Kettunen P. FGF signaling in craniofacial development and developmental disorders. *Oral Disease*. 1996; 12: 102-111.
- Nishimura I, Drake TA, Lusk AJ, Lyons KM, Nadeau JH, Zernik J. ENU large-scale mutagenesis and quantitative trait linkage (QTL) analysis in mice: novel technologies for searching polygenetic determinants of craniofacial abnormalities. *Critical Review of Oral Biology Medicine*. 2003; 14(5):320-330.
- Nwoku AL. Rehabilitating children with temporomandibular joint ankylosis. *Int J Oral Surg* 1979; 8:271-5.
- Oh J, Wang CJ, Poole M, Kim E, Davis RC, Nishimura I, Pae EK. A genome segment on mouse chromosome 12 determines maxillary growth. *Journal of Dental Research*. 2007; 86(12):1203-1206.
- Oliveira-Neto LA, Melo Mde F, Franco AA, Oliveira AH, Souza AH, Valença EH, Britto IM, Salvatori R, Aguiar-Oliveira MH. Cephalometric features in isolated growth hormone deficiency. *Angle Orthodontist*. 2011 Jul; 81(4):578-83.
- Ornitz and Itoh. Fibroblast growth factors. *Genome Biology*. 2001; 2:1-12.
- Paschetta C, Azevedo S, Castillo, Martí'nez-Abadías N, Herná'ndez M, Lieberman DE, González-José R. The Influence of Masticatory Loading on Craniofacial Morphology: A Test Case Across Technological Transitions in the Ohio Valley. *AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY*. 2010; 141:297–314.
- Passos-Bueno MR, Wilcox WR, Jabs EW, Sertié AL, Alonso LG, Kitoh H. Clinical spectrum of

- fibroblast growth factor receptor mutations. *Human Mutation*. 1999; 14(2):115-125.
- Passos-Bueno MR, Serti Eacute AE, Jehee FS, Fanganiello R, Yeh E. Genetics of craniosynostosis: genes, syndromes, mutations and genotype-phenotype correlations. In: Rice D, editor. *Craniofacial Sutures: Development, Disease and Treatment*. 2008; Vol 12. Basel: Karger. p 107-143.
- Paternoster L, Zhurov AI, Toma AM, Kemp JP, St Pourcain B, Timpson NJ, McMahon G, McArdle W, Ring SM, Smith GD, Richmond S, Evans DM. Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. *American Journal of Human Genetics*. 2012; 90(3):478-485.
- Penfold JL, Simpson DA. Premature craniosynostosis- A complication of thyroid replacement therapy. *The Journal of Pediatrics*. 1975; 86: 360-363.
- Peng S, Tan J, Hu S, Zhou H, Guo J, Jin L, Tang K. Detecting genetic association of common human facial morphological variation using high density 3D image registration. *PLoS Computational Biol*. 2013; 9(12).
- Persson EC, Engström C, Thilander B. *Eur J Orthod*. The effect of thyroxine on craniofacial morphology in the growing rat. Part I: A longitudinal cephalometric analysis. 1989; 11(1):59-6.
- Persson M. The role of sutures in normal and abnormal craniofacial growth. *Acta Odontologica Scandinavia*. 1995; 53:152–161.
- Pirinen S, Majurin A, Lenko HL, Koski K. Craniofacial features in patients with deficient and excessive growth hormone. *Journal of Craniofacial Genetics and Developmental Biology*. 1994; 14: 144–152.
- Pirinen S. Endocrine regulation of craniofacial growth. *Acta Odontologica Scandinavia*. 1995; 53:179–185.
- Pitteloud N, Acierno JS Jr., Meysing A, Eliseenkova AV, Ma J, Ibrahim OA, Metzger DL, Hayes FJ, Dwyer AA, Hughes VA, Yialamas M, Hall JE, Grant E, Mohammadi M, Crowley WF Jr. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proceedings of the National Academy of Sciences of the U.S.A*. 2006; 103, 6281–6286.
- Pretorius E, Steyn M, Scholtz Y. Investigation into the usability of geometric morphometric analysis in assessment of sexual dimorphism. *America Journal of Physical Anthropology*. 2006; 129(1):64–70.
- Proffit WR, Fields HW, Sarver DM. *Contemporary Orthodontics* 5th Ed. 2013.
- Pucciarelli HM. Craniofacial development of the rat with respect to vestibular orientation. *Acta Anatomica*. 1978; 32: 65-86.

- Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dodé C, Mohammadi M, Marazita ML, Murray JC. Impaired FGF signaling contributes to cleft lip and palate. *Proceedings of the National Academy of Sciences of the U.S.A.* 2007; 104, 4512–4517.
- Robin NH, Falk MJ, Haldeman-Englert CR. *FGFR-Related Craniosynostosis Syndromes*. 1998. Gene Reviews [Internet].
- Ronning O. Alterations in craniofacial morphogenesis induced by parentally administered papain. *Sumalainen Hammar Laakeri Toim.* 1971. Suppl I. III 67.
- Ronning O. Basicranial synchondroses and the mandibular condyle in craniofacial growth. *Acta Odontologica Scandinavia.* 1995; 53: 162–166.
- Rosas A, Bastir M. Thin-plate spline analysis of allometry and sexual dimorphism in the human craniofacial complex. *Am J Phys Anthropol* 2002; 117(3):236–45.
- Ross AH, Williams S. Testing repeatability and error of coordinate cranial landmark data acquired from skulls. *Journal of Forensic Sciences.* 2008; 53:782Y785.
- Ross, AH, Williams, SE. Craniofacial Growth, Maturation, and Change: Teens to Midadulthood. *Journal of Craniofacial Surgery.* Issue: Volume 21(2), March 2010, pp 458-461.
- Salvatori R, Hayashida CY, Aguiar-Oliveira MH, et al. Familial dwarfism due to a novel mutation of the growth hormone releasing hormone receptor gene. *Journal of Clinical Endocrinology Metabolism.* 1999; 84: 917–923.
- Sardi ML, Novellino PS, Pucciarelli HM. Craniofacial morphology in the Argentine Center-West: consequences of the transition to food production. *American Journal of Physical Anthropology.* 2006; 130: 333–343.
- Sarnäs KV, Solow B. Early adult changes in the skeletal and soft-tissue profile. *European Journal of Orthodontics.* 1980; 2:1-12.
- Savoye, R. Loos, C. Carels, C. Derom, R. Vlietinck. A genetic study of anteroposterior and vertical facial proportions using model-fitting. *Angle Orthodontist.* 68 (1998), pp. 467–470.
- Schlessinger, J and Ullrich, A. Growth factor signalling by receptor tyrosine kinases. *Neuron* 9. 1992; 383-391.
- Schumacher G-H. Principles of skeletal growth. In: Dixon A, Hoyte D, Ronning O, eds. *Fundamentals of Craniofacial Growth.* Boca Raton, FL: CRC Press, 1997:21.
- Segal DG, Pescovitz OH, Schaefer GB, DiMeglio LA. Craniofacial and acral growth responses in growth hormone- deficient children treated with growth hormone. *Journal of Pediatrics.* 2004; 144:437–443.

- Sherwood RJ, Duren DL, Havill LM, Rogers J, Cox LA, Towne B, Mahaney MC. A genomewide linkage scan for quantitative trait loci influencing the craniofacial complex in baboons (*Papio hamadryas* spp.). *Genetics*. 2008; 180(1):619-628.
- Simonis N, Migeotte I, Lambert N, Perazzolo C, de Silva DC, Dimitrov B, Heinrichs C, Janssens S, Kerr B, Mortier G, Van Vliet G, Lepage P, Casimir G, Abramowicz M, Smits G, Vilain C. *FGFR1* mutations cause Hartsfield syndrome, the unique association of holoprosencephaly and ectrodactyly. *Journal of Medicine and Genetics*. 2013 Sep; 50(9):585-92.
- Sperber GH. *Craniofacial Development*. London: BC Decker Inc, 2001.
- Spiegel RN, Sather AH, Hayles AB. Cephalometric study of children with various endocrine diseases. *American Journal of Orthodontics*. 1971; 59: 362–375.
- Stachowiak MK, Fang X, Myers JM, Dunham SM, Berezney R, Maher PA, Stachowiak EK. Integrative nuclear *FGFR1* signaling (INFS) as a part of a universal "feed-forward-and-gate" signaling module that controls cell growth and differentiation. *J Cell Biochem*. 2003 Nov 1; 90(4):662-91.
- Stockard C. The genetic and endocrinic basis for differences in form and behaviour, as elucidated by studies of contrasted pure-line dog breeds and their hybrids. Philadelphia: The Wistar Institute of Anatomy and Biology. 1941.
- Tanner JM. *Growth adolescence*. 2nd edn. Oxford: Blackwell Scientific Publications; 1962.
- Thilander B. Basic mechanisms in craniofacial growth. *Acta Odontologica Scandinavica*. 1995; 53:144–151.
- Toma AM, Zhurov A, Playle R, Richmond S. *Orthod Craniofac Res*. A three-dimensional look for facial differences between males and females in a British-Caucasian sample aged 151/2 years old. 2008 Aug; 11(3):180-5.
- Toma Arshed M, Zhurov Alexei I, Playle Rebecca, Marshall David, Rosin Paul L, Richmond Stephen. The assessment of facial variation in 4747 British school children. *European Journal of Orthodontics*; 2011.
- VanErum R, Mulier G, Carels C, deZegher F. Craniofacial growth and dental maturation in short children born small for gestational age: effect of growth hormone treatment—own observations and review of the literature. *Hormone Research*. 1998; 50:141–146.
- Veldhuis JD, et al. Endocrine control of body composition in infancy, childhood, and puberty. *Endocrine Reviews* 2005; 26:114-146.
- Vilmann H, Moss ML. Studies on orthocephalization 2. Flexions of the rat head in the period between 14 and 60 days after gestation. *Gegenbaurs morphologische Jahrbuch, Leipzig*. 1979; 125: 572-582.

- Wagner GP. Homologues, natural kinds and the evolution of modularity. *American Zoology*. 36:36–43. 1996.
- Wagner GP, Altenberg L. Complex adaptations and the evolution of evolvability. *Evolution* 50:967–976. 1996.
- Walicke, PA. Novel neurotrophic factors, receptors and oncogenes. *Ann Rev. Neurosci.* 1989; 12, 103-126.
- Wanaka, A, Johnson, EM, Jr. and Milbrandt, J. Localisation of FGF receptor mRNA in the adult rat central nervous system by in situ hybridisation. *Neuron* 5. 1990; 267-281.
- Wang C, Chang JY, Yang C, Huang Y, Liu J, You P, McKeehan WL, Wang F, Li X. Type 1 fibroblast growth factor receptor in cranial neural crest cell-derived mesenchyme is required for palatogenesis. *Journal of Biology and Chemistry*. 2013 Jul 26; 288(30):22174-83.
- Weinberg SM, Naidoo S, Govier DP, Martin RA, Kane AA, Marazita ML. 2006. Anthropometric precision and accuracy of digital three-dimensional photogrammetry: comparing the Genex and 3dMD imaging systems to one another and to direct anthropometry. *J Craniofac Surg* 17(3):477-483.
- Weinberg SM, Parsons TE, Marazita ML, Maher BS. Heritability of face shape in twins: a preliminary study using 3D stereophotogrammetry and geometric morphometrics. *Dentistry* 3000. Vol 1, No 1 (2013).
- Wilkie AO, Morriss-Kay GM, Jones EY, Heath JK. Functions of fibroblast growth factors and their receptors. *Current Biology*. 1995; 5:500-507.
- Williams EJ, Furness J, Walsh FS, Doherty P. Characterisation of the second messenger pathway underlying neurite outgrowth stimulated by FGF. *Development*. 1994 Jun; 120(6):1685-93.
- Wong JY, Oh AK, Ohta E, Hunt AT, Rogers GF, Mulliken JB, Deutsch CK. 2008. Validity and reliability of craniofacial anthropometric measurement of 3D digital photogrammetric images. *Cleft Palate Craniofac J* 45(3):232-239.
- Yamada K, Kimmel D B. The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Archives of Oral Biology* 36. 1991; 129-138.
- Yamamoto S. The effects of food consistency on maxillary growth in rats. 6. *European Journal of Orthodontics*. 1996 Dec; 18(6):601-15.