GENETIC AND PHENOTYPIC EVALUATION OF THE CLASS III DENTOFACIAL DEFORMITY: COMPARISONS OF THREE POPULATIONS

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ABSTRACT

KEISHA N. ALEXANDER:
Genetic and Phenotypic Evaluation of the Class III Dentofacial Deformity: Comparisons of Three Populations
(Under the direction of Dr. Sylvia A. Frazier-Bowers)

The etiology of skeletal Class III malocclusion is multifactorial, complex and likely results from mutations in numerous genes. In this study, we sought to understand the phenotype/genotype correlation of the Class III trait in 3 specific populations, a Colombian cohort, Amelogenesis Imperfecta (AI) cohort and a Caucasian cohort. The phenotype was evaluated using multiple statistical comparisons of 3 populations followed by genetic analysis of 2 populations. Phenotypic analysis indicated a difference between the z-scores of 10 cephalometric variables among the 3 groups. Pedigree analysis by inspection supported an autosomal dominant mode of inheritance with incomplete penetrance. A Genome-wide scan and linkage analysis of members in 2 cohorts revealed 3 regions suggestive of linkage for the Colombian cohort but was inconclusive for the AI cohort. Our phenotypic and genetic analysis highlights that each group is unique, and that differences between them could be due to specific craniofacial morphologic features.

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ABBREVIATIONS

saddle	Saddle/sella angle (SN-Ar)
gonang	Gonial/Jaw angle (Ar-Go-Me)
chinang	Chin angle (Id-Pg-MP)
acb	Length of ant cranial base (SN)
pcb	Length of post cranial base (S-Ar)
ramht	Ramus height (Ar-Go)
mdlgth	Length of Mn base (Go-Pg)
factap	Facial Taper
artang	Articular angle
facang	Facial angle (FH-NPo)
convex	Convexity angle (NA-APo)
abfp	A-B to facial plane angle
fpsn	Facial plane to SN (SN-NPog)
midface	Midface Length (Co-A)
pafaceht	P-A Face height (S-Go/N-Me)
yang	Y-Axis angle (SGn-SN)
sna	SNA
snb	SNB
anb	ANB
anvt	A to N Vert (True Vert) (mm)
bnvt	B to N Vert (True Vert) (mm)

pgnv	Pg to N Vert (True Vert) (mm)
anperp	A-N Perpendicular (mm)
bnperp	B-N Perpendicular (mm)
pgnp	Pog-N Perpendicular (mm)
mxul	Mx Unit Length (Co-ANS) (mm)
mdul	Mand Unit length (Co-Gn) (mm)
unitdif	Mx-Mand Unit Length (mm)
u1sndeg	U1 - SN (º)
u1nadeg	U1 - NA (º)
u1namm	U1 - NA (mm)
u1fhdeg	U1 - FH (º)
impa	IMPA (L1-MP) (º)
l1nbdeg	L1 - NB (º)
l1nbmm	L1 - NB (mm)
liprot	L1 Protrusion (L1-APo) (mm)
l1apo	L1 to A-Po (º)
wits	Wits Appraisal (mm)
interang	Interincisal Angle (U1-L1) (º)
oj	Overjet (mm)
pgnbmm	Pog - NB (mm)
hold	Holdaway Ratio (L1-NB:Pg-NB) (%)
fmia	FMIA (L1-FH) (º)
tfh	Total Anterior Face Ht (N-Me) (mm)

ufh	Upper Face Height (N-ANS) (mm)
lfh	Lower Face Height (ANS-Me) (mm)
nasht	Nasal Height (%)
pfh	Post Facial Ht (Co-Gn) (mm)
pfhafh	PFH:AFH (%)
fma	FMA (MP-FH) (º)
sngogn	SN - GoGn (º)
opsn	Occ Plane to SN (º)
opfh	Occ Plane to FH (º)
fhsn	FH - SN (º)
u1ppmm	U1 - PP (UADH) (mm)
l1mpmm	L1 - MP (LADH) (mm)
u6ppmm	U6 - PP (UPDH) (mm)
l6mpmm	L6 - MP (LPDH) (mm)
obite	Overbite (mm)
uleplane	Upper Lip to E-Plane (mm)
lleplane	Lower Lip to E-Plane (mm)
softnvtul	STissue N Vert (True Vert) to Upper Lip (mm)
softnvtll	STissue N Vert (True Vert) to Lower Lip (mm)
softnvtpg	STissue N Vert (True Vert) to ST Pogonion (mm)
softnpul	STissue N Vert (N Perp) to Upper Lip (mm)
softnpll	STissue N Vert (N Perp) to Lower Lip (mm)
softnppg	STissue N Vert (N Perp) to ST Pogonion (mm)

CHAPTER 1

INTRODUCTION

Skeletal Class III malocclusion is a general morphological description of a diverse group of dentofacial conditions in which the mandibular teeth are forward in relationship to the maxillary teeth, resulting in an anterior crossbite or underbite. The term skeletal implies that the positions of the teeth are the result of underlying jaw relationships. This type of skeletal occlusal pattern is also referred to as true Class III or true mesiocclusion. These conditions are developmental to the extent that they are not recognizable at birth and by definition, until the individual is dentate, it is not possible to make a diagnosis of skeletal Class III malocclusion. As one would expect there is a higher incidence of this condition in the transitional and adult dentitions than there is in the primary dentition. The antero-posterior dental discrepancy often becomes more significant during growth and does not reach its complete expression until the individual is fully mature. In acromegaly the mandible continues to grow even after most other somatic growth has ceased.

Skeletal Class III malocclusions are among the few orthodontic conditions in which there are often physiologic and psychosocial symptoms associated with the physical signs of the condition. When an underlying skeletal dysplasia becomes great enough, the individual is said to have a dentofacial deformity. The facial skeleton of an individual with a true Class III malocclusion can have one or more of three possible jaw configurations. The first, and perhaps most common is maxillary hypoplasia, midface deficiency or

retrusion of the maxillary complex. The second is mandibular prognathism and the third is increased cranial base flexure and or a shortened anterior cranial base. Since the mandible articulates (hinges) with the rest of the skull at the temporal bones, the positions of the glenoid fossae have a major influence on maxillo-mandibular relationships. A Class III deformity can be an attribute of a syndrome, as in achondroplasia with associated midface deficiency resulting from a failure in the development of the cartilaginous nasal capsule or can merely be a manifestation of normal morphologic variation. Where a growth effect is responsible for the skeletal Class III problem, the affect can be primary and active, such as in acromegaly where an increased production of pituitary growth hormone acts on the condylar cartilage creating exuberant mandibular growth.

It has long been known that some skeletal Class III malocclusions have a familial history. Many of the Hapsburgs, a famous ruling family in Europe for nearly six centuries, had characteristically large lower jaws. Unfortunately, there have been relatively few studies of families with a high frequency of skeletal Class III malocclusions. Although the Hapsburg cohort is likely autosomal dominant, this pedigree structure is undoubtedly confounded by known consanguinity, thus not ruling out the possibility of an autosomal recessive inheritance pattern. Environmental factors have been implicated as contributing factors for the development of skeletal Class III malocclusion; however, there is little evidence to support this hypothesis. Recent gene mapping and linkage analysis of individuals with achondroplasia and acromegaly have identified some of the responsible genes. Since skeletal Class III malocclusion is one of the manifestations of these two disorders it gives hope that the genetic determinants of facial development in general and facial deformity in particular will be better understood in the near future.

While research in humans holds great promise, animal models have led the way. In particular, multiple studies have been completed in transgenic mice that manifest mandibular prognathism (Machicek S.L., et al 2007). Moreover, the advent of the U.S. Human Genome Project (HGP) in 1990 focused attention on the construction of comprehensive genetic maps for locating and identifying genes underlying susceptibility to disease. This increasingly detailed knowledge of the human genome at the DNA level forms the basis of our understanding of genetic transmission and gene action. These advances in molecular biology and human genetics have made it possible to study the genetics of craniofacial disorders with more precision. The Insulin-like Growth Factor 1 gene (IGF1), which mediates growth hormone (GH), which acts on the growth and development of bones and muscles postnatally, has been shown in previous studies to be major contributors in the body size in small dogs and in synthetic cattle breed.

Skeletal Class III malocclusions are perhaps the most challenging orthodontic problems to diagnose and treat. One of the likely reasons for the difficulty is that the etiology of a jaw disproportion for a specific individual is rarely known. Surely, there is nothing more essential in establishing a treatment plan for a patient with this problem than the consideration of future growth. Treatment decisions should be based on the direction, amount, duration and pattern of craniofacial growth and particularly its completion. The efficacy of utilizing dentofacial orthopedics to modify or redirect facial growth in skeletal Class III patients is controversial and the determination of the borderline between those patients who can be treated non-surgically (orthopedically) and those who require surgery is poorly defined. With the recent introduction of temporary bone anchors in orthodontics,

it is possible that orthodontists will have the ability to gain a greater orthopedic affect than previously possible.

If it were possible to identify subgroups of the current non-specific morphological classification of skeletal Class III malocclusions and if these subgroups could be defined genetically, rather than phenotypically we would be a great deal further toward our goal of being able to treat these conditions in a more rational and effective fashion. If it were possible to firmly establish the genetic nature of the problem it might reduce the uncertainty regarding future growth and therapeutic modifiability. In this study we hypothesize that the Class III dentofacial deformity is clinically and genetically heterogeneous presenting with a distinct subphenotype and genotype in 3 cohorts.

We will test the above hypothesis with the following specific aims below:

1) Based on radiographic cephalometric measurements, utilize multivariate analysis of variance (MANOVA) to phenotypically characterize the Class III trait in 3 specific populations (Colombian/Hispanic, AI/Enriched and Caucasian families).
2) Conduct genome-wide scans followed by linkage analysis to identify the genetic loci associated with the Class III trait in the Colombian and AI populations.

CHAPTER 2
BACKGROUND

In discussing the phenotypic trait, skeletal Class III malocclusion, the development of this disorder must first be considered in the context of the embryology and growth of the craniofacial skeleton. The bony skull is formed from two components. The neurocranium, which surrounds and protects the brain and sense organs, which include the frontal parietal, temporal, occipital and sphenoid bones, and the viscerocranium includes the bones of the face (mandible, maxilla, zygoma and nasal), and the palatal, pharyngeal, temporal and auditory bones. The entire viscerocranium and part of the neurocranium are formed from the neural crest – a mesenchymal tissue that migrates from the lateral edges of the epithelial neural plate to form a great variety of cell types (Wilkie et al 2001). There are two distinct developmental processes involved in the formation of skeletal elements. Intramembranous ossification gives rise to the flat bones that comprise the cranium and medial clavicles. Endochondral ossification gives rise to long bones that comprise the appendicular skeleton, facial bones, vertebrae, and the lateral medial clavicles. These two types of ossification involve an initial condensation of mesenchyme and eventual formation of calcified bone. Intramembranous bone formation accomplishes this directly, whereas endochondral ossification incorporates an intermediate step where a cartilaginous template regulates the growth and patterning of the developing skeletal element (Ornitz D., et al 2002). The development of the cranial vault is a complex process involving cells of neural crest

origin and paraxial mesoderm that contribute to intramembranous bones of the cranial vault and sutures (Ornitz D., et al 2002).

Genes involved in the regulation of growth of the skeleton have already been identified. Approximately a half-century ago, Daughaday et al introduced the somatomedin hypothesis which aided in the improvement of our knowledge of the insulin-like growth factor (IGF) system (Roith 1999). Growth in animals is controlled by a complex system, where the somatotropic axis plays an important role in postnatal growth. IGF-1 mediates the direct action of growth hormone on the regulation of growth and development of bones and muscles postnatally. IGF-1 is responsible for the stimulation of protein metabolism and plays a key role in the function of some organs and is considered a factor of cellular proliferation and differentiation (Pereira 2005). The Insulin-like Growth Factor 1 gene (IGF1) has been shown to be involved in postnatal growth and development of bones and muscles in previous studies involving small dogs (Sutter et al 2007).

The etiology of skeletal Class III malocclusion is clearly wide ranging and complex. It is a multifactorial, polygenic trait which most likely results from mutations in numerous genes. Skeletal Class III malocclusion can occur among various groups of people such as those possessing syndromic conditions with a genetic etiology, such as achondroplasia, acromegaly and Crouzon syndrome. Other dental anomalies such as Amelogenesis Imperfecta (AI) can occur during the stages of enamel has been noted for specific craniofacial features including Class III malocclusion. Genetic mutations have been identified in the development of these conditions. Mutations in the FGFR3 (Fibroblast Growth Factor Receptor 3) gene, results in achondroplasia, while mutations in the MEN-1, results in acromegaly. Crouzon syndrome, or craniofacial dystosis is a rare

deformity that is closely related to Apert syndrome. Although many of the physical deficiencies associated with Apert are not present in the Crouzon syndrome patient, both are thought to have similar genetic origins. Crouzon syndrome patients have three distinct features: Craniosynostosis (premature fusion of the cranial sutures) most often of the coronal and lambdoid, and occasionally sagittal sutures; underdeveloped midface with receded cheekbones or exophthalmos (bulging eyes) and ocular proptosis which is a prominence of the eyes due to very shallow orbits. The patient may have crossed eyes and/or wide-set eyes. In both Apert and Crouzon syndromes, inheritance is autosomal dominant and results from the mutations of the fibroblast growth factor receptors (FGFR) 1 – 3 genes (Preising M., et al 2003). Genetic characterization of AI has also led to the identification of several mutations. Mutations in the amelogenin gene (AMELX) cause X-linked amelogenesis imperfecta, while mutations in the enamelin gene (ENAM) cause autosomal-inherited forms of amelogenesis imperfecta (Ravassipour et al 2005).

Skeletal Class III malocclusion represents a very small proportion of the total incidence of malocclusion, and is most prevalent in Oriental populations with a range reported from 3-23% in Asian Mongoloid populations of Taiwanese, Japanese, Korean and Chinese (Susami 1972, Tang 1994). Certain X-chromosome aneuploidal conditions can also lead to mandibular prognathism and are predominantly an inherited trait (Jena et al 2005). Environmental factors that have been suggested as contributing to the development of Class III malocclusion include enlarged tonsils, difficulty in nasal breathing, congenital anatomic defects, disease of the pituitary gland, hormonal disturbances, premature loss of the maxillary six year molars and irregular eruption of permanent incisors or premature loss of deciduous incisors. Other factors such as the size

and relative positions of the cranial base, maxilla and mandible, the position of the temporomandibular articulation and any displacement of the lower jaw also affect both the sagittal and vertical relationships of the jaws and teeth. The position of the foramen magnum, spinal column and habitual head position may also influence the eventual facial pattern.

The morphological mechanisms involved in the etiology of Class III malocclusions are an important consideration in the development of this trait. Singh (1999) inferred that an acute cranial base angle may affect the articulation of the condyles in their glenoid fossae resulting in their forward displacement, and he also inferred that the reduction in the anterior cranial base size may affect the position of the maxilla. Recent studies have supported this morphologic feature in a transgenic mouse model as well (Machicek et al 2007). In particular, studies have been carried out in an achondroplastic mouse model. These mice have a phenotype that resembles human achondroplasia, including a domed skull, hypoplastic midface and nasal bone, anteriorly displaced foramen magnum, and a prognathic mandible. Achondroplasia is defined as a defect of cartilage and results from either a genetic mutation of the fibroblast growth factor receptor 3 (FGFR3) gene located on chromosome 4, or it can be inherited from a parent with the condition, where one copy of the altered gene in each cell is sufficient to cause the disorder (Machicek 2007).

Currently, the timing of treatment for the Class III patient is difficult, but a greater understanding of the relationship between the genotype and phenotype of this disorder may improve the outcome of treatment. In addition to the fact that the phenotype is difficult to define precisely, craniofacial growth, and particularly the growth of the

mandible, is highly variable and is reported to continue into the late teens and well beyond the third decade of life. An emphasis should be placed on devising an effective method of not only diagnosing mandibular prognathism, but also investigating the heritable patterns of each skeletal morphologic characteristic that may contribute to it. Once this definitive method of phenotypic classification is developed, whereby homologous phenotypes and not analogous ones are considered part of the same group, this study could be expanded to other populations.

Establishing the genetic etiology of skeletal Class III malocclusion may provide hope for improvements in the management of such patients and allow the clinician to elect an early intervention aimed at intercepting the development of Class III malocclusions. Molecular genetic information may be used in the future to accurately predict long-term growth changes, and may ultimately lead to the utilization of gene therapy. Understanding the specific genetic factors contributing to the risk for mandibular prognathism would be a major advancement in dentofacial orthopedics and potentially reduce the need for oral and maxillofacial surgery in the treatment of skeletal Class III patients.

Current technological tools have provided the opportunity to study the molecular and environmental origins of Class III malocclusion. These tools include linkage, but are not limited to, SNP (Single Nucleotide Polymorphism) markers, microsatellite markers, and 3-Dimensional Computed Tomography (3-D CT). Information from these technological advances can aid in further understanding the growth and development of Class III malocclusion.

In order to completely understand the genetic component of skeletal Class III malocclusion, one must first establish a clear definition of the phenotype. The phenotype can be thought of as a clinical expression of an individual's specific genotype. In the study reported here, we initially used Cephalometric analysis to characterize the phenotype. After characterizing the phenotype, the multivariate analysis of variance (MANOVA) is used to distinguish the variations in the phenotype of each of the groups. The first step in elucidating the genetic components in the development of mandibular prognathism was a genome wide linkage study in two groups. The genotype refers to an organism's exact genetic makeup, that is, the particular set of genes it possesses. Two organisms whose genes differ at even one locus (position in their genome) are said to have different genotypes. The transmission of genes from parents to offspring is under the control of precise molecular mechanisms. The discovery of these mechanisms and their manifestations began with Mendel and comprises the field of genetics. The term "genotype" refers, then, to the full hereditary information of an organism. The inheritance of physical properties occurs only as a secondary consequence of the inheritance of genes (Wikepedia). The Human Genome Project (HGP) in 1990 focused attention on the construction of comprehensive genetic maps for locating and identifying genes underlying susceptibility to disease. Increasingly detailed knowledge of the human genome at the DNA level forms the basis of our understanding of genetic transmission and gene action. The Human Genome Project has mapped 30,000 genes thus far, and therefore provides the basis for genetic diagnosis and therapy. These advances in molecular biology and human genetics have made it possible to study the genetics of craniofacial disorders with more precision.

The advancement in the field of molecular genetics should make it possible to identify relevant genetic markers for such traits as skeletal Class III malocclusion. The existence of familial aggregation of mandibular prognathism (MP) suggests that genetic components play an important role in its etiology and several studies have demonstrated this (Jena et al 2005, Litton et al 1970). Mandibular prognathism has been shown to be an autosomal dominantly inherited trait.

Amelogenesis imperfecta (AI) is an inherited enamel dysplasia involving both dentitions with no other systemic effects. The hereditary pattern is autosomal or X-related dominant or recessive. Its prevalence is approximately 1:14,000-1:16,000. It can be classified as hypocalcified, hypoplastic and hypomatured according to clinical, radiological, histological and hereditary findings (Turkun 2005). Amelogenesis Imperfecta (AI) serves as an interesting model for studying the genetics of Class III skeletal pattern because it has preliminarily been shown to be associated with a higher incidence of skeletal Class III malocclusion relative to the general population (F-B., et al, unpublished). By comparing the genes involved in the etiology of Amelogenesis Imperfecta, it is possible that further clues to the genetic etiology of Class III malocclusion can be ascertained.

Establishing the genetic etiology of skeletal Class III malocclusion may not have a direct clinical application in the immediate future, however, detection of the gene(s) involved may provide hope for improvements in the management of such patients. This information may be used to accurately predict long-term growth changes, and may ultimately lead to potential gene therapies. In the studies described in this publication, we aim to first understand the phenotypic variation of the Class III malocclusion (or

dentofacial deformity) and then to begin to embark upon unraveling the genetic basis of this common problem.

CHAPTER 3

III. REVIEW OF THE LITERATURE

The genetic etiology of Class III malocclusion has been demonstrated in several studies. In 2005, Bui, et al., demonstrated that the Class III trait was inherited in an autosomal dominant fashion in the 12 families that they studied. This has been previously suggested by other studies (Mossey, et al. 1999).

Certain syndromic conditions with a genetic etiology, such as Crouzon syndrome, acromegaly and achondroplasia, have been described as presenting with skeletal Class III malocclusion (Preising M., et al 2003, Machicek et al., 2007, Yagi et al., 2004). Normal growth and development of the craniofacial complex is affected by the function of the endocrine glands and by the hormones they produce. Acromegaly is caused by an anterior pituitary tumor that secretes growth hormone. Growth hormone is a potent anabolic agent secreted by the somatotropic cells of the anterior lobe of the pituitary gland. The primary action of growth hormone is to stimulate somatic growth through increased protein deposition from chondrocytes and osteogenetic cells. The resultant epiphyseal cartilage growth leads to bone length increase. The increased proliferation rate of somatotropic cells and the transformation of chondrocytes into osteogenetic cells lead to deposition of new bone over the surface of older bone (Tsaousoglou, et al., 2006). Postpubertal overproduction of growth hormone leads to highly disproportionate growth of the jaws and facial bones, which is mainly a result of periosteal bone apposition due to reactivation of the subcondylar growth zones. Some of the most noticeable profile

characteristics of patients with acromegaly include the enlargement of the ascending ramus, prominence of the mandible, chin and lips. Even though the tumor may be removed or irradiated, though the excessive growth may stop, the skeletal deformity will continue and require orthognathic surgery (Yagi 2004).

Studies have been conducted in animals to demonstrate the effect of acromegaly. In 2004, Iikubo, et al., investigated the time course of mandibular enlargement in acromegaly to determine the most suitable period for occlusal treatment in this disease. Continuous subcutaneous infusion of human recombinant insulin-like growth factor-I (IGF-I) (640 μg/day) was used in six 10 week old male rats for 4 weeks to induce mandibular enlargement. A control group of 6 rats were injected with saline. The length of the experimental group of rats mandible, maxilla, and femur all demonstrated a significant increase as compared to the control group (Iikubo, et al., 2004). In 2002, Tamura et al reported that acromegaly results from the mutation of the MEN-1 locus on chromosome 11q13.

In 2007, Machicek et al, reported that mutations in the FGFR3 (Fibroblast Growth Factor Receptor 3) gene, results in achondroplasia. Amelogenin gene (AMELX) mutations resulted in X-linked amelogenesis imperfecta, while mutations in the enamelin gene (ENAM) cause autosomal-inherited forms of amelogenesis imperfecta (Ravassipour et al 2005). These recent advances have fallen on the heels of the Human Genome Project (HGP) that began in 1990. As a result of the HGP comprehensive genetic maps have been created that locate and identify genes underlying susceptibility to disease. Increasingly detailed knowledge of the human genome at the DNA level forms the basis of our understanding of genetic transmission and gene action. The HGP has mapped

30,000 genes thus far, and therefore provides the basis for genetic diagnosis and therapy. These advances in molecular biology and human genetics have made it possible to study the genetics of craniofacial disorders with more precision.

Of the nearly 16,000 disorders annotated in Online Mendelian Inheritance in Man: (OMIM), an estimated 900 contain an oral and/or craniofacial component (http://www.ncbi.nih.gov/Omim) (Bui et al., 2006). Even though there has been numerous advancements in the field of molecular biology, the etiology of numerous anomalies remain to be discovered. The growth and development of the craniofacial complex is yet to be discovered, in particular, skeletal Class III malocclusion (mandibular prognathism OMIM # 176700). As the field of molecular genetics continues to improve and advance, it should be possible to identify relevant genetic markers for such traits.

Skeletal Class III malocclusion or mandibular prognathism has been analyzed genetically. Huang, et al (1981), conducted a study on mandibular prognathism in the rabbit to discriminate between single-locus and multifactorial models of inheritance. The results indicated a simple autosomal recessive inheritance with incomplete penetrance for this condition. In a study conducted by Sutton et al in 2007, the breed structure of dogs was investigated to determine the genetic basis of size. Moreover, a genome-wide scan revealed a quantitative trait locus (QTL) on chromosome 15, which was reported to influence the size variation within a single breed of dogs. Sutton et al also examined the genetic variation on chromosome 15 and discovered significant evidence for a selective sweep on a single gene (IGF-1). They also found that the IGF-1 single nucleotide polymorphism is common to all small breeds and absent from giant breeds, thereby suggesting that the mutation of this gene is a major contributor to the body size in all small

dogs (Sutton et al 2007). In another study by Pereira et al in 2005, the effects of growth hormone (GH) and insulin-like growth factor 1 (IGF-1) in 688 animals were examined. Genotyping effects on expected breeding values for birth weight, weaning weight and yearling weight were investigated and significant effects were found for the GH genotype on yearling weight, with positive effects associated with the leucine/valine genotype. The IGF-1 genotypes revealed significant effects on birth weight and yearling weight (Pereira 2005).

Human studies have also played a major role in the developing hypothesis that Class III malocclusion is at least in part due to genetic factors. Orofacial structures are significant in the development of the craniofacial complex and have been shown to be under genetic control, hence they should be considered in the etiology of the development of skeletal Class III malocclusion (Mossey 1999). Horowitz et al (1960) studied both fraternal and identical twins using linear cephalometric measurements and showed significant variation in the anterior cranial base, mandibular body length, lower face height, and total face height. Mossey described previous work done by Hunter in 1965 that used linear measurements on lateral cephalometric radiographs as well and demonstrated a stronger genetic component of variability for vertical measurements, instead of measurements in the anteriorposterior plane of space. Mossey also described work by Harris (1963) who stated that multivariate analysis is required in order to examine genetic variation utilizing lines and angles. A study conducted by Singh et al (1999) discussed the influence of the cranial base morphology with a more acute cranial base and shortened posterior cranial base resulting in a more posterior glenoid fossa, thus contributing to mandibular prognathism. Singh also stated that the skeletal Class III

could be due to the failure of the cranial base to flatten antero-posteriorly rather than the flexure of the anterior cranial base (Singh 1999). Singh referenced a study conducted by Vilmann and Moss in 1979, who reported that in rats, the angle between the cranial base and viscerocranium becomes more obtuse between 14-60 days postnatally. Singh cited another study done by Zelditch in 1993, which suggests that in young mammals, the cranial base straightens by an increase in the ventral angle between the basioccipatal and the basispheniodal bones (Singh 1999).

Studies have been conducted to examine the role of heredity in the development of Angle's Class III malocclusion. Nakasima, et al (Aug 1982), compared the craniofacial morphologic differences between parents with Class II offspring and those with Class III offspring and by analyzing the parent-offspring correlations within each Class II and Class III malocclusion group. Lateral and frontal roentgenographic cephalograms were obtained for ninety-six patients with Class II malocclusion, and 104 patients with Class III malocclusion, and their respective parents. Their cephalograms were superimposed between the two groups of parents as well as between their offspring. Nakasima showed that there was a hereditary pattern of inheritance for skeletal Class II and Class III malocclusions (Nakasima A, et al 1982).

The epidemiology of the Class III dentofacial deformity

Class III malocclusion represents a very small proportion of the total malocclusion, and is most prevalent in Oriental populations (3-23%) (Susami 1972, Tang 1994). Environmental factors have also been suggested as contributing to skeletal Class III malocclusion. Some authors have also suggested that other factors affect both the sagittal and vertical relationships of the jaws and teeth such as the size and relative

positions of the cranial base, maxilla and mandible, the position of the temporomandibular articulation and any displacement of the lower jaw. The position of the foramen magnum, spinal column and habitual head position may also influence the eventual facial pattern (Singh 1999). These facts further support the premise that the etiology of Class III malocclusion is wide ranging and complex.

The prevalence skeletal Class III malocclusion depends upon the population and the type of Class III problem. The prevalence varies by race, with a higher prevalence in East Asians, Africans, and Caucasians, respectively. It also varies by age, ranging from an approximate prevalence of 0.5% in children 6-14 years old to a range of 2-4 % in adults (El-Gheriani 2003). According to Jena and co-workers, Class III malocclusions are most prevalent in Oriental populations (3-5% in Japan and 1.75% in China). Susami and Tang all reported a relatively high prevalence of Class III malocclusion from 15% to 23%, in Asian Mongoloid populations of Taiwanese, Japanese, Korean and Chinese. Other studies reported an incidence of this class of malocclusion in American, European and African Caucasian populations below 5% (Thailander 1973; Jacobson 1974; Graber 1977). Class III malocclusion is a common clinical problem in orthodontic patients of Asian or Mongoloid descent.

In a Finnish study conducted by Keski-Nisula (2003), et al., the occlusions of 489 children at the onset of the mixed dentition period (mean age 5.1 years, range 4.0-7.8 years) were analyzed. This study found the frequencies of mesial step, flush terminal plane, and distal step were 19.1%, 47.8%, and 33.1%, respectively. The canine relationship was Class I in 46.1%, Class II in 52.4%, and Class III in 1.5% of the sides examined. A Nigerian study by Onyeaso (2004), of the prevalence of malocclusion

among 636 secondary school Yoruba adolescents in Ibadan, Nigeria, (334 boys and 302 girls), aged 12-17 years (mean age, 14.72), reported 24% of the subjects had normal occlusions, 50% had Class I malocclusions 14% had Class II malocclusions, and 12% Class III malocclusions. Class I malocclusion is the most prevalent occlusal pattern among these Nigerian students, as well as in other ethnic populations. Different patterns of Class II and Class III might be present for the dominant ethnic groups.

A study conducted by Basdra et al in 2001, investigated the relationships between different malocclusions such as Class III and Class II division 1, and congenital tooth anomalies. Two-hundred Class III and 215 Class II division 1 patients were examined for the presence of any of the following congenital tooth anomalies: maxillary incisor hypodontia, maxillary canine impaction, transpositions, supernumerary teeth, and tooth agenesis. The result revealed no statistical difference in the occurrence rates of upper lateral incisor agenesis, peg-shaped laterals, impacted canines, or supernumerary teeth between Class III and Class II division 1 malocclusions. When the occurrence rate of all congenital tooth anomalies was compared between the two malocclusions, Class III subjects showed significantly higher rates. Basdra et al concluded that subjects with Class III and Class II division 1 malocclusions show patterns of congenital tooth anomalies similar to those observed in the general population. Amelogenesis imperfecta hence provides a unique and original discovery for our long term goal to map the chromosomal locus.

The Class III problem in terms of major categories

Certain syndromic conditions such as Crouzon syndrome, acromegaly and achondroplasia possess the features of skeletal Class III malocclusion. The genes

involved in the development of these syndromic conditions have already been identified. Among these genes include the FGFR3 (Fibroblast Growth Factor Receptor 3) gene, which results in achondroplasia, the MEN-1 gene which results in acromegaly (Machicek 2007, Tamura et al. 2002).

Studies have been conducted on transgenic achondroplastic mice, creating a phenotype that resembles human achondroplasia, having a domed skull, hypoplastic midface and nasal bone, anteriorly displaced foramen magnum, and a prognathic mandible. Achondroplasia, the most common and best known skeletal dysplasia, is the most common form of human short limbed dwarfism, and is due to a mutation in the gene for fibroblast growth factor receptor 3 (FGFR3) gene located on chromosome 4, or it can be inherited from a parent with the condition, where one copy of the altered gene in each cell is sufficient to cause the disorder (Machicek 2007). FGFR3 signaling occurs via the mitogen-activated protein kinase (MAPK) pathway and plays an important role in the regulation of endochondral ossification. FGFR3 is a negative regulator of bone growth. Binding of fibroblast growth factors to the FGFR3 receptor stimulates its tyrosine kinase activity in the cell. This activates a signal transduction pathway that regulates endochondral ossification by inhibition of cell division and stimulation of cell maturation and differentiation. Mutations in the FGFR3 gene give rise to activation of the receptor in the absence of growth factors, thus causing abnormal long bone development. Position and type of mutation in the FGFR3 gene determine the extent of overactivation and thus the severity of the skeletal abnormality. (Ravenswaaij 2001).

Acromegaly is a rare disorder caused by an anterior pituitary tumor that secretes growth hormone (GH). Overproduction of growth hormone during post-pubescent years

can result in highly disproportionate growth of the jaws and facial bones, which is mainly a result of periosteal bone apposition due to reactivation of the subcondylar growth zones. This results in the enlargement of the ascending ramus and prominence of the mandible, chin, and lips (Yagi et al 2003). Some authors have reported that mutations in the MEN-1gene, can also result in acromegaly. MEN 1 is an autosomal dominantly inherited disorder that results from the inactivation of germ-line mutations of the MEN-1 tumor suppressor gene, which is located on chromosome 11q13 (2, 3). It includes tumors of parathyroid glands, pituitary gland, pancreatic islets and adrenal cortex and nueroendocrine carcinoid tumors, at a young age (Dreijerink 2005).

Class III malocclusion is thought to be an inherited trait and few studies of Class III subjects have included data from genetic analysis. Certain dental syndromes possessing a genetic etiology, such as Amelogenesis Imperfecta (AI), exhibit distinct skeletal features such as open bite (Ravassipour, Powell et al. 2005) and based on our preliminary studies, Class III malocclusion. In our study we wish to better understand the relationship between Class III and AI.

Amelogenesis imperfecta (AI) is an inherited enamel dysplasia involving both dentitions with no other systemic effects. The hereditary pattern is autosomal or X-related dominant or recessive. Its prevalence is approximately 1:14,000-1:16,000. It can be classified as hypocalcified, hypoplastic and hypomatured according to clinical, radiological, histological and hereditary findings (Turkun 2005). Normal enamel formation can be divided into three distinct developmental stages including translation, secretion of an extracellular matrix, mineralization of the matrix, and final matrix removal and crystallite growth or maturation of enamel (Robinson, Kirkham J et al.

1982). The major forms of AI are thought to primarily affect at least one of the three stages of enamel formation. Although the most appropriate classification system for the AI disorders is not universally accepted, the most commonly accepted nosology identifies three main AI types: hypoplastic (HPAI), hypocalcified (HCAI), and hypomatured (HMAI) (Witkop and Sauk 1976). HPAI is thought to result primarily from a secretory defect in enamel formation. However, HPAI enamel can be poorly mineralized making classification difficult. HCAI is characterized by a normal width of enamel which has a deficient mineral content and is believed to result from a defect in the initial nucleation of enamel crystallites. HMAI is considered to be a defect in the removal of extracellular protein resulting in decreased mineral deposition and increased matrix retention.

The molecular defects are not known for most AI types, but it has been accepted that the AI related genes are primarily, if not exclusively, involved in amelogenesis (Cartwright, Kula et al. 1999). Several amelogenin gene mutations have been identified and are known to cause at least some types of X-linked AI. The amelogenin gene has been known to be expressed in ameloblasts and the mutations results in defects that are apparently limited to enamel. Other developmental defects in tissues other than enamel, such as pulp calcifications and abnormal tooth eruption, have been associated with various AI types (Cartwright, Kula et al. 1999).

Other studies have shown the association between AI and other craniofacial anomalies, such as Ravassipour, et al. (2005), who reported that AI is associated with dental and/or skeletal open bite malocclusions and may be related to craniofacial development. Persson and Sundell (1982), reported that 40% of the AI affected individuals had skeletal open bite. Pamukcu, et al (2001), reported a case involving an

AI patient with craniofacial anomalies such as severe anterior open bite, long face, facial asymmetry, high angle, and Class III skeletal pattern. This patient was treated with a multidisciplinary approach and the study looked at improving the patient's quality of life (Keles A, et al. 2001 Winter). Our preliminary studies revealed that there was a 16 fold increase in Class III diagnosis in the AI population when compared to the caucasian norms.

By comparing the genes involved in the etiology of Amelogenesis Imperfecta, further clues in to the genetic etiology of Class III malocclusion can be ascertained. Establishing the genetic etiology of skeletal Class III malocclusion may not have a direct clinical application in the immediate future, however, detection of the gene(s) involved may provide hope for improvements in the management of such patients. This information may be used to accurately predict long-term growth changes, and may lead to pharmacological interventions.

The etiology of Class III dentofacial disorder is controversial

Genetics has been frequently cited as the etiology of Class III dentofacial problem

The existence of familial aggregation of mandibular prognathism (MP) suggests that genetic components play an important role in its etiology. A genetic etiology of class III malocclusion is suggested by many lines of evidence (Jena et al, Litton et al, El-Gheriani). However, there has been a wide range of environmental factors suggested as contributing factors for the development of class III malocclusion. The familial aggregation of mandibular prognathism has also been described and ascribed to a variety of genetic models, including autosomal recessive, autosomal dominant, and a polygenic model of transmission (El-Gheriani). Jena in her article entitled, "Class – III

malocclusion: Genetics or environment? A twins study," discussed the fact that a twin study is one of the most effective methods available for investigating genetically determined variables of malocclusion. She also states that discordancy for class III malocclusion is a frequent finding in dizygotic twins, however, that class III discordancy in monozygotic twins is a rare finding. Her study examined monozygotic twins in an effort to assess the genetic and environmental components of variation within the cranio-dento-facial complex.

For investigation of genetically determined variables in orthodontics, twin study method is the most effective. Baker reported a case in which monozygotic twins were concordant for mandibular prognathism. Korkhaus also reported two cases of monozygotic twins; one pair was concordant and another pair was discordant for class III malocclusion. In Jena's report, a pair of monozygotic female twins were presented. The girls exhibited a marked similarity in facial appearance. They both had a similar dentition, but their occlusions were dissimilar to some extent. Twin 1, reverse overjet, overbite and class III molar relations were more severe than twin 2. Both twins had bilateral posterior crossbite. The cephalometric parameters did not reveal a very significant difference in skeletal morphology. The cephalometric analysis revealed the class III maxillo-mandibular relationship in both twins was more severe in twin 1. Twin 1 when compared to twin 2 had flat cranial bases. The position of the maxilla was more backward and the position of the mandible more forward in twin 1 as compared to twin 2. Height of the anterior face was similar in both the twins, but posterior facial height was more in twin 2.

Position of mandible in relation to anterior cranial base and Frankfort-horizontal plane was significantly different among the twins (Jena et al., 2005). In this study, the concavity of the face (Angle of convexity) in twin 1 was more compared to twin 2. Relatively more backward position of the maxilla (Angle SNA, N Perpendicular to point-A) and forward position of the chin (Angle SNB, N Perpendicular to Pog) contributed to such difference in the severity of the facial concavity. The antero-posterior position of the mandible in the twin study was influenced significantly by environmental factors. However, in a previous study undertaken, a report was made that the anterior-facial posterior position of the mandible is genetically determined. Anterior facial height of both twins was apparently equal. It showed that the height of the anterior face is genetically determined and did not play any role in the discordance of class III malocclusion. This is in agreement with the result from a study done by Townsend and Richards (Townsend et al., 1990). The shape of the cranial base (Saddle angle) was different among the twins. This characteristic played a major role in the discordance of class III malocclusion. It was suggested that the form of the cranial base is least genetically controlled and strongly influenced by environmental factors. The relative position of the maxilla (Angle SNA), temporomandibular joint (Articular angle) and effective length of the mandible and maxilla were different in both twins. These characteristics played a significant role in the severity of class III malocclusion as described by many authors. Vertical position of the mandible in relation to the Frankfort-horizontal plane (FMA) was identical in both twins, but he interesting difference as the position of the mandible in relation to the anterior cranial base (SN-GoGn). Such severe spatial discrepancy of mandible in twin 1 was due to more upward tipping of the anterior

cranial base. Positions of the upper incisors were more variable than the lower incisors. Proclination of the lower incisors was relatively more in twin 2. Such dento-alveolar compensation was considered as an important environmental factor in the variation of severity of class III incisor relationship among the twins. From this twin study, it was concluded that genetics is not the sole controlling factor for the etiology of the class III malocclusion. The multifactorial etiology of class III malocclusion was hence confirmed (Jena et al 2005).

Another study investigated the role of genetic influences in the etiology of class III malocclusion (El-Gheriani 2003). In this study, a segregation analysis of 37 families of patients that were treated for mandibular prognathism, was performed. Mandibular prognathism was treated as a qualitative trait, with cephalometric radiographs, dental models, and photographs used to verify diagnosis. Segregation analysis of a prognathic mandible in the entire dataset supported a transmissible Mendelian major effect, with a dominant mode of inheritance determined to be the most parsimonious. El-Gheriani's study aimed to apply modern methods of segregation analysis to examine specific genetic models of the familial transmission of mandibular prognathism in a series of large Libyan families. El-Gheriani, et al, identified 37 probands with mandibular prognathism from the patient base of several dental clinics in Benghazi, Libya. They then completed family histories for each proband and the affection status of other individuals in each family were confirmed by cephalometric, photographic, and/or dental models. The study sample of 37 families comprised of 1013 individuals. Mandibular prognathism was determined by assessing one or more of the orthodontic records. All 37 probands had a lateral cephalometric radiograph as part of their treatment record, and a confirmed

negative ANB angle was a prerequisite for enrollment in the study. The data were stratified by age and sex, hence pooled sex measurements were chosen at age 12 as the mean value for each measurement for comparative purposes (El-Gheriani et al 2003).

The results from the study performed by El-Gheriani et al (2003), supported the previous findings that there is a hereditary component to the expression of this phenotype. They were able to conclude that, among the autosomal dominant, recessive, and additive models, the autosomal dominant model was the most parsimonious. Their conclusion of autosomal-dominant inheritance was in agreement with Wolff et al (1993), who used pictures or authentic descriptions to determine affection status, but disagreed with the polygenic conclusion of Litton el al (1970). The weakness of the El-Gheriani study is that they failed to completely characterize the phenotype.

A study conducted by Yamaguchi, et al, in 2005, utilized a genome-wide linkage analysis to identify loci susceptible to MP with 90 affected sibling-pairs in 42 families, comprised of 40 Korean sibling-pairs and 50 Japanese sibling-pairs. Two non-parametric linkage analyses, GENEHUNTER-PLUS and SIBPAL, were applied and detected nominal statistical significance of linkage to MP at chromosomes 1p36, 6q25, and 19p13.2. The best evidence of linkage was detected near D1S234 (maximum $Z_{lr} = 2.51$, $P = 0.0012$). In addition, evidence of linkage was observed near D6S305 (maximum $Z_{lr} = 2.23$, $P = 0.025$) and D19S884 (maximum $Z_{lr} = 1.93$, $P = 0.0089$). This study while helpful relied on sibling pairs, which is less powerful than the family studies that we report in this publication. The identification of the susceptible genes in the linkage regions will pave the way for insights into the molecular pathways that cause MP,

especially overgrowth of the mandible, and may lead to the development of novel therapeutic tools.

Common craniofacial morphological features noted in the development of skeletal Class III malocclusion

Some of the craniofacial features usually noted in the development of skeletal Class III malocclusion include a steep mandibular plane angle, obtuse gonial angle, overdeveloped mandible, underdeveloped maxilla, and a small cranial base angle which may displace the glenoid fossa anteriorly to cause a forward positioning of the mandible (Sato, 1994). These factors are generally thought to contribute to the development of skeletal malocclusion as well as facial deformities, and are believed to originate from genetic and/or environmental factors. The posterior discrepancy is an important etiological factor in the development of a skeletal Class III malocclusion because it affects the occlusal plane.

Previous Clinical Studies

Traditionally, Class III malocclusion was thought to be due mainly to a prognathic mandible (Guyer et al 1986). A study conducted by Proffit, et al. in 1990, indicated that 20% of Class III cases is accounted for by mandibular prognathism, maxillary deficiency accounts for 20%, and a combination of maxillary deficiency and mandibular prognathism accounts for the other 50-60% of Class III cases.

Studies have shown that components of the craniofacial complex, such as cranial base length, can also affect the position of the jaws (Battagel, 1993; Dhopatkar et al., 2002). Many Class III patients have a shorter anterior cranial base when compared with

Class I controls (Battagel, 1993), which results in a more anteriorly positioned glenoid fossa, which then positions the mandible further anteriorly.

The prevalence of Class III malocclusion is more common among Asian than Caucasians, however, the information in the literature is contradicting as to the phenotypic variation. Several investigators reported that Asian Class III subjects are more often characterized by a hypoplastic midface and a deficient maxillary development associated with a short anterior cranial base (Miyajima, et al 1997, and Ishii, et al 2004, and Kao, et al 1995). Another study comparing the racial differences between British and Japanese females with severe Class III malocclusions, showed that the Japanese females had a significantly reduced anterior cranial base, more retrusive, midfacial component, increased lower anterior facial height, more obtuse gonial angle, and more proclined upper incisors than their Caucasian counterparts (Ishii, et al 2002). Ishii et al., reported no significant differences in the mandibular dimensions between the British and Japanese groups, the Japanese females had a relatively larger mandible due to the characteristics mentioned above. Ishii also reported that the Japanese subjects have a high-angle facial pattern with a steeper mandibular plane compared to the British sample. Another study conducted by Singh et al., in 1998, found that the skeletal components of Korean American children with Class III malocclusion also consisted of a smaller skeletal anterior cranial base and midfacial dimensions as well as increased mandibular length when compared to their Caucasian counterparts. When comparing Japanese, Koreans exhibited acute mandibular angles. Hence, these studies illustrate the morphological differences between Asian and Caucasian Class III groups as well as variation among the different Asian populations.

The most widely used method to describe and classify the Class III facial pattern morphologically is cephalometric analysis, which consists of linear and angular measurements. In order to achieve more statistical detail, other methods have been described to analyze cephalometric parameters. These methods include multivariate analyses, such as discriminate, cluster and principal component analysis, to distinguish between Class I and Class III subjects and in predicting growth and treatment outcome (Tahimna et al., 2000; Biscotti et al., 1998; Bagatelle, 1993; Stellzig-Eisenhauer et al., 2002).

Treatment of Class III malocclusion

Mandibular prognathism or skeletal Class III malocclusion with a prognathic mandible is one of the most severe maxillofacial deformities. Facial growth modification can be an effective method of resolving skeletal Class III jaw discrepancies in growing children with dentofacial orthopedic appliances including the face mask, maxillary protraction combined with chincup traction and the Frankel functional regulator III appliance. Orthognathic surgery with orthodontic treatment is required for the correction of adult mandibular prognathism.

Interceptive Orthodontics and Timing of Treatment

Timing of orthodontic treatment for the Class III problem has always been somewhat controversial. Many practitioners elect to postpone most orthodontic treatment until all permanent teeth are present. Many different functional appliances have proven to be very useful in correcting Class II conditions in the growing patient. However, this enthusiasm for interceptive treatment in the developing Class III patient has not gained such popularity. Most of the treatment of Class III malocclusion is done utilizing a

combined orthodontic/surgical correction. Currently, many orthodontists will not treat Class III patients until they feel that active growth has been completed (Campbell 1983).

The early interception of Class III malocclusion has been advocated for many years. Angle (1907) suggested that: *"Deformities under this class begin at about the age of the eruption of the permanent molars, or even much earlier, and are always associated at this age with enlarged tonsil and the habit of protruding the mandible, the latter probably affording relief in breathing. So in harmony being once established, it usually progresses rapidly, only a few years being necessary to develop by far the worst type of deformities the orthodontist is called on to treat, and when they have progressed until the age of 16 or 18, or after the jaws have become developed in accordance with the malpositions of the teeth, the patient has usually passed beyond the boundaries of malocclusion only, and into the realm of bone deformities, for which, with our present knowledge, there is little possibility of affording relief through orthodontic operations."*

Angle was also one of the first to suggest that a combined orthodontic and surgical approach was the only way to correct true mandibular prognathism, once fully developed (Campbell 1983). Tweed (1966) divided Class III malocclusions into a category A for pseudo-Class III malocclusions with normally shaped mandibles and underdeveloped maxillae, and a category B for skeletal Class III malocclusions with large mandibles. Tweed expressed the fact that category A, should be treated during the mixed dentition stage of growth (7 to 9 years of age). He also stated that if the malocclusion occurred in the primary dentition, it should be treated as early as 4 years of age. Tweed also stated that those in category B, where the condition is pronounced and the patient is 14 years of age or older, it is, perhaps, best not to attempt to treat them orthodontically.

Such treatment should be postponed until has been consummated, at which time it surgery could be attempted (Campbell 1983).

Salzmann, (1966) suggested that treatment in Class III malocclusion should be instituted as soon as the abnormality is diagnosed. He also suggested a chin cup to influence the vector of mandibular growth. Graber (1966) advocates that since Class III malocclusions are among the most difficult to treat by the specialist and since surgical intervention is contemplated more frequently for this type of problem than any other malocclusion, it just make good common sense that at least a chin cup should be tried early to intercept the developing malocclusion and basal malrelationship. He also suggests that extraoral force as an interceptive or at least palliative procedure may serve to prevent a worsening malocclusion at the very least. Graber also suggested that since Class III faces tend to become more prognathic and result in unfavorable muscle and tooth adjustments, it is good interceptive dentofacial orthopedics to place appliances early where there is Class III malocclusion. Turpin (1981), placed the incidence of Class III malocclusion at 1 to 2 percent of the population with Japanese and Scandinavian populations being somewhat higher. Jacobson (1974) and associates, in a summary of such studies, show a range from 1 percent to 12.2 percent but most studies reflect an incidence below the 5 percent level. Bell, Proffit and White (1980) stated that in most patients with skeletal Class III malocclusions, there is some degree of maxillary deficiency in addition to the more obvious mandibular excess. They further suggest that although most Class III patients have excess mandibular development, the component of maxillary deficiency is strong enough in at least 30 to 40 percent to make it a significant part of the problem. These authors also suggest that although some maxillary protraction

may be achieved with interceptive reverse-pull mechanics, significant downward repositioning of the chin and forward repositioning of the maxillary teeth likewise occurred. They concluded that although some forward repositioning of the maxilla can be achieved by orthopedic forces, it is not yet possible to do this without having a greater effect on the mandible than on the maxilla and expressed hope for improved appliance design to allow more downward and forward repositioning of the maxilla.

Turpin (1981) developed guidelines for deciding when to intercept Class III malocclusion. He suggested that if the patient discloses characteristics such as a convergent facial type, anterior-posterior functional shift, symmetrical condylar growth, young, with growth remaining, mild skeletal disharmony (ANB < -2), good cooperation, no familial prognathism, and good facial esthetics, early treatment should be considered. Conversely, if the patient had characteristics such as divergent facial type, no anterior-posterior shift, asymmetrical growth, growth complete, severe skeletal disharmony (ANB >-2), poor cooperation, familial pattern established, poor facial esthetics, then delaying treatment until condylar growth has ceased may be the better alternative. Turpin further stated that after evaluating the characteristics of Class III malocclusions, it is apparent that the early interception of developing prognathism is often valid. Turpin also stated that caution is advised, however, not to undertake procedures that will compromise the need for orthognathic surgery later on if the mandible grows excessively during adolescence. Early treatment can prevent the problem from becoming more severe. It can occasionally reduce the need for surgery and it can reduce potential psychosocial problems. The literature definitely reveals a definite trend toward the need for at least an attempt at early interception of developing Class III malocclusions (Campbell 1983).

Protraction Facemask/Reverse pull headgear

The severity of Class III malocclusion ranges from dentoalveolar problems with anterior posturing of the mandible to true skeletal problems with significant maxillomandibular discrepancies. The interception of a Class III malocclusion requires a long-term growth prediction in order to estimate the subject's evolution from the prepubertal phase to adulthood. It is important for the orthodontic clinician have early interception of Class III malocclusion included in his armamentarium. It is also obvious that correction of this complex problem must be a long-term procedure.

Class III patients present with some maxillary deficiency as well as possible mandibular excess, hence mechanics applied early to protract the maxillary structures and apply reciprocal retractive forces to the mandible appear to have significant validity. Campbell conducted a clinical study of early Class III treatment in fourteen patients, with emphasis on the reverse-pull face crib. The conclusion from his study was the important benefits of early treatment should not be denied because of concerns that a few may still require further treatment later. Campbell used the reverse-pull face crib (RPFC), in combination with the necessary fixed appliances, which provided the force system. Campbell's data confirmed the same response in several patients, as observed by other authors using these forces. In 2006, Wells et al reported on the long-term efficacy of reverse pull headgear therapy, and demonstrated that up to age 10, the time at which RFHG treatment started does not appear to be a major factor in long-term success in maintaining positive overjet. Wells et al in 2006 also suggested another aspect of Class III early treatment with the use of RPHG to bone anchors in the maxilla, to decrease forward movement of the maxillary teeth. In 1998, Baccetti et al reported on treated and

untreated samples of individuals with skeletal Class III malocclusion and divided them into early and late mixed-dentition groups to aid identification of the optimum timing of the orthopedic treatment of the underlying skeletal disharmony. The results from his study indicate that the combination of a bonded maxillary expander and face-mask therapy is more effective in the early mixed dentition than in the late mixed dentition.

Treatment timing with early interceptive Class III treatment is most important. These patients need to be seen at the earliest possible date in order to plan for the future. Treatment should not be initiated until the maxillary first molars, centrals and lateral incisors are present. According to Campbell, the goals of early interception of Class III malocclusions are to help provide a more favorable environment for normal growth; to achieve as much relative maxillary advancement as possible by sutural growth; to improve occlusal relationships; to improve facial esthetics for more normal psychosocial development.

Ngan discussed the reason for the reluctance of some clinicians to render early orthopedic treatment in Class III patients through the use of a protraction face mask therapy is the inability to predict mandibular growth. He stated that patients who have received early orthodontic or orthopedic treatment might need surgical treatment at the end of the growth period. Ngan investigated whether or not it is worth the burden to treat a Class III malocclusion early. It was shown by Melsen and Melsen in histological findings that the midpalatal suture is broad and smooth during the infantile stage (8-10 years of age), and the suture became more squamous and overlapping in the juvenile stage (10-13 years of age). Clinically, studies have shown that maxillary protraction is effective in the deciduous, mixed, and early permanent dentitions (Merwin, et al 1997;

Kapust, et al 1998; Yuksel, et al 2001). Several studies suggested that more anterior maxillary displacement can be found when treatment begins in the deciduous or early mixed dentition (Nartallo-Turley, et al 1998; Kapust, et al 1998; Baccetti, et al 1998).

The study conducted by Ngan of 20 patients successfully treated with facemask therapy and 20 patients who were unsuccessfully treated with facemask therapy showed that some Class III patients with mild to moderate Class III skeletal patterns can be successfully camouflaged with orthodontic treatment. However, other Class III patients with excessive mandibular growth should be warned of the need for future orthognathic surgery.

Accurate diagnosis and understanding of the individual growth pattern is crucial in determining the proper timing of Class III treatment. Optimal treatment timing for facemask therapy is in the deciduous or early mixed dentition. Early treatment with a facemask allows for favorable sutural response and improvement in facial profile and self-esteem (Ngan 2006).

Surgical intervention

Orthognathic surgery in conjunction with orthodontic treatment is required for the correction of adult mandibular prognathism. The two most commonly applied surgical procedures to correct mandibular prognathism are sagittal split ramus osteotomy (SSRO) and intraoral vertical ramus osteotomy. Both procedures are suitable for patients in whom a desirable occlusal relationship can be obtained with a setback of the mandible, and each has its own advantages and disadvantages. In bilateral SSRO, the intentional ostectomy of the posterior part of the distal segment can offer long-term positioned stability. This

may be attributable to reduction of tension in the pterygomasseteric sling that applies force in the posterior mandible (Chang 2006).

Future Perspectives on Pharmacological Intervention

In 2006, Chang stated that future work will employ molecular genetics to identify candidate genes within the human genome to predict those individuals most likely to develop mandibular prognathism. Further studies in molecular biology are needed to disclose the gene-environment interactions associated with the phenotypic diversity of mandibular prognathism and the heterogenic developmental mechanisms thought to be responsible for them. Identification of candidate genes will permit early clinical diagnosis and intervention, as the growing craniofacial complex may be amenable to prophylactic treatments. Identification of the susceptible genes in the linkage regions will pave the way for insights into the molecular pathways that cause mandibular prognathism, especially overgrowth of the mandible, and may lead to the development of novel therapeutic tools (Chang 2006).

Hypothesis

The Class III dentofacial deformity is clinically and genetically heterogeneous presenting with a distinct subphenotype and genotype in 3 cohorts.

Goals of study

The specific goals of this study:

1) Based on radiographic cephalometric measurements, utilize multivariate analysis of variance (MANOVA) to phenotypically characterize the Class III trait in 3 specific populations (Colombian/Hispanic, AI/Enriched and Caucasian families).

2) Conduct genome-wide scans followed by linkage analysis to identify the genetic loci associated with the Class III trait in the Colombian and AI populations.

CHAPTER 4

PART I: PHENOTYPIC ANALYSIS

Materials and Methods

Subjects

This study consisted of 100 participants derived from 3 cohorts of subjects with the clinical diagnosis of skeletal Class III malocclusion. There were 54 female and 46 male subjects, with 27 unaffected and 73 affected with skeletal Class III malocclusion. The average age was 35.04 years with a range from 6-90 years. A summary of the demographic characteristics of these groups can be found in Table 16.

One cohort of 48 patients, termed Colombian cohort, was derived from collaboration with Dr. Rincon-Rodriguez, in Medellin, Colombia, South America and 1 family was recruited through the University of North Carolina at Chapel Hill, School of Dentistry. This cohort consisted of 19 male, 29 female subjects. There were 32 subjects affected with the skeletal Class III malocclusion. The age range was from 6 – 76 years, with an average age of 34.77 years. A second cohort consisting of 25 AI patients, was derived from Dr. Wright's laboratory at the University of North Carolina, Chapel Hill. This cohort consisted of 17 male and 8 female subjects. Of the AI cohort, 20 subjects were affected with skeletal Class III malocclusion. The age range of this group was from 4-79 years, with an average age of 40.84 years. The third cohort of patients was termed, Caucasian group. This sample consisted of 27 patients, 10 male and 17 female with 21

affected with skeletal Class III malocclusion. The age range was from 6- 90 with an average age of 30.15 years.

Inclusion criteria

In order to be included in the study, the subjects had to be diagnosed with skeletal Class III malocclusion based on an ANB angle less than 1 degree, and/or overjet (OJ was not used for edentulous subjects) of less than or equal to 0mm, and a concave profile. Inclusion criteria are listed in Table 1. The subjects who had an ANB angle of greater than or equal to 1 degree, *could* be included in the sample if they had a decreased maxillary unit length, or an increased mandibular unit length, so as not to exclude those subjects who may nonetheless have had a skeletal Class III malocclusion based on other valid criteria. This study was reviewed and approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board. The IRB numbers for both the AI and the Colombian studies respectively are as follows: 96-0981 (formerly DENT-3127), and 03-1640. Consent was obtained for each individual who participated in the study and by parents in the case of a minor.

Exclusion criteria

Subjects were excluded from the study if they had previous orthodontic treatment, or any craniofacial anomalies (eg., cleft lip/palate), or any cephalogram of non-diagnostic quality.

Cephalometric Analysis

Lateral cephalometric radiographs were taken in natural head position with posterior teeth in maximum intercuspation. The lateral cephalograms were traced by one investigator using the Dolphin Imaging version 9 software program (Dolphin Imaging

Systems, Chatsworth, California), for digitization in order to perform cephalometric analyses on the two groups using sixty-seven variables to phenotypically characterize the skeletal Class III trait. Among the measurements calculated, 38 were linear, 25 angular, and 4 were proportional.

Reliability of the measurements

The Method Error (ME) was calculated once all the cephalometric tracings were completed. Ten randomly selected cephalograms were traced and digitized on three occasions at two week intervals by the same observer. The ME calculations were performed using the Intraclass Correlation(ICC) method (SPSS for Windows, version 14, Chicago, IL). The formula for the Intraclass Correlation is:

$$ICC = \frac{Var\ (T)}{Var\ (T)\ +\ Var\ (E)}$$

Where Var (T) is the variance due to true differences among subjects, and Var (E) is the error variance. This method describes how much of the total observed score variance is due to true score variance between subjects.

Data Normalization

In order to perform the statistical analysis in the study, all measured values were adjusted according to age and gender using standard normative data and converting them to z-scores. The normative values have been established on various reference groups. Reference groups have generally been chosen to represent excellent occlusion and facial proportion. The composite of normative standards used in this analysis were derived from the following sources: lateral cephalograms of the children comprising the Bolton standards, selected values from a group of untreated children from the Burlington Growth Center and several smaller growth studies, along with numerous specific samples

collected in university projects to develop standards for specific racial and ethnic groups (Proffit 2000).

Factor Analysis

A factor analysis was conducted to identify and eliminate variables that are redundant in order to reduce the variable set to a manageable size for the multivariate analysis of variance.

In order to perform the factor analysis, the data was first coded using the Microsoft Excel 2003 Program, then entered into a computer and analyzed with the Statistical Package for Social Sciences (SPSS for Windows, version 14, Chicago, IL). Initially, the variables were divided into 2 groups according to linear and angular measurements. Then each of the groups was further sub-grouped according to the theoretical similarity of each measurement. The first principle factor was extracted for each set and the variable with the highest correlation with this factor was chosen to represent the set in the final analysis.

MANOVA (Multivariate Analysis of Variance)

A Single Factor MANOVA (multivariate analysis of variance) was used to phenotypically characterize the Class III trait within three cohorts and to assess the statistical significance between the three groups (Colombian, AI, and Caucasian), while taking p values of less than .05 as statistically significant (SPSS for Windows, version 14, Chicago, IL). The single factor refers to one independent variable, the grouping variable with 3 levels. The 3 levels are the 3 cohorts. The term multivariate refers to the multiple dependent (outcome) variables.

The multivariate analysis of variance (MANOVA) was performed six times. First on the entire sample from each of the three groups using the 18 reduced variable set from the factor analysis, not adjusted for Skeletal Class III affection status, second on the dataset of the three groups without Skeletal Class III affection status, third on the three groups controlled for affection status. The other three times were performed on each of the families within each cohort controlled for affection status to observe the statistically significant differences between families within each cohort.

Inter-familial comparisons

Affected family members within each of the 3 group were analyzed using the multivariate analysis of variance based on their z-scores to identify statistical significant differences between the means of the variables between each of the families in each of the cohorts.

RESULTS FROM PHENOTYPIC ANALYSIS

Reliability of measurements

The method error was first computed using the intra-class correlation coefficient which ranged from 68% to 99.5% for the inter-time reliability of measurements for a single rater on 52 repeated measurements from 10 randomly selected cephalometric radiographs. Only 2 variables had intra-class correlation statistics of 68% and 89%, posterior face height to anterior face height and Frankfurt's horizontal plane to sella nasion. All other variables had intra-class correlation statistics above 90%. This implies high inter-time score consistency for all variables.

Factor Analysis

A factor analysis was performed to eliminate redundancy of cephalometric measurements. This very critical step addresses our primary goal to determine cephalometric variables that can distinguish statistically significant differences between the 3 cohorts described. Sixty-seven cephalometric variables that are commonly used in cephalometric analyses were reduced to the following 18 variables (Table 17). We performed a factor analysis on a subset of variables into groups that should be highly correlated. We extracted the first principle factor and released a representative from the group with the variable having the highest correlation with the factor.

Multivariate Analysis of Variance (MANOVA)

Table 1. Z-score and P values of three cohorts (n = 100)

Variable	Colombian (z-score) (n = 48)	AI (z-score) (n = 25)	Caucasian (z-score) (n = 27)	P-value
SNA	-0.298 [A]	-0.212 [A]	-0.600 [A]	0.435
SNB	0.158 [A]	0.628 [A]	0.222 [A]	0.422
ANB	-0.742 [A]	-1.380 [A]	-1.163 [A]	0.170
SN to GoGn	0.142 [A]	-0.148 [A]	-0.081 [A]	0.627
FH to SN	1.363 [A]	0.852 [A]	1.522 [A]	0.137
Chin Angle	0.402 [A]	-1.268 [B]	-0.959 [B]	0.000
Articular Angle	0.135 [A]	0.604 [A]	-0.256 [B]	0.032
Facial Taper	-1.123 [A]	-1.348 [A]	-0.941 [A]	0.617
Facial Plane to SN	-0.263 [A]	-0.144 [A]	0.159 [A]	0.311
Mx Unit Length	-5.571 [A]	-0.452 [B]	-1.381 [B]	0.000
Mn Unit Length	-2.692 [A]	2.692 [B]	1.770 [B]	0.000
B to N Perp	0.421 [A]	0.060 [A]	0.459 [A]	0.259
LFH	-2.021 [A]	0.228 [B]	-0.233 [B]	0.000
PFH	-1.988 [A]	1.268 [B]	0.726 [B]	0.000
Upper lip to E plane	0.065 [A]	-3.460 [B]	-1.619 [C]	0.000
Lower lip to E plane	0.346 [A]	-2.588 [B]	-1.341 [C]	0.000
Midface Length	-4.406 [A]	-0.240 [B]	-1.493 [B]	0.000
Anterior Cranial Base	-4.254 [A]	1.644 [B]	-0.711 [C]	0.000

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

Table 2. Z-score and P values of three cohorts unaffected with Class III (n = 27)

Variable	Colombian (z-score) (n = 16)	AI (z-score) (n = 5)	Caucasian (z-score) (n = 6)	P-value
SNA	-0.681 [A]	0.600 [A]	-0.033 [A]	0.129
SNB	-1.000 [A]	0.940 [B]	-0.400 [B]	0.025
ANB	-0.400 [A]	-0.300 [B]	-0.467 [C]	0.071
SN to GoGn	0.469 [A]	0.340 [A]	0.633 [A]	0.905
FH to SN	1.788 [A]	1.040 [A]	1.683 [A]	0.603
Chin Angle	1.081 [A]	-0.340 [B]	0.267 [B]	0.040
Articular Angle	0.469 [A]	1.020 [A]	-0.033 [A]	0.448
Facial Taper	-0.594 [A]	-2.060 [B]	-1.133 [B]	0.054
Facial Plane to SN	-1.19 [A]	0.18 [B]	-0.45 [B]	0.049
Mx Unit Length	-5.563 [A]	0.720 [B]	-1.250 [B]	0.000
Mn Unit Length	-3.713 [A]	4.560 [B]	1.017 [B]	0.000
B to N Perp	-0.213 [A]	0.300 [A]	-0.033 [A]	0.471
LFH	-2.294 [A]	1.420 [B]	-0.167 [B]	0.000
PFH	-2.619 [A]	1.620 [B]	0.833 [B]	0.000
Upper lip to E plane	1.350 [A]	-2.200 [B]	-0.083 [B]	0.022
Lower lip to E plane	0.675 [A]	-1.000 [A]	0.100 [A]	0.204
Midface Length	-4.344 [A]	0.720 [B]	-1.617 [B]	0.000
Anterior Cranial Base	-4.081 [A]	2.220 [B]	-1.300 [C]	0.000

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

Table 3. Z-score and P values of three cohorts affected with Class III (n = 73)

Variable	Colombian (z-score) (n = 32)	AI (z-score) (n = 20)	Caucasian (z-score) (n = 21)	P-value
SNA	-0.106 [A]	-0.415 [A]	-0.762 [A]	0.114
SNB	0.738 [A]	0.550 [A]	0.400 [A]	0.685
ANB	-1.313 [A]	-1.650 [A]	-1.629 [A]	0.607
SN to GoGn	-0.022 [A]	-0.270 [A]	-0.286 [A]	0.740
FH to SN	1.150 [A]	0.805 [A]	1.476 [A]	0.207
Chin Angle	0.063 [A]	-1.500 [B]	-1.310 [B]	0.001
Articular Angle	0.031 [A]	0.500 [A]	-0.319 [A]	0.055
Facial Taper	-1.388 [A]	-1.170 [A]	-0.886 [A]	0.531
Facial Plane to SN	-0.200 [A]	0.135 [A]	0.333 [A]	0.887
Mx Unit Length	-5.575 [A]	-0.745 [B]	-1.419 [B]	0.001
Mn Unit Length	-2.169 [A]	2.225 [B]	1.986 [B]	0.001
B to N Perp	0.738 [A]	0.000 [B]	0.600 [B]	0.030
LFH	-1.884 [A]	-0.070 [B]	-0.252 [B]	0.009
PFH	-1.672 [A]	1.180 [B]	0.695 [B]	0.001
Upper lip to E plane	-0.578 [A]	-3.775 [B]	-2.057 [C]	0.000
Lower lip to E plane	0.181 [A]	-2.985 [B]	-1.752 [B]	0.000
Midface Length	-4.438 [A]	-0.480 [B]	-1.457 [B]	0.000
Anterior Cranial Base	-4.341 [A]	1.500 [B]	-0.543 [B]	0.000

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

Table 4. A comparison of the 3 cohorts in regard to the statistically significant differences between the 10 reduced cephalometric variables (n = 100)

Variable	Colombian	AI	Caucasian
Chin Angle	slightly increased	moderately decreased	very slightly decreased
Articular Angle	slightly increased	increased	decreased
Mx Unit Length	very decreased	slightly decreased	moderately decreased
Mn Unit Length	very decreased	very increased	moderately increased
LFH	very decreased	slightly decreased	Decreased
PFH	Decreased	increased	slightly increased
Upper lip to E plane	slightly decreased	very decreased	moderately decreased
Lower lip to E plane	slightly decreased	very decreased	Decreased
Midface Length	very decreased	slightly decreased	Decreased
Anterior Cranial Base	very decreased	Increased	slightly decreased

Table 5. A comparison of the 3 unaffected cohorts in regard to the statistically significant differences between the 10 reduced cephalometric variables (n = 27)

Variable	Colombian	AI	Caucasian
SNB	mn more posteriorly positioned relative to anterior cranial base	mn slightly anteriorly positioned relative to anterior cranial base	mn slightly posteriorly positioned relative to anterior cranial base
Chin Angle	Increased	slightly decreased	slightly decreased
Facial Plane to SN	moderately decreased	slightly increased	slightly decreased
Mx Unit Length	very decreased	slightly increased	moderately decreased
Mn Unit Length	very decreased	very increased	moderately increased
LFH	very decreased	slightly increased	decreased
PFH	very decreased	moderately increased	slightly increased
Upper lip to E plane	moderately increased	very decreased	slightly decreased
Lower lip to E plane	slightly increased	moderately decreased	slightly increased
Midface Length	very decreased	slightly increased	moderately decreased
Anterior Cranial Base	very decreased	very increased	moderately decreased

Table 6. A comparison of the 3 affected cohorts in regard to the statistically significant differences between the 10 reduced cephalometric variables (n = 73)

Variable	Colombian	AI	Caucasian
Chin Angle	slightly increased	decreased	decreased
Mx Unit Length	very decreased	slightly decreased	moderately decreased
Mn Unit Length	moderately decreased	moderately increased	moderately increased
B to N Perp	slightly increased	normal	slightly increased
LFH	moderately decreased	slightly decreased	slightly decreased
PFH	moderately decreased	moderately increased	decreased
Upper lip to E plane	slightly decreased	very decreased	moderately increased
Lower lip to E plane	slightly increased	very decreased	slightly decreased
Midface Length	very decreased	slightly decreased	moderately decreased
Anterior Cranial Base	very decreased	increased	slightly decreased

Inter-familial Comparisons

Comparison between families within each of 3 cohorts individuals affected with Class III

Table 7. Z-scores and P values of affected Colombian Families (n=32)

Variable	Family #1 (z-score) (n = 4)	Family #2 (z-score) (n = 8)	Family #3 (z-score) (n = 14)	Family #4 (z-score) (n = 6)	P-value
SNA	-0.325 [A]	-0.363 [A]	0.136 [A]	-0.183 [A]	0.825
SNB	-0.975 [A]	0.738 [A]	1.036 [A]	-0.117 [A]	0.337
ANB	-1.950 [A]	-1.675 [A]	-1.464 [A]	-0.050 [A]	0.048
SN to GoGn	-0.375 [A]	0.113 [A]	-0.514 [A]	1.483 [A]	0.011
FH to SN	0.925 [A]	0.813 [A]	0.936 [A]	2.250 [A]	0.117
Chin Angle	-1.200 [A]	-0.300 [A]	0.036 [A]	1.450 [B]	0.048
Articular Angle	-0.100 [A]	-0.225 [A]	-0.200 [A]	-0.267 [A]	0.743
Facial Taper	-1.300 [A]	-1.400 [A]	-1.086 [A]	-2.133 [A]	0.469
Facial Plane to SN	-0.775 [A]	0.313 [A]	-0.471 [A]	-0.967 [A]	0.079
Mx Unit Length	-4.750 [A]	-9.838 [A]	-4.364 [A]	-3.267 [A]	0.155
Mn Unit Length	-0.625 [A]	-5.625 [A]	-1.607 [A]	0.100 [A]	0.220
B to N Perp	-0.950 [A]	0.688 [A]	0.721 [A]	0.700 [A]	0.936
LFH	-1.175 [A]	-3.625 [A]	-2.007 [A]	0.250 [A]	0.080
PFH	-1.275 [A]	-3.675 [A]	-1.400 [A]	0.100 [A]	0.225
Upper lip to E plane	-3.050 [A]	-0.938 [A]	-0.257 [B]	0.800 [C]	0.016
Lower lip to E plane	-2.125 [A]	-0.088 [B]	0.321 [C]	1.750 [C]	0.001
Midface Length	-3.975 [A]	-7.413 [A]	-3.621 [A]	-2.683 [A]	0.160
Anterior Cranial Base	-3.175 [A]	-7.463 [A]	-3.479 [A]	-2.967 [A]	0.219

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

Table 8. Z-scores and P values of affected AI Families (n=20)

Variable	Family #19 (z-score) (n = 5)	Family #33 (z-score) (n = 5)	Family #18 (z-score) (n = 10)	P-value
SNA	-0.130 [A]	-0.280 [A]	-1.120 [A]	0.079
SNB	-0.920 [A]	0.840 [A]	-0.480 [A]	0.149
ANB	-1.830 [A]	-1.720 [A]	-1.220 [A]	0.821
SN to GoGn	0.020 [A]	0.280 [A]	-1.400 [B]	0.048
FH to SN	0.520 [A]	0.600 [A]	1.580 [A]	0.138
Chin Angle	-2.3901 [A]	-1.060 [A]	-0.160 [A]	0.052
Articular Angle	0.500 [A]	0.800 [A]	0.200 [A]	0.729
Facial Taper	-2.110 [A]	-1.980 [A]	1.520 [B]	0.001
Facial Plane to SN	0.460 [A]	0.220 [A]	-0.600 [A]	0.290
Mx Unit Length	-0.270 [A]	-1.660 [A]	-0.780 [A]	0.873
Mn Unit Length	-3.320 [A]	1.880 [A]	0.380 [A]	0.543
B to N Perp	0.050 [A]	0.100 [A]	-0.200 [A]	0.941
LFH	0.390 [A]	0.340 [A]	-1.400 [A]	0.314
PFH	-1.710 [A]	0.020 [A]	1.280 [A]	0.575
Upper lip to E plane	-4.530 [A]	-3.280 [A]	-2.760 [A]	0.504
Lower lip to E plane	-3.750 [A]	-2.360 [A]	-2.080 [A]	0.433
Midface Length	-0.130 [A]	-1.080 [A]	-0.580 [A]	0.909
Anterior Cranial Base	-1.570 [A]	0.680 [A]	1.570 [A]	0.843

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

Table 9. Z-scores and P values of affected Caucasian Families (n=20)*

Variable	Family #1 (z-score) (n = 4)	Family #2 (z-score) (n = 2)	Family #3 (z-score) (n = 5)	Family #4 (z-score) (n = 3)	Family #6 (z-score) (n = 4)	Family #8 (z-score) (n = 2)	P-value
SNA	-0.850 [A]	0.600 [A]	-0.780 [A]	-1.133 [A]	-0.825 [A]	-1.100 [A]	0.632
SNB	-0.425 [A]	2.200 [A]	-0.680 [A]	0.267 [A]	0.400 [A]	-0.400 [A]	0.566
ANB	-0.800 [A]	-2.300 [A]	-1.880 [A]	-2.000 [A]	-1.800 [A]	-0.900 [A]	0.603
SN to GoGn	1.000 [A]	-2.100 [A]	-0.820 [A]	-1.000 [A]	-0.325 [A]	-0.600 [A]	0.255
FH to SN	2.025 [A]	1.050 [A]	1.900 [A]	0.367 [A]	0.875 [A]	2.600 [A]	0.331
Chin Angle	-1.100 [A]	-1.700 [A]	-2.220 [A]	-0.600 [A]	-0.875 [A]	-0.750 [A]	0.362
Articular Angle	0.400 [A]	0.900 [A]	-0.840 [B]	-1.033 [B]	1.100 [A]	-1.250 [B]	0.008
Facial Taper	-1.975 [A]	-0.50 [A]	-0.640 [A]	-0.367 [A]	-0.725 [A]	-1.050 [A]	0.173
Facial Plane to SN	-0.600 [A]	2.100 [A]	-0.800 [A]	0.233 [A]	0.225 [A]	-0.550 [A]	0.444
Mx Unit Length	-0.625 [A]	-0.900 [A]	-0.880 [A]	0.267 [A]	-3.625 [A]	-2.300 [A]	0.140
Mn Unit Length	2.400 [A]	1.850 [A]	3.060 [A]	1.867 [A]	0.450 [A]	0.900 [A]	0.676
B to N Perp	0.400 [A]	1.950 [A]	0.820 [A]	0.067 [A]	0.075 [A]	0.800 [A]	0.100
LFH	0.650 [A]	-1.250 [A]	-0.060 [A]	-1.000 [A]	-0.500 [A]	-0.250 [A]	0.285
PFH	0.100 [A]	0.900 [A]	1.980 [A]	0.733 [A]	0.325 [A]	-0.250 [A]	0.514
Upper lip to E plane	-0.725 [A]	-4.150 [B]	-3.760 [B]	-1.700 [A]	-0.975 [A]	-2.000 [A]	0.038
Lower lip to E plane	0.500 [A]	-3.950 [B]	-4.360 [B]	-2.067 [B]	-0.675 [A]	-0.400 [A]	0.001
Midface Length	-0.95 [A]	-0.90 [A]	-1.12 [A]	-0.50 [A]	-3.05 [A]	-1.80 [A]	0.169
Anterior Cranial Base	-0.600 [A]	-0.750 [A]	-0.460 [A]	-0.567 [A]	-1.950 [B]	-0.750 [A]	0.045

Z-scores with the same superscript (ie., A, B or C) are not significantly different from one another ($p > 0.05$)

* Family #7 was excluded in this intra-family comparison due to < 2 family members

Table 10. A comparison of the 4 affected families within the Colombian cohort with regard to the statistically significant differences between the 3 reduced cephalometric variables (n = 32)

Variable	Family #1 (mean) (n = 5)	Family #2 (mean) (n = 5)	Family #3 (mean) (n = 6)	Family #4 (mean) (n = 6)
ANB	very decreased	very decreased	very decreased	slightly decreased
SN to GoGn	slightly decreased	slightly increased	slightly decreased	moderately increased
Chin Angle	moderately decreased	decreased	slightly increased	moderately increased
Upper lip to E plane	very decreased	slightly decreased	slightly decreased	slightly increased
Lower lip to E plane	very decreased	slightly decreased	slightly increased	moderately increased

Table 11. A comparison of the 3 affected families within the AI cohort with regard to the statistically significant differences between the 2 reduced cephalometric variables (n = 20)

Variable	Family #19 (mean) (n = 5)	Family #33 (mean) (n = 5)	Family #18 (mean) (n = 10)
SN to GoGn	slightly increased	increased	moderately decreased
Facial Taper	very decreased	moderately decreased	moderately increased

Table 12. A comparison of the 3 affected families within the Caucasian cohort with regard to the statistically significant differences between the 4 reduced cephalometric variables (n = 21)*

Variable	Family #1 (mean) (n = 4)	Family #2 (mean) (n = 2)	Family #3 (mean) (n = 5)	Family #4 (mean) (n = 3)	Family #6 (mean) (n = 4)	Family #8 (mean) (n = 2)
Articular Angle	slightly increased	slightly increased	slightly decreased	moderately decreased	moderately increased	moderately decreased
Upper lip to E plane	slightly decreased	very decreased	very decreased	moderately decreased	slightly decreased	very decreased
Lower lip to E plane	slightly increased	very decreased	very decreased	very decreased	slightly decreased	slightly decreased
Anterior Cranial Base	slightly decreased	slightly decreased	slightly decreased	slightly decreased	moderately decreased	slightly decreased

* Family #7 was excluded in this inter-family comparison due to < 2 family members

Findings from Phenotypic Analysis

Unaffected combined group comparison

The results from the factor analysis indicated that there were redundant and highly correlated cephalometric variables which have been reduced to 18 variables. The multivariate analysis of variance (MANOVA) of the combined groups, not adjusted for Skeletal Class III affection status, revealed that the following 10 measurements were significantly different among the three groups: Chin angle, articular angle, maxillary unit length, mandibular unit length, lower face height (LFH), posterior face height (PFH), upper lip to E Plane, midface length and anterior cranial base. There were no statistically significant differences when comparing the three cohorts, when not controlling for skeletal Class III affection status for following variables: SNA, SNB, ANB, SN to GoGn, FH to SN, facial plane to SN, facial taper and B to N Perp.

Unaffected non-combined group comparison

The results from the MANOVA of the 3 cohorts unaffected with skeletal Class III malocclusion revealed 11 cephalometric variables showed statistically significant differences between the means of the three cohorts. These variables included SNB, chin angle, facial plane to SN, maxillary unit length, mandibular unit length, lower face height, posterior face height, upper lip to E plane, lower lip to E plane, midface length and anterior cranial base. Upon comparing the affected with the unaffected groups, SNB, facial plane to SN and upper lip to E plane all indicated a difference. Based on the results, the Colombian and Caucasian cohorts appeared to have more posteriorly positioned mandibles relative to the anterior cranial base, while the AI group had a slightly more anteriorly positioned mandible relative to the anterior cranial base. The

facial plane to SN was moderately decreased in the Colombian cohort, while it was slightly increased in the AI cohort and slightly decreased in the Caucasian cohort. The upper lip to E plane was also different between the affected and unaffected groups. The unaffected skeletal Class III Colombian cohort had a moderately increased upper lip to E plane, while the upper lip to E plane was slightly decreased in the affected skeletal Class III Colombian cohort, which was consistent with their more retrusive maxilla.

There were no statistically significant differences between the means (z-scores) of the following 7 variables: SNA, ANB, SN to GoGn, FH to SN, Articular Angle, Facial Taper and B to N Perp, in the 3 cohorts.

Affected group comparison

The results from the multivariate analysis of variance of the combined groups, adjusted for Skeletal Class III affection status, revealed that the following 10 measurements were significantly different between the three groups: chin angle, maxillary unit length, mandibular unit length, B to N Perp mm, lower face height, posterior face height, upper lip to E plane, lower lip to E plane, midface length and anterior cranial base. There were no statistically significant differences among the following cephalometric variables: SNA, SNB, ANB, SN to GoGn, FH to SN, articular angle, facial taper, and facial plane to SN, when comparing the three cohorts and controlling for skeletal Class III affection status. The maxillary unit length was greatly decreased in the Colombian group when compared to the AI and Caucasian cohort. Mandibular unit length was moderately decreased in the Colombian cohort, and moderately increased in the AI and Caucasian cohorts. Lower face height was moderately decreased in the Colombian cohort and slightly decreased in the AI and

Caucasian cohort. Posterior face height was moderately decreased in the Colombian and moderately increased in the AI, and decreased in the Caucasian cohort. Upper lip to E plane was slightly decreased in the Colombian, very decreased in the AI and moderately increased in the Caucasian cohorts. Lower lip to E plane was greatly decreased in the AI cohort compared to the other cohorts. Anterior cranial base was greatly decreased in the Colombian cohort, increased in the AI and slightly decreased in the Caucasian cohorts.

The results from the multivariate analysis of variance indicate that a significant difference exists between the residual values of the means of the 18 variables between the two groups. It also indicates that the Colombian and AI groups are not alike. The differences between the three groups indicate that the three populations differ from one another, which could be due to distinctly different craniofacial developmental morphology.

Inter-familial Comparisons

The MANOVA was used to compare families within each of the 3 cohorts to detect any statistically significant differences that may exist between families. The results from the MANOVA demonstrated that the greatest differences in the affected Colombian cohort existed between families 1 and 4. Within the Colombian cohort of 4 families, the cephalometric variables ANB, SN to GoGn, chin angle, upper and lower lips to E plane, indicated statistically significant differences between the 4 families examined. Family # 4 had a more of an increase in SN to GoGn than the other 3 families, whereas families 1 and 2 both had a slight decrease in SN to GoGn than families 3 and 4 who both had an increase. Family #1 had a more decreased chin angle when compared to family #4, who had a more increased chin angle. Family #1 also had a decreased upper lip to E

plane when compared to family #4 whose upper lip to E plane appeared to be increased. Family #1 also had more of a decreased lower lip to E plane when compared to Family #4 whose lower lip to E-plane appeared to have increased.

The affected AI cohort of 3 families indicated a statistically significant difference between families #18 and #33. Family #18 demonstrated a decrease in the SN to GoGn angle, while family #33 indicated an increase. Family #18 also indicated an increase in facial taper and family #33 indicated a decrease.

Within the affected Caucasian cohort of 7 families, (only 6 were examined in this section due to insufficient family members in family #7), families #4, 6 and 8 showed the greatest variation when compared with families #1, 2 and 3. These statistically significant differences existed when comparing the cephalometric variables articular angle, upper and lower lips to E plane and anterior cranial base. Family #4 had an articular angle which was decreased relative to the other families, while family #6 exhibited an articular angle which was increased relative to the other families. Families #2, 3 and 8 all had very decreased upper lips to E plane when compared to the other families whose upper lips to E plane were only slightly to moderately decreased. Families #2, 3, 4 had very decreased lower lips to E plane, when compared to the other groups and family #1 had a slightly increased lower lip to E plane. Family # 6 had the greatest decrease in the anterior cranial base when compared to the other groups.

After determining the average of each cohort mathematically, we then selected the individual that was closest to the average and used that as an example for each cohort as represented in figures 3-5. The Colombian group exhibited a skeletal Class III of the

maxillary retrognathism type, the AI and Colombian groups exhibited more of a mandibular prognathic skeletal Class III type.

CHAPTER 5

PART II: GENETIC ANALYSIS

Materials and Methods

Recruitment and Pedigree Analysis

Subjects from the Colombian and AI cohorts as described in Table 14, were also analyzed for their genetic makeup using genome-wide scan and linkage analysis (described below). Each participant was recruited through the Wright and/or Frazier-Bowers laboratory. This study was reviewed and approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board. The IRB numbers for both the AI and the Colombian studies respectively are as follows: 96-0981, formerly DENT-3127 and 03-1640. Consent was obtained for each individual who participated in the study and by parents in the case of a minor.

Family members were interviewed, their dental records obtained and participants were categorized as affected or unaffected. Pedigrees of the families recruited were then constructed and analyzed using visual inspection technique to determine the pattern of inheritance. Constructed pedigrees were stored in Cyrillic version 2.1 (Oxford, UK 1997) and judged for inheritance pattern using the following guidelines listed in Table 5.

DNA Extraction from Samples

Consenting individuals were sent kits through the mail to collect saliva or buccal cells to provide DNA for subsequent extraction. DNA cell samples of both affected and

unaffected family members were prepared for DNA extraction using a Gentra Systems PUREGENE® DNA Purification Kit (Gentra Systems, Minneapolis, MN). The PUREGENE® DNA Purification Kit contains all the reagents necessary to purify high molecular weight genomic DNA from its source.

Once the samples were obtained from each participant, they were then stored at room temperature prior to purification. In order to perform cell-lysis, the collection brush was removed from the handle using a sterile scissor or razor blade and placed into a 1.5 ml microfuge tube containing 300 µl of Cell Lysis Solution. The sample was then incubated at 65^{o} C for 15-60 minutes, or if maximum yield was required, 1.5 µl of Proteinase K Solution (20 mg/ml) was added to the cell lysate. The brush was then removed from Cell Lysis Solution, scraping the brush on the sides of the tube to facilitate the removal of the lysate from the collection brush head. The RNase Treatment was then performed by adding 1.5 µl RNase Solution to the cell lysate. The sample was then mixed by inverting the tube 25 times and incubated at 37 ^{o} C for 15-60 minutes, then cooled to room temperature. The tube was then placed in an ice bath for 5 minutes and centrifuged at 13,000-16,000 x g for 3 minutes. The precipitated proteins then formed a tight, white pellet. If the precipitated protein pellet was not tight, the previous steps were repeated.

For the purposes of DNA Precipitation, the supernatant containing the DNA was poured into a clean 1.5 ml microfuge tube containing 300 µl 100% Isopropanol and 0.5 µl Glycogen Solution. The sample was then inverted gently and centrifuged to precipitate the DNA. At this point, the DNA may or may not have been visible as a small white pellet depending on the yield. DNA was washed and spun again in the centrifuge.

A DNA Hydration Solution was added of 20µl to each sample after it was dried and the DNA rehydrated by incubating the samples for 1 hour at 65 ^{o} C and/or overnight at room temperature. The DNA was then stored at 4 ^{o} C. Samples in need of long term storage were placed at -20 ^{o} C or -80 ^{o} C. Quantification of the DNA in the samples was performed using a nanodrop spectrophotometer.

Genome wide scan and linkage analysis

The Colombian cohort consisting of 48 individuals in 4 families was genotyped using 500 microsatellite markers prior to the refinement of the Single Nucleotide Polymorphism (SNP) genotyping methods. In order to amplify the DNA at these loci, polymerase chain reaction (PCR) was carried out using primers surrounding a previously identified locus. The initialization step took place at 95°C for 15 minutes and the DNA denaturation step took place at 95°C for 30 seconds. The annealing step took place at 62°C for 30 seconds. The elongation step took place at 72°C for 1 minute.

Chromatography was then used to analyze the gene fragment. A chromatogram was analyzed to determine the size of the fragments (bp) as well as whether subjects were heterozygous or homozygous for a given allele. A chromatogram of a heterozygous individual typically exhibits two defined peaks with smaller leading and lagging peaks, while that of a homozygous individual typically exhibits one defined peak.

The GeneChip Mapping 10K 2.0 Assay® version by Affymetrix (Santa Clara, CA 2004), is a mapping tool designed to identify regions of the genome that are linked to or associated with a particular trait or phenotype. It is also useful for determination of allele frequencies in various populations and for mapping regions with chromosomal copy

number changes during cancer progression. Data was entered into a Microsoft Access database developed and maintained at UNC.

Genotyping using the 10K SNP Chip Mapping Assay® was carried out for 25 individuals in 3 families (AI syndromic cohort). This array system provides genotypes for 10,000 human single nucleotide polymorphisms (SNPs) on a single array. Genome-wide scan will be performed for the Caucasian families in the near future. Once the genomic DNA was extracted from each of the AI samples, they were then digested with a restriction endonuclease enzyme and ligated to adaptors recognizing the cohesive four base overhangs. All fragments resulting from the restriction enzyme digestion, regardless of size, were substrates for adaptor ligation. A generic primer, which recognizes the adaptor sequence, was used to amplify ligated DNA fragments and PCR conditions were optimized to preferentially amplify fragments in the 250-1000 base pair size range. The amplified DNA was labeled and hybridized to GeneChip arrays. The arrays were then washed and stained on a GeneChip fluidics station and scanned on a GeneChip Scanner 3000.

Linkage Mapping & Analysis

Parametric and non-parametric linkage analyses were run to analyze the marker data obtained for both the Colombian and AI cohorts. Parametric linkage analysis assumes a model for inheritance (i.e., autosomal dominant or autosomal recessive), while non-parametric linkage analysis (Model Free Analysis), does not make this assumption. Non-parametric linkage (NPL) analysis has been described as a powerful approach to pedigree analysis, due to the lack of certainty about mode of inheritance, and is much more powerful than commonly used nonparametric methods, and loses little power

relative to parametric linkage analysis. NPL has been referred to as the method of choice for pedigree studies of complex traits (Kruglyak L. et al., 1996). In this study, both parametric and non-parametric models were run since the mode of inheritance of skeletal Class III malocclusion has not been completely confirmed.

Alleles were then designated using known allele frequencies from a Ceph database. Allegro software version 2.0 (Allegro Microsystems, Inc., Worcester, MA) was used to examine the transmittance of alleles from one family generation to the next. Logarithm of the odds (LOD) of linkage scores were then calculated using the Allegro software to indicate the genetic loci where mutations are most likely to occur. A LOD score of 3.0 or greater was considered significant evidence in favor of linkage. Any LOD score that falls between -2.0 and 3.0 was inconclusive, and a LOD score of -2.0 or lower was considered significant evidence in favor of non-linkage (Current Protocols in Human Genetics, Volume 1, 2001).

RESULTS FROM GENETIC ANALYSIS

Pedigree Analysis by Inspection

Family pedigrees and the analyses are shown in figures 6-19. A square indicates a male and a circle indicates a female. A shaded square or circle indicates an affected individual. An arrow beneath the shape indicates the family proband. A divorced couple is indicated by two slashes through the connecting line. A deceased individual is indicated by a slash through the square or circle. An analysis by inspection revealed an autosomal dominant mode of inheritance among all the families.

Colombian family #1 (figure 6), is composed of 3 generations of both affected and unaffected individuals. Each generation exhibits an approximately equal number of affected and unaffected family members. In this family, more females than males exhibit the phenotype. This family appears to exhibit autosomal dominant mode of inheritance of the Class III trait. Colombian family #2 (figure 7), comprises 4 generations of individuals affected and unaffected with the skeletal Class III trait. This family has twice as many females exhibit the phenotype. This family also appears to exhibit an autosomal dominant mode of inheritance. Colombian family #3 (figure 8), is composed of 4 generations with approximately males and females affected the trait equally, exhibiting an autosomal dominant mode of inheritance. Colombian family #4 (figure 9), comprises 2 generations with twice as many female affected as males and all are affected with the trait. The mode of inheritance is autosomal dominant.

AI family #19 (figure 10) and #33 (figure 11) are both composed of 6 and 7 generations respectively, with an equal number of males and females affected with the skeletal Class III trait, thereby exhibiting an autosomal dominant mode of inheritance.

Caucasian family #1 (figure 12), is made up of three generations of both affected and unaffected individuals. Each generation exhibits an approximately equal number of affected and unaffected family members. This family has twice as many males as females exhibiting the phenotype and appears to exhibit an autosomal dominant mode of skeletal Class III malocclusion. Family #2 in the Caucasian cohort (figure 13), is composed of 2 generations with an equal amount of males affected with the skeletal Class III trait as females. This family appears to exhibit autosomal dominant inheritance of the skeletal Class III malocclusion. Caucasian family # 3 (figure 14), has 5 generations with 4 generations affected. Males seem twice as likely to inherit the condition as females. This family appears to exhibit autosomal dominant inheritance of this trait. Family #4 (figure 15), has 2 generations with both generations affected with skeletal Class III malocclusion. Twice as many males are likely to be affected than females. This family appears to exhibit an autosomal dominant mode of inheritance of the skeletal Class III phenotype. Caucasian Family # 5 (figure 16), has 3 generations with 1 generation of affected individuals. More males were affected than females. There was some uncertainty regarding the skeletal Class III affection status of some of the individuals, hence a statement regarding the mode of inheritance was not made for this family. Caucasian family #6 (figure 17), had 3 generations with 2 generations of affected individuals and an equal number of affected females as males. This family also had a few individuals where the affection status of the skeletal Class III trait was uncertain, however, with the information currently available, this family appears to have an autosomal dominant mode of inheritance. Caucasian family #7 (figure 18) had 2 generations with only females affected with the trait. Family # 8 in the Caucasian cohort

(figure # 19), had 2 generations of affected individuals with twice as many females affected as males, revealing an autosomal dominant mode of inheritance.

Linkage Analysis for Colombian Cohort

The parametric linkage results from the gene fragment analysis for the Colombian cohort yielded LOD scores > 1.0 at a specific locus on chromosome 1, while the non-parametric linkage results revealed positive LOD scores at specific loci on chromosomes 1, 3 and 12. The loci in the parametric linkage analysis correspond to the regions including and between markers D1S2865 and D1S435. The loci in the non-parametric linkage analysis correspond to the regions including and between markers D1S435 and D1S206, D3S3725 and DS3041, and D12S368 and D12S83. LOD score data are summarized in the tables 13 and 14 below.

Table 13. Parametric Linkage Analysis

MARKERS	CHROMOSOME	LOD SCORE
D9S1843 – D9S307	9	0.7590
D12S83 – D12S1294	12	0.7699
D13S1243 – D13S221	13	1.1277
D1S2865 – D1S435	1	1.8554

Table 14. Non-Parametric Linkage Analysis

MARKERS	CHROMOSOME	LOD SCORE
D17S922 – D17S839	17	1.0168
D5S2031 – D5S674	5	1.1873
D1S435 – D1S206	1	1.6382
D12S368 – D12S83	12	1.7820
D3S3725 – D3S3041	3	1.9136

Figure 1. Diagrammatic Representation of 23 Chromosomes and Relative Location of Markers D1S2865 – D1S435 using Parametric Linkage Analysis for Colombian Cohort

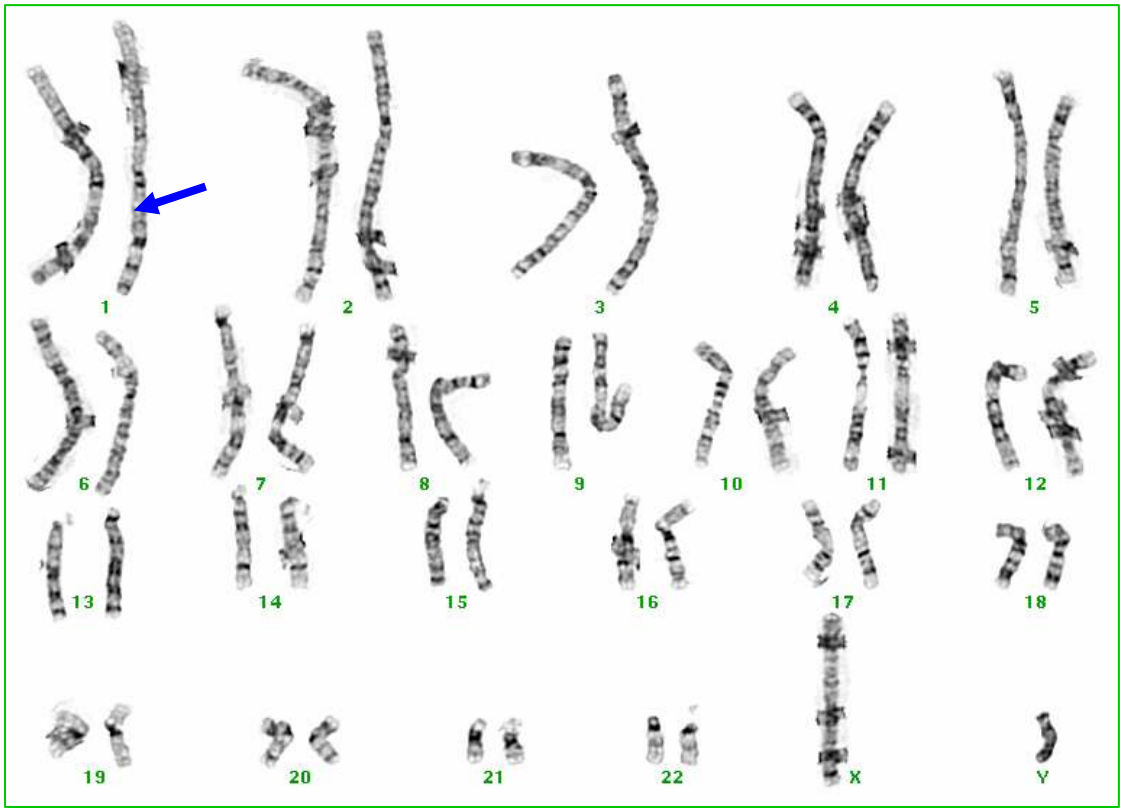
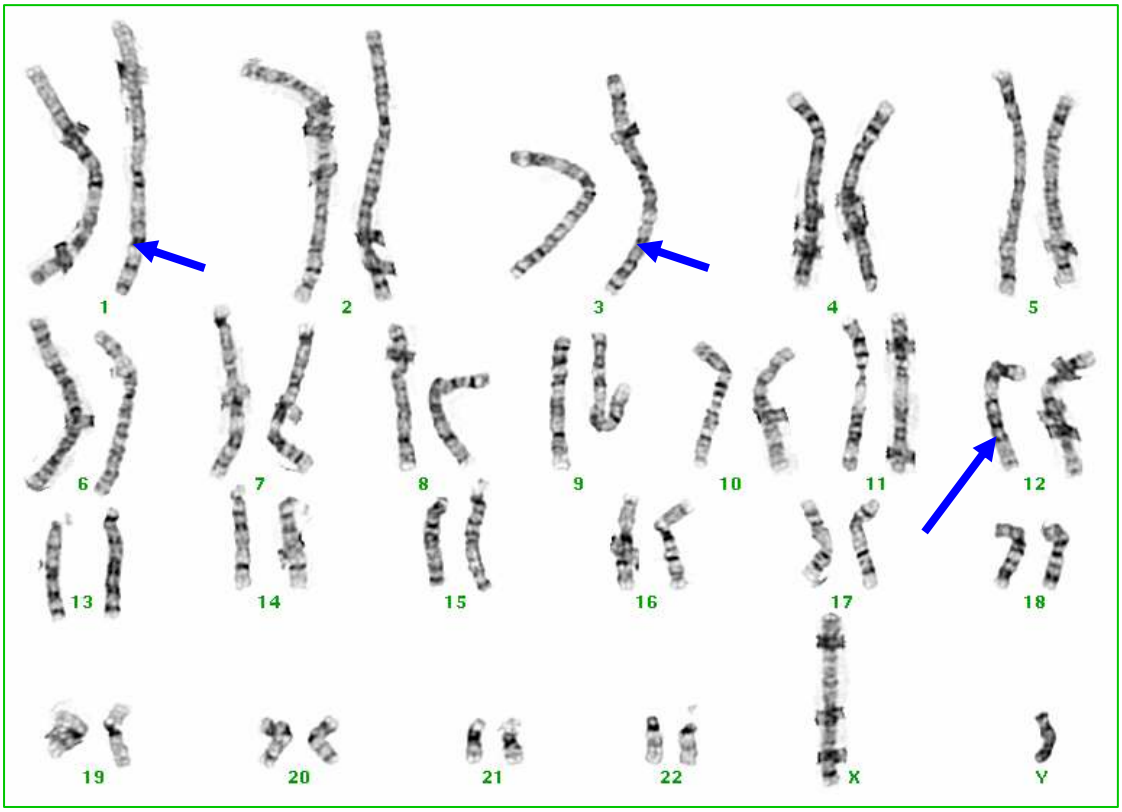


Figure 2. Diagrammatic Representation of 23 Chromosomes and Relative Location of Markers D1S435 – D1S206, D3S3725 – D3S3041 and D12S368 – D12S83, using Non-Parametric Linkage Analysis for Colombian Cohort



Linkage Analysis for AI Cohort

Data for the AI cohort was inconsistent due to insufficient power of family structure (ie., family #33), and missing DNA samples. The results for this cohort could therefore not be included here and is forthcoming.

CHAPTER 6

DISCUSSION

Phenotypic Analysis

This study was conducted to examine the phenotypic and genetic components that contribute to the development of skeletal Class III malocclusion. We compared 3 cohorts affected with this trait, using Cephalometric analysis and genome wide scan. Cephalometrics was used to investigate the morphology of the craniofacial complex in each of the individuals involved in this study. Factor analysis was then used to reduce the cephalometric variables to a manageable amount for the multivariate analysis of variance (MANOVA). The MANOVA was then used to detect statistically significant differences between each of the 3 cohorts and between families within each of the 3 cohorts, to identify overall morphological variations in the craniofacial complex.

The results of the factor analysis revealed 18 cephalometric variables for use in the MANOVA. The results from the MANOVA when comparing the 3 groups, indicated that each of the 3 groups are unique, and that the differences between them could be due to specific craniofacial morphologic features.

In order to complete the intra-familial comparisons within each of the three groups, z-scores of cephalometric variables were compared using the MANOVA which revealed similarities among relatives. Affected family members appeared to be similar. The differences that exist between families 1 and 4 within the Colombian cohort may be due to a slight morphological difference including an increase in the pogonion projection

in this family, hence an increase in chin angle, when compared to the other families within this cohort. The differences between families #18 and 33 within the AI cohort were most evident when examining the SN to GoGn and facial taper cephalometric variables. Within the Caucasian cohort, differences also existed between the families among the following cephalometric variables: articular angle, upper and lower lips to E plane and anterior cranial base. These differences may be due to slightly different morphologic characteristics that exist within these families.

In our study, we found that each cohort presented unique craniofacial characteristics and that each of the families were similar within each cohort. Specifically, the Colombian cohort exhibited a greater decrease in lower facial height, increased anterior cranial base length and decreased posterior face height than the other groups, while the AI cohort was found to have a more increased anterior cranial base length and increased posterior face height. The Caucasian cohort was found to have a more increase in mandibular unit length and an increase in the upper lip to E plane than the other groups.

Genetic Analysis

Genetic mutations have been suggested in previous studies as contributing to the development of skeletal Class III malocclusion and that this mutation can be transmitted from one generation to the next. The parametric linkage analysis results revealed a LOD score suggestive of linkage on chromosome 1. The regions between markers D1S2865 to D1S435 yielded the greatest LOD score. However, non-parametric linkage analysis results revealed a LOD score suggestive of linkage on chromosomes 1, 3, 12. The regions between markers D1S435 to D1S206, D3S3725 to D3S3041, and D12S368 to

D12S83 yielded the greatest LOD scores on chromosomes 1, 3 and 12. The difference between the parametric and non-parametric linkage analysis results for the Colombian cohort could be due to the assumption of the autosomal dominant mode of inheritance of the trait, hence this trait would affect both male and females equally and does not skip a generation and should be represented in the pedigree. Whereas the non-parametric linkage analysis does not calculate the LOD scores based on inheritance patterns of the trait, but on their transmission through generations. Hence, once the constraint of mode of inheritance is removed, it is then possible to measure the fraction of the total inheritance information extracted by the available marker data and is able to indicate the regions in which typing additional markers might be most useful and consider LOD scores that are significant.

Furthermore, single nucleotide polymorphism chip technology (SNP) has been used to identify regions associated with skeletal Class III malocclusion in the AI families. The Affymetrix GeneChip Mapping Assay (Affymetrix, Inc., Santa Clara, CA) has been used to analyze SNP's in both affected and unaffected individuals. We anticipate the results from the AI linkage analysis will reveal other genes in addition to those found from the results of the Colombian linkage analysis.

Although the findings of this study are suggestive of linkage at chromosome 1 for the skeletal Class III trait, the answers regarding the genetic etiology of true mandibular prognathism might be found in the IGF system which would suggest that the mutation could be on chromosome 15. If the somatomedin hypothesis introduced by Daughaday almost a half-century ago is correct, the regulation of the growth of the skeleton would be the key to the entire genetic study on skeletal Class III malocclusion.

Limitations of previous studies

Limitations of previous studies include the lack of classification of the skeletal Class III phenotype into specific types of class III malocclusion, namely, true mandibular prognathism, maxillary retrognathism, or a combination of both. This resulted in a wide range of Class III prevalence reported in the literature (El-Gheriani et al 2003, Litton 1970). It is important therefore to appropriately define the class III phenotype based on the morphologic characteristics of the craniofacial complex, not just based on the first molar relationship.

Another limitation of previous studies involved the lack of control for genetics versus environment in the determination of the etiology of class III malocclusion. This was particularly seen in the twin studies conducted by Jena et al 2005. In twin studies it is difficult to determine where the phenotypic similarities seen are due to genetics or from the environment.

Limitations of this study

A limitation of this study was missing DNA in the AI cohort, hence inconclusive data due to insufficient power of family structure. Other limitations include the limited sample size. Inclusion of edentulous family members was another limitation of this study, as this could potentially affect the mandibular position. Changes in the vertical dimension usually accompanies edentulous subjects, that may in turn result in changes in sagittal direction and hence these subjects may have a false presentation of skeletal Class III malocclusion.

CHAPTER 7

CONCLUSIONS

Phenotypic Analysis

A comparison of the three cohorts in this study, Colombian, AI and Caucasian, revealed that all three groups are different and that theses differences could be attributed to morphological characteristics that are not the same from cohort to cohort. A comparison of the families within each of the cohorts revealed that they tended to be more alike with the exception of specific cephalometric variables such as chin angle, upper and lower lips to E plane, SN to GoGn, facial taper and anterior cranial base.

Genetic Analysis

A visual inspection of the pedigrees suggests an autosomal dominant mode of inheritance of skeletal Class III malocclusion. Results from the linkage analysis in this study suggest that chromosomes 1, 3 and 12 are suggestive of linkage to the skeletal Class III trait. In light of other genetic studies currently being done with improved technology, these results are not consistent with some of the other previous studies which would suggest that the IGF-1 gene located on chromosome 15, is involved in the regulation of growth hormone and hence the development of the skeleton.

Once the skeletal Class III trait is phenotypically characterized according to type, i.e., maxillary hypoplasia, mandibular prognathism, or a combination of both, it may be possible to utilize the candidate genes identified for other syndromes that have a skeletal

Class III component in the identification of the genes involved in the development of this trait.

CHAPTER 8

FUTURE DIRECTIONS OF STUDY AND CLINICAL IMPLICATIONS

Upon completion of the linkage analysis for the AI cohort, these results can be compared with the results from the linkage analysis for the Colombian cohort. Conceivably, a prospective association study can be designed in the future with the selection of subjects divided into 3 groups. These groups could be: individuals affected with true mandibular prognathism i.e., with a reverse overjet of at least -5mm, those affected with maxillary retrognathism, and a group with the combination of both mandibular prognathism and maxillary retrognathism. These subjects could be derived from ethnic groups such as Japanese or Korean populations. Once each of these subjects are identified and classified into each of the three groups, it may be possible to conduct the phenotypic and genetic analysis on each of the three groups as was conducted in this study. The gene IGF-1 involved in the regulation of growth, and the gene MEN 1 involved in the development of acromegaly could be used as potential candidate genes in the search for a mutation for the group affected with true mandibular prognathism, in particular, chromosome 15. The genes involved in syndromic conditions such as Crouzon's syndrome, FGFR 1-3, and the gene involve in FGFR3 could be used as potential candidate genes involved in the development of maxillary hypoplasia. It is difficult however to obtain subjects for this type of study. We will therefore continue our collaboration with the Colombian, and Japanese populations, in hope of increasing our recruitment efforts to include other populations.

Due to the tremendous complexity of this skeletal jaw disharmony, it is conceivable that in the future, it may be necessary for a clinician to classify this trait distinctly, not only based on its morphology, but primarily based on its molecular genetic composition.

Table 15. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
ANB < 1mm	Previous orthodontic treatment
Overjet ≤ 0 *	Congenital abnormalities
Concave Profile	Craniofacial anomalies

* Not used for edentulous subjects

Table 16. Descriptive Statistics for Study Groups

VARIABLES	Colombian	AI	Caucasian
Sample Size	48	25	27
Race [1]			
Caucasian	0 (0.00)	25 (100)	27 (100)
Hispanic	48 (100.00)	0 (0.00)	0 (0.00)
Gender [1]			
Male	19 (39.6)	17 (68.00)	10 (37.00)
Female	29 (60.4)	8 (32.00)	17 (63.00)
Age [2]	34.77 (+/- 18.488)	40.84 (+/-17.296	30.15 (+/- 21.162
Range	6-76	SD)	SD)
		14-79	6-90
Class III Affectivity[1]	N = 16 (33.3) Y = 32 (66.6)	N = 5 (28.00) Y = 20 (72.00)	N = 6 (22.2) Y = 21 (72.8)

[1] n (%)

[2] Mean (years) +/- Standard Deviation

Table 17. Results from the Factor Analysis - Variable List

VARIABLE
SNA [1]
SNB [1]
ANB [1]
SN to GoGn [1]
FH to SN[1]
Chin Angle[1]
Articular Angle[1]
Facial Taper[1]
Facial Plane to SN[1]
Mx Unit Length [2]
Mn Unit Length [2]
B to N Perp [2]
LFH [2]
PFH [2]
Upper lip to E plane[2]
Lower lip to E lane[2]
Midface Length [2]
Anterior Cranial Base [2]

[1] degrees (angular measurement)

[2] mm (linear measurement)

Table 18. Summary of Modes of Inheritance

	Autosomal Dominant Inheritance	Autosomal Recessive Inheritance	Sex-linked Inheritance
Males and Females Affected	Equally	Equally	Males more than females
Phenotype Appearance	Every generation	Typically appears in one generation and not in the individual's offspring or parents.	-----
Probability of Inheritance	Offspring have a 50% chance of inheriting the trait	Offspring have a 25% chance of inheriting the trait if both parents are carriers	Carrier females have a 50% chance of having an affected son and a 50% chance of having a carrier daughter

Figure 3. Representative Cephalometric Tracing of Colombian Cohort

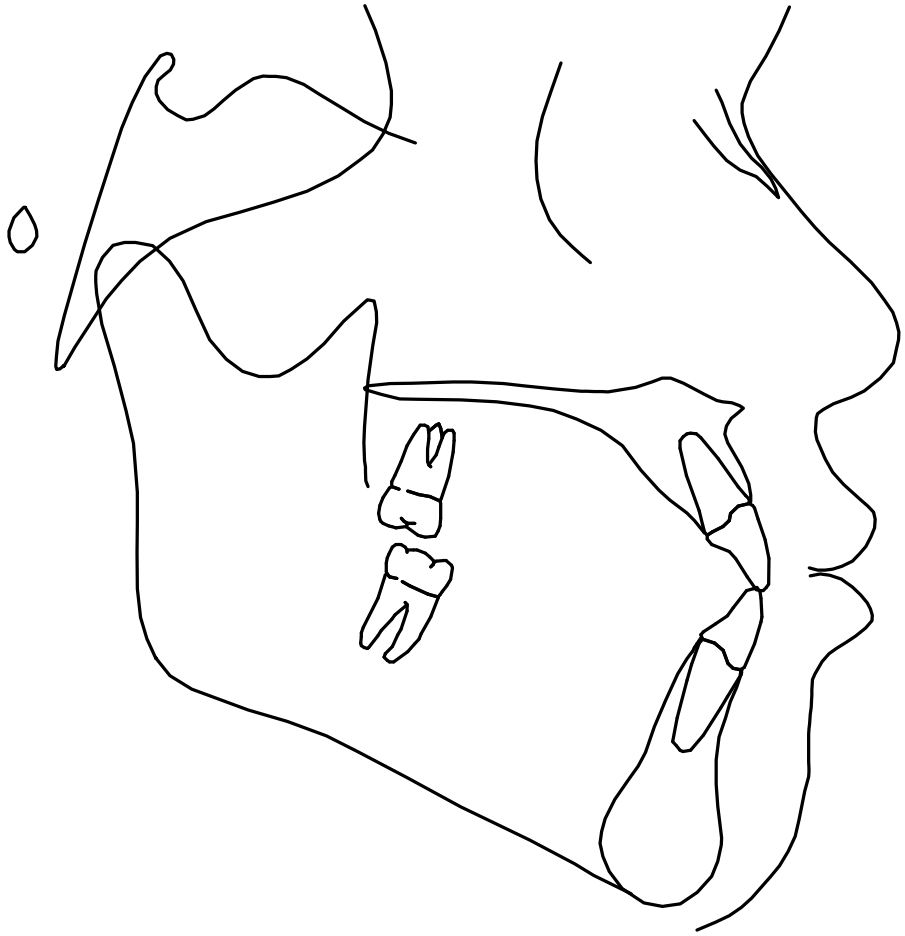


Figure 4. Representative Cephalometric Tracing of AI Cohort

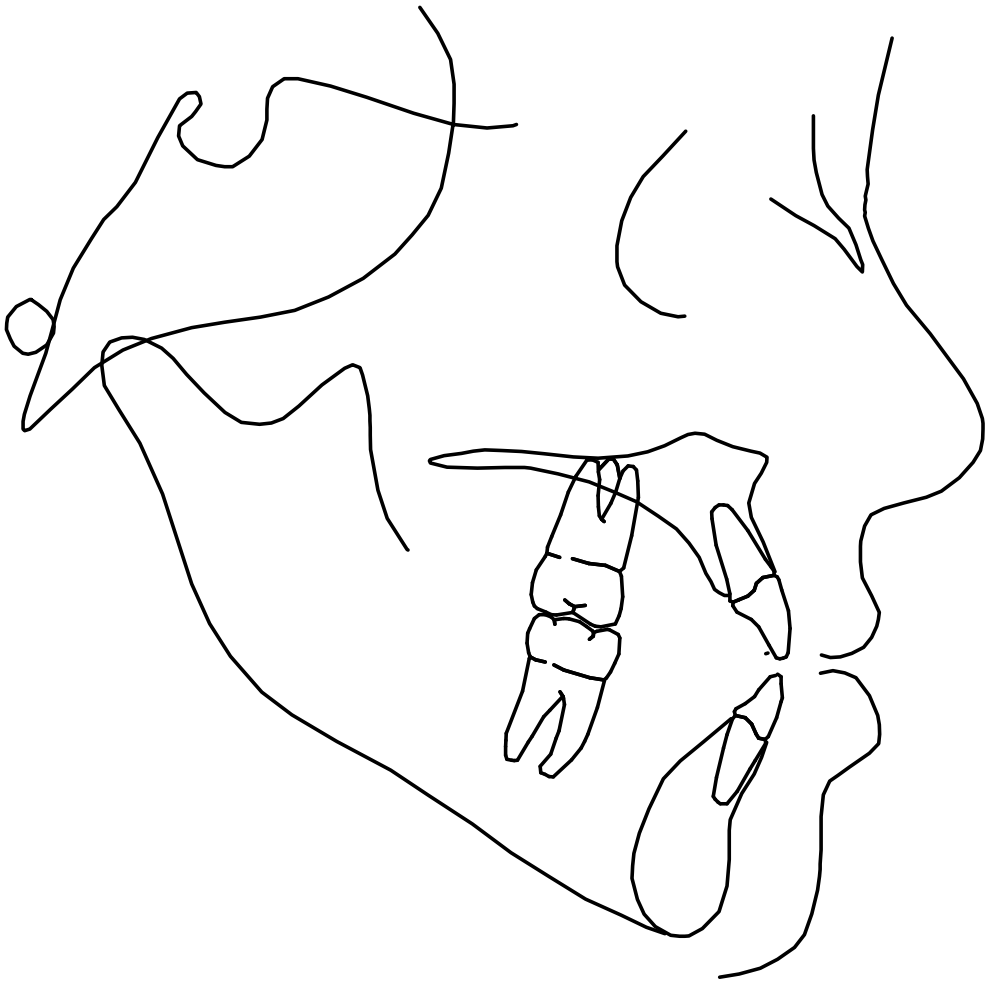


Figure 5. Representative Cephalometric Tracing of Caucasian Cohort

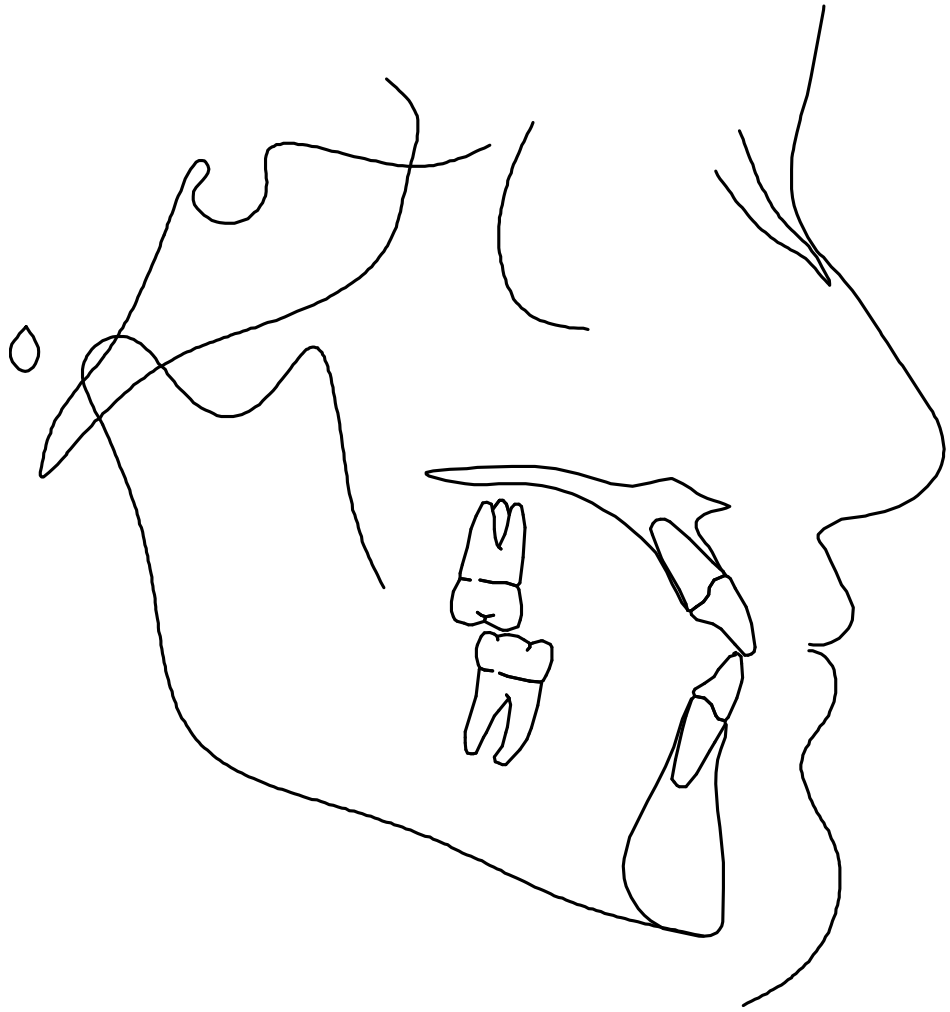


Figure 6. Pedigree – Colombian Family #1

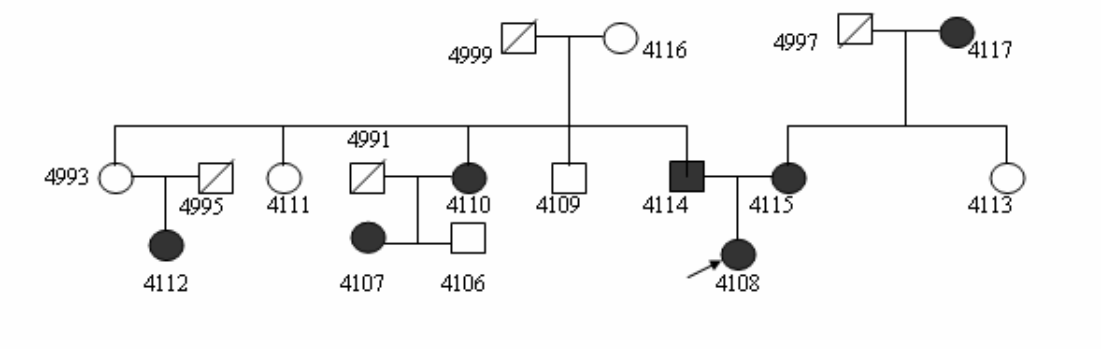


Figure 7. Pedigree – Colombian Family #2

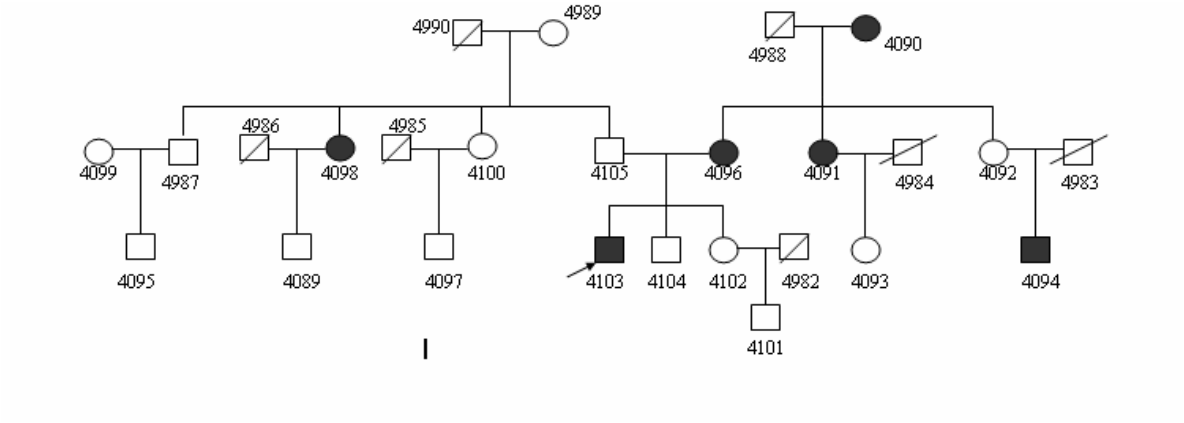


Figure 8. Pedigree – Colombian Family #3

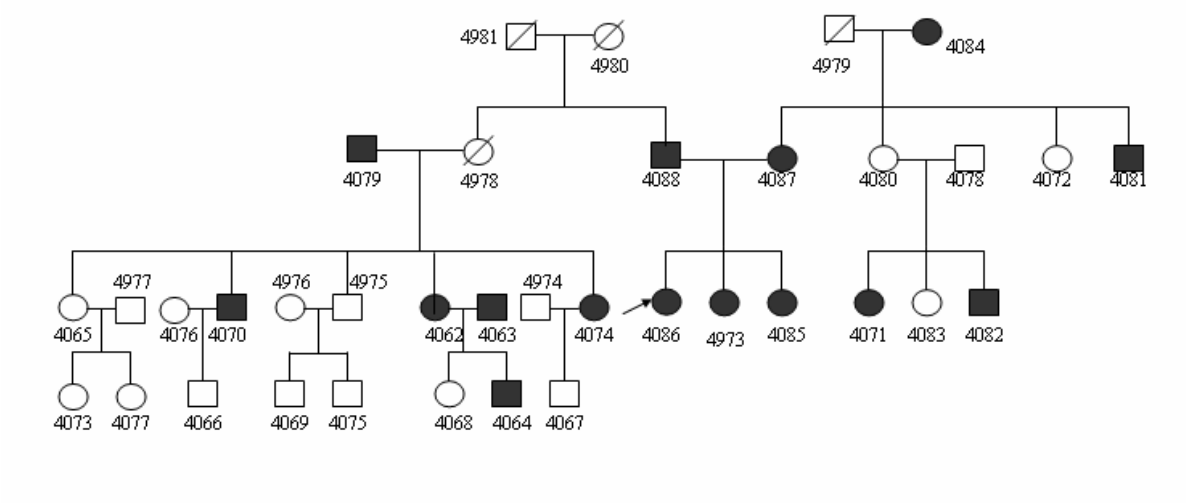


Figure 9. Pedigree – Colombian Family #4

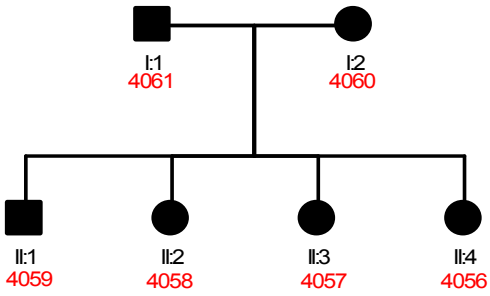


Figure 10. Pedigree – AI Family #19

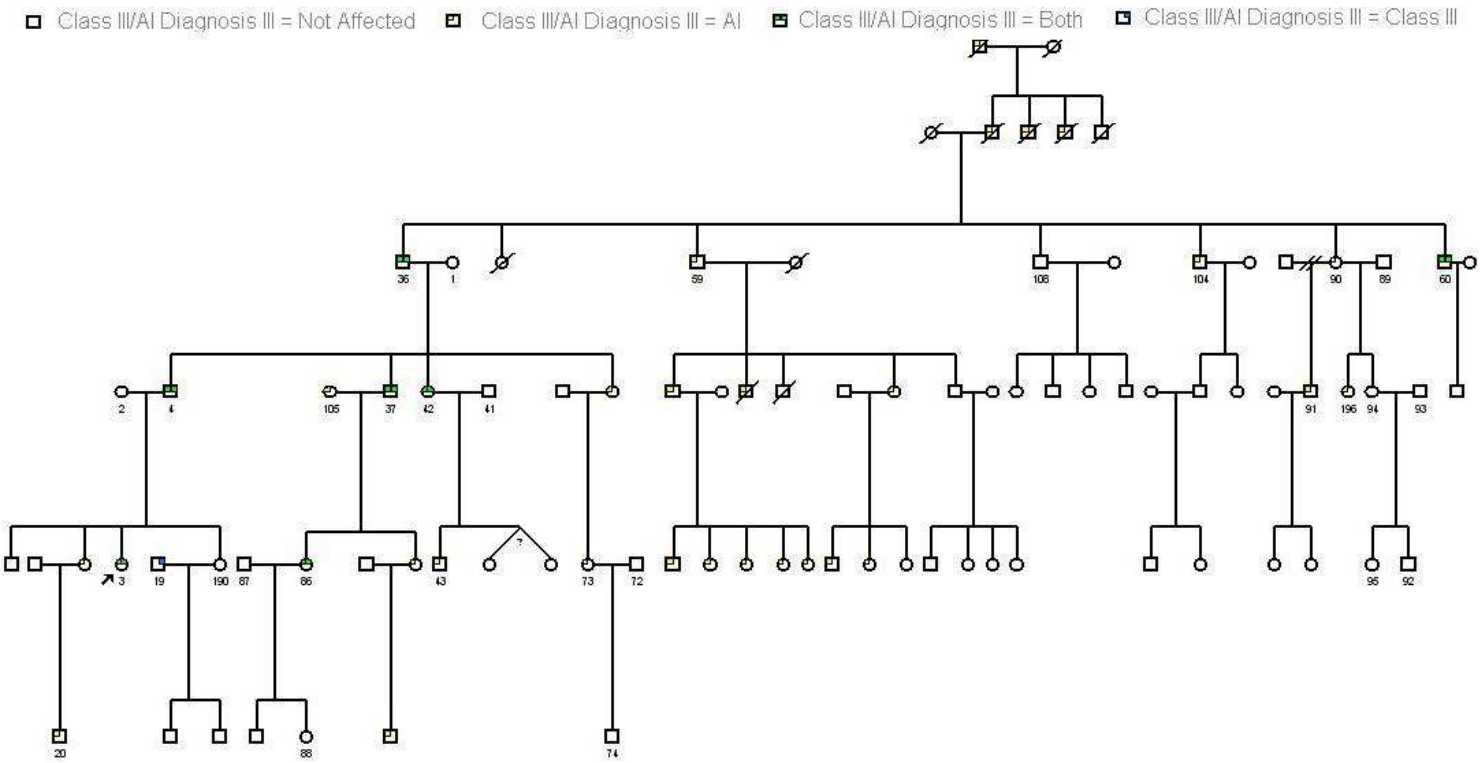


Figure 11. Pedigree – AI Family #33

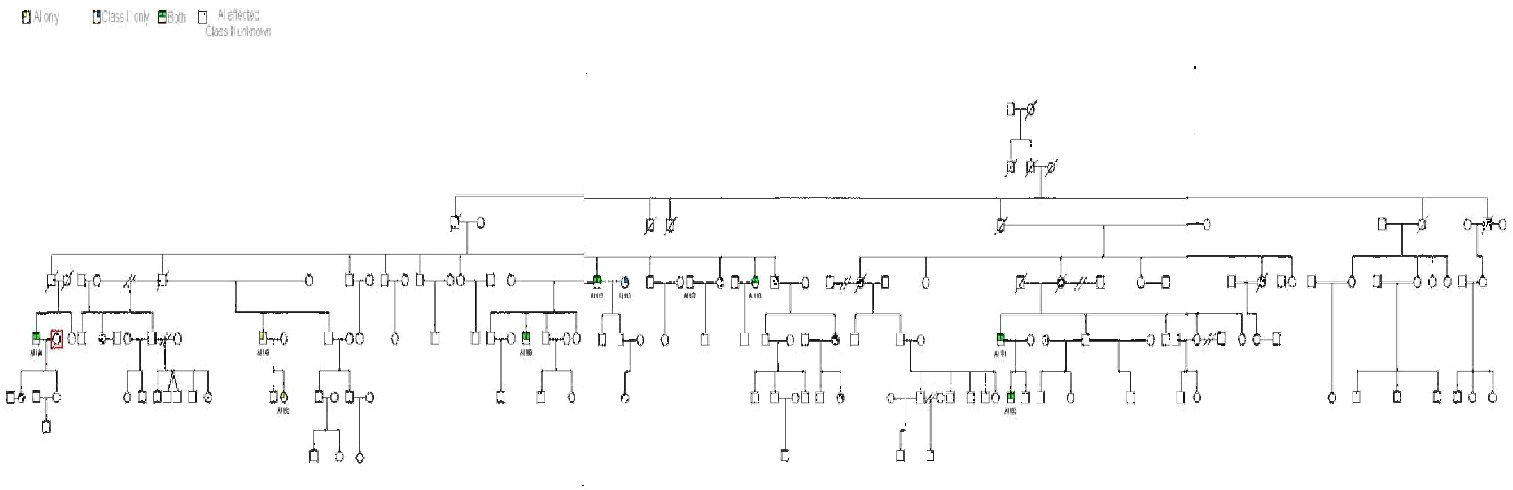


Figure 12. Pedigree – Caucasian Family #1

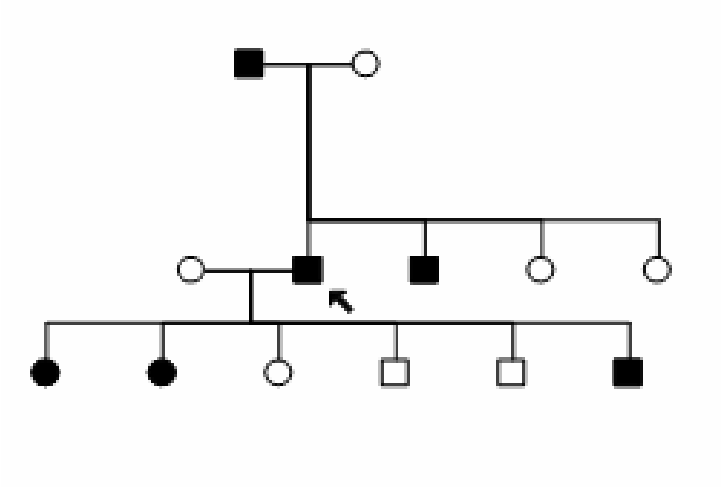


Figure 13. Pedigree – Caucasian Family #2

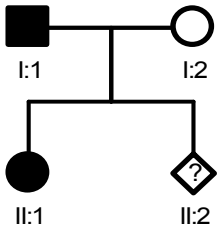


Figure 14. Pedigree – Caucasian Family #3

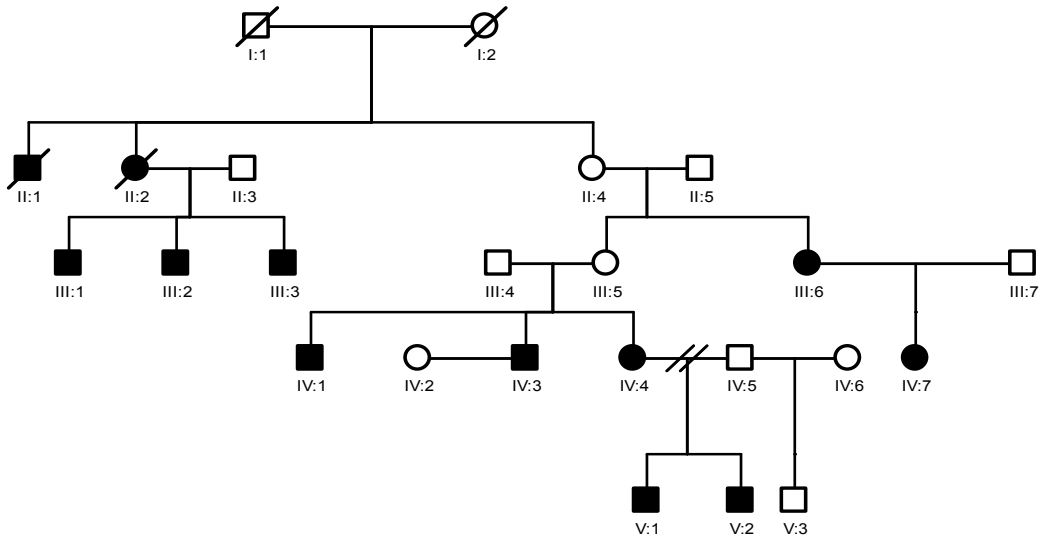


Figure 15. Pedigree – Caucasian Family #4

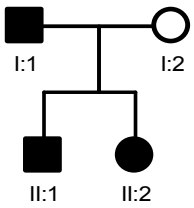


Figure 16. Pedigree – Caucasian Family #5

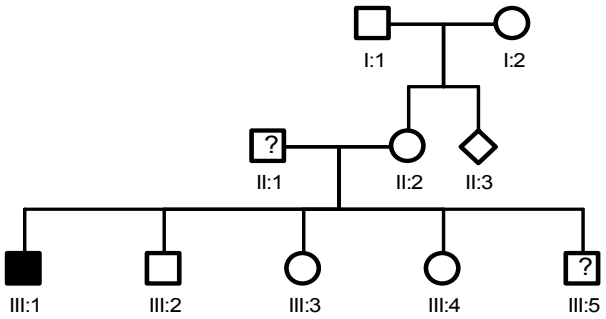


Figure 17. Pedigree – Caucasian Family #6

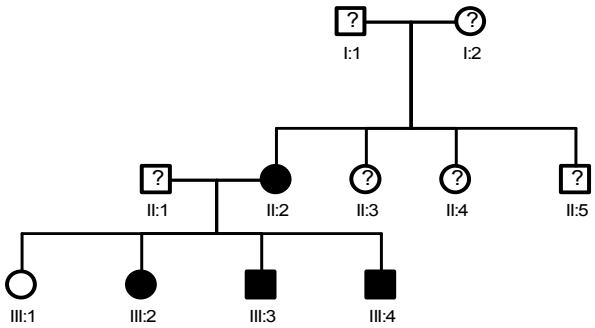


Figure 18. Pedigree – Caucasian Family #7

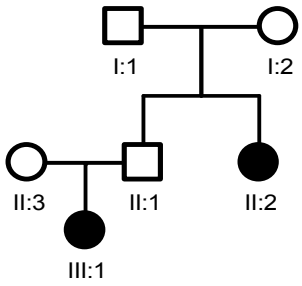
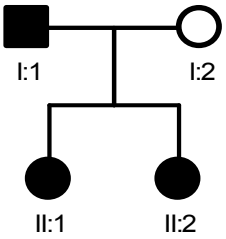


Figure 19. Pedigree – Caucasian Family #8



APPENDIX A

Intraclass Correlation Results

Obs	icc	Variable
1	.	NasalHeight
2	.	HoldawayRatio
3	0.68110	PFHtoAFH
4	0.89069	FHtoSN
5	0.90772	ArticularAngle
6	0.92687	AtoNPerpmm
7	0.94363	GonialJawAngle
8	0.94571	STissueNPerptoUpperLip
9	0.95310	MxUnitLengthCotoANSmm
10	0.95362	FMA
11	0.95541	STissueNPerptoLowerLip
12	0.95971	SNA
13	0.96233	L6toMP
14	0.96422	MidfaceLength
15	0.96447	BtoNPerpmm
16	0.96508	OccPlanetoFH
17	0.96513	PFH
18	0.96521	L1toMP
19	0.96688	PogtoNPerpmm
20	0.96695	FacialAngle
21	0.96755	RamusHeight
22	0.96803	UFH
23	0.96933	SaddleSellaAngle
24	0.97342	LFH
25	0.97890	STissueNPerptoSTPg
26	0.97911	FacialTaper
27	0.97917	U1toNAmm
28	0.98096	PAFaceHeight
29	0.98224	AnteriorCranialBase
30	0.98399	U1toPP
31	0.98498	AtoNVertmm
32	0.98630	PogtoNB
33	0.98735	U6toPP
34	0.98754	L1protrusionL1toAPomm
35	0.98821	ABtoFacialPlane
36	0.98975	IMPAL1toMP
37	0.99025	L1toFH
38	0.99029	L1toAPodegree
39	0.99075	ChinAngle
40	0.99090	ANB

41	0.99256	L1toNBdegree
42	0.99260	LengthofMandBase
43	0.99279	U1toFHdegree
44	0.99297	MxMnDiffCotoGntoCotoANSmm
45	0.99303	SNB
46	0.99315	MnUnitLengthCotoGNmm
47	0.99338	PosteriorCranialBase
48	0.99394	Convexity
49	0.99436	SNtoGoGn
50	0.99473	OJ
51	0.99490	TAFH
52	0.99511	FacialPlanetoSN

APPENDIX B

Factor Analysis Results

Factor Analysis #1
Rotated Component Matrix

Variable	Component	
	1	2
SNA	***0.990***	0.124
SNB	0.838	-0.540
ANB	-0.024	***0.999***

2 components extracted

Factor Analysis #2
Rotated Component Matrix

Variable	Component	
	1	2
U1 to SN	0.067	0.949
U1 to NA degree	0.063	0.937
U1 to FH degree	0.091	***0.948***
IMPA L1 to MP	0.902	0.031
L1 to NB degree	***0.968***	-0.016
L1 to APo degree	0.848	0.311
Interincisal Angle	-0.874	-0.437
L1 to FH	-0.949	0.134

2 components extracted

Factor Analysis #3
Rotated Component Matrix

Variable	Component	
	1	2
FMA	***0.896***	0.111
SN to Go Gn	0.727	0.639
Occ Plane to SN	0.785	-0.048
Occ Plane to FH	0.720	-0.495
FH to SN	-0.052	***0.929***

2 components extracted

Factor Analysis #4
Rotated Component Matrix

Variable	Component	
	1	2
Saddle Sella Angle	0.398	0.826
Gonial Jaw Angle	-0.811	0.101
Chin Angle	***0.784***	0.108
Articular Angle	0.311	***0.874***

2 components extracted

Factor Analysis #5
Rotated Component Matrix

Variable	Component	
	1	2
Facial Taper	-0.019	***0.967***
Y Axis	-0.905	-0.296
Facial Plane to SN	***0.951***	-0.039
AB to Facial Plane	0.713	-0.356
Facial Angle	0.811	-0.093

2 components extracted

Factor Analysis #6
Rotated Component Matrix

Variable	Component	
	1	2
A to N Vert mm	0.928	-0.002
B to N Vert mm	***0.929***	0.264
Pg to N Vert mm	0.880	0.274
A to N Perp mm	0.293	0.600
B to N Perp mm	0.137	***0.974***
Pg to N Perp mm	0.058	0.962

2 components extracted

Factor Analysis #7
Rotated Component Matrix

Variable	Component 1
Mx Unit Length (Co to ANS mm)	0.915
Mn Unit Length (Co to Gn mm)	***0.992***
Mx Mn Diff Co to Gn to ANS mm	0.875

2 components extracted

Factor Analysis #8
Rotated Component Matrix

Variable	Component	
	1	2
U1 to NA degree	0.067	***0.713***
L1 to NB degree	0.816	0.277
L1 protrusion (L1 to APomm)	***0.920***	0.131
OJ	-0.208	0.689
Pog to NB	-0.787	0.318

2 components extracted

Factor Analysis #9
Rotated Component Matrix

Variable	Component	
	1	2
U1 to PP	0.903	0.047
L1 to MP	***0.929***	0.053
U6 to PP	0.861	0.145
L6 to MP	0.895	-0.243
OB	0.001	***0.989***

2 components extracted

Factor Analysis #10
Rotated Component Matrix

Variable	Component		
	1	2	3
Upper lip to E plane	0.961	-0.034	0.074
Lower lip to E plane	***0.963***	0.078	0.073
S Tissue N True Vertical to Upper Lip	0.302	0.898	0.242
S Tissue N True Vertical to Lower Lip	0.028	***0.971***	0.195
S Tissue N True Vertical to STPg	-0.476	0.850	0.136
S Tissue N Perp to Upper Lip	0.329	0.167	0.901
S Tissue N Perp to Lower Lip	0.020	0.293	***0.935***
S Tissue N Perp to STPg	-0.598	0.112	0.766

3 components extracted

Factor Analysis #11
Rotated Component Matrix

Variable	Component
	1
Anterior Cranial Base	0.964
Posterior Cranial Base	0.930
Ramus Height	0.915
Convexity	-0.290
Midface Length	***0.976***

1 component extracted

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