

***ENPPI AND ESRI* GENOTYPE ASSOCIATE WITH
CRANIOFACIAL ASYMMETRY AND SEVERITY OF TMD**

A Thesis
Submitted to
The Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
MASTER OF SCIENCE in ORAL BIOLOGY

by
Kay (Ki Yoon) Chung, D.D.S.
August 2018

Thesis Approval(s):

James J. Sciote, D.D.S., M.S., Ph.D.

Thesis Advisor, Temple U. Kornberg School of Dentistry, Dept. of Orthodontics

Jeffrey H. Godel, D.D.S., M.S.

Committee Member, Temple U. Kornberg School of Dentistry, Dept. of Orthodontics

Elizabeth B. Spannhake, D.D.S., M.S., M.P.H.

Committee Member, Temple U. Kornberg School of Dentistry, Dept. of Orthodontics

ABSTRACT

Introduction:

There are many approaches for classification of skeletal asymmetry using either PA cephalograms or SMV radiographs; however, there is no universally accepted one. We developed a new classification system to remove much of the previous diagnostic uncertainty that is also useful in genotyping single nucleotide polymorphisms (SNPs) associated with asymmetry. Also, we investigated whether *ACTN3*, *ENPP1*, *ESR1*, *PITX1*, and *PITX2* genes which contribute to sagittal and vertical malocclusions also contribute to facial asymmetries and temporomandibular disorders (TMD) before and after orthodontic and orthognathic surgery treatment.

Methods:

One hundred seventy-four patients with a dentofacial deformity were diagnosed as symmetric or subdivided into 4 asymmetric groups according to posteroanterior cephalometric measurements. 13 SNPs in *ACTN3*, *ENPP1*, *ESR1*, *PITX,1* and *PITX2* were selected for genotyping to determine whether specific allelic variants were overrepresented in subjects with malocclusion subclassifications. TMD examination diagnosis and jaw pain and function (JPF) questionnaires assessed the presence and severity of TMD.

Results:

Fifty-two percent of the patients were symmetric, and 48% were asymmetric. The asymmetry classification demonstrated significant cephalometric differences between the

symmetric and asymmetric groups, and across the 4 asymmetric subtypes: group 1, mandibular body asymmetry; group 2, ramus asymmetry; group 3, atypical asymmetry; and group 4, C-shaped asymmetry. *ENPP1* SNP-rs6569759 was associated with group 1 ($p=0.004$), and rs858339 was associated with group 3 ($p=0.002$). *ESRI* SNP-rs164321 was associated with group 4 ($p=0.019$). Diagnoses of disc displacement with reduction, masticatory muscle myalgia, and arthralgia were highly prevalent in the asymmetry groups, and all had strong statistical associations with *ENPP1* rs858339. The average JPF scores for asymmetric subjects before surgery (JPF, 7) were significantly higher than for symmetric subjects (JPF, 2). Patients in group 3 had the highest preoperative JPF scores, and groups 2 and 3 were most likely to be cured of TMD 1 year after treatment.

Conclusions:

1. A new posteroanterior cephalometric analysis using 6 measurements to detect differences in facial sides has been developed to distinguish 4 main classifications of asymmetry that are common in patients with dentofacial deformity.
2. TMD prevalence is much higher in patients with asymmetry compared with patients with dentofacial deformity without asymmetry.
 - a. The most common TMD presentations were disc displacement with reduction, masticatory muscle myalgia, and arthralgia.
 - b. Two of the 4 asymmetry groups had both high positive diagnoses for TMD and subjective patient reporting of symptoms.
3. SNP genotype rs6569759 in *ENPP1* was associated with asymmetry group 1, and rs858339 was associated with asymmetry group 3.

4. SNP genotype rs1643821 in *ESRI* was associated with asymmetry group 4. rs3020318 in *ESRI* was associated with PC1 and PC2, which relate to maxillary canting and menton deviation.
5. SNP genotype rs858339 in *ENPP1* was associated with disc displacement with reduction, masticatory muscle myalgia, and arthralgia.
6. Orthodontic and orthognathic treatment of asymmetry alleviates TMD symptoms for at least 1 year into retention in most patients.

ACKNOWLEDGEMENT

First, I would like to acknowledge all the investigators who devoted their efforts and time into this research project and collaborated with me, and they include: Dr. Alexandre Vieira from University of Pittsburgh for genotyping, Drs. Romain Nicot, Gwenael Raoul, and Joel Ferri from University of Lille for data collection of the patients, and Dr. James Sciote from Temple University for supervising the project as the principal investigator.

Also, I thank you Dr. Jeffrey Godel and Dr. Elizabeth Spannhake for your guidance and support with my research project and orthodontic education. I am honored to be given the opportunity to begin my journey in orthodontics at Temple University.

Last but not least, I would like to extend a heartfelt thanks to my family for their continued support and encouragement in my educational journey.

TABLE OF CONTENTS

	Page
ABSTRACT.....	i
LIST OF FIGURES	vii
LIST OF TABLES	viii
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: REVIEW OF THE LITERATURE	4
2.1: Facial Asymmetry	4
2.2: Causes of Facial Asymmetry	6
2.3: Genetic Influence on Facial Asymmetry and TMD	7
2.4: Diagnosis and Classification of Facial Asymmetry	12
2.5: The Association of Temporomandibular Joint Disorders (TMD) and Facial Asymmetry ..	14
CHAPTER 3: AIMS OF INVESTIGATION	16
CHAPTER 4: MATERIALS & METHODS	17
4.1: Patient Population	17
4.2: Assessment of Asymmetry	18
4.3: Genotype Assessment	22
4.4: Assessment of TMD	22
4.5: Statistical Analysis.....	24
CHAPTER 5: RESULTS	26
5.1 Cephalometric Analysis	26
5.2 Genotype Analysis	28
5.3. Temporomandibular Disorder Analysis.....	31
CHAPTER 6: DISCUSSION.....	35
6.1 General Statements	35
6.2 Potential Errors	40
6.3 Future Directions	41
CHAPTER 7: CONCLUSIONS	42
BIBLIOGRAPHY	43
APPENDICES	52
APPENDIX A: SUMMARY OF SAGITTAL/VERTICAL MALOCCLUSION DIAGNOSIS OF SUBJECTS	52
APPENDIX B: SUMMARY OF SUBJECTS & PA CEPH RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MAXILLARY MEASUREMENTS).....	53

APPENDIX C: PA RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MANDIBULAR MEASUREMENTS).....	56
APPENDIX D: PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MAXILLARY MEASUREMENTS).....	59
APPENDIX E: PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MANDIBULAR MEASUREMENTS).....	62
APPENDIX F: PRE- AND POST-TX JPF SCORE DATA COLLECTION.....	65

LIST OF FIGURES

Figure	Page
Figure 1: Prototypes for four asymmetry subtypes and illustration of PA cephalometric tracing.....	20
Figure 2: Example Jaw Pain and Function Questionnaire.....	24
Figure 3: Histogram of cephalometric measurement comparisons by different asymmetry groups.....	28
Figure 4: One-year posttreatment changes in JPF scores by asymmetry classification...	34

LIST OF TABLES

Table	Page
Table 1: Etiologic factors of facial asymmetry in three categories.....	6
Table 2: Mendelian and complex traits summary.....	9
Table 3: Currently identified genes and SNPs associated with malocclusion.....	10
Table 4: Landmarks used for PA cephalometric analysis.....	21
Table 5: Measurements used for evaluation of facial asymmetry in maxilla and mandible.....	21
Table 6: Cephalometric measurement comparison between symmetric and asymmetric subjects and statistical significance.....	27
Table 7: Comparison of SNP genotypes by symmetry subclassification – <i>ACTN3</i>	29
Table 8: Comparison of SNP genotypes by symmetry subclassification – <i>ENPP1</i>	30
Table 9: Comparison of SNP genotypes by symmetry subclassification – <i>ESR1</i>	30
Table 10: Comparison of SNP genotypes by symmetry subclassification – <i>PITX1</i> & <i>PITX2</i>	31
Table 11: Percentage of TMD diagnosis among symmetric and asymmetric classification groups.....	31
Table 12: <i>ENPP1</i> rs858339 genotypes by DC-TMD Classification.....	32
Table 13: Pre- and post-surgical JPF scores of symmetric and asymmetric patients.....	33

CHAPTER 1:

INTRODUCTION

Bilaterally perfect face and body symmetry in living organism exists as a theoretical concept, however, seldom exists (Cheong & Lo, 2011). This inevitably applies to human faces as well, and a perfectly symmetric face is rare (Shin et al., 2016). Previous studies reported that proportions of facial asymmetry ranging from 25% up to 50% in the United States (Proffit et al., 1990). Many factors have been identified in contributing to facial asymmetry, including but not limited to, hemifacial macrosomia, unilateral craniosynostosis, disharmony of masticatory muscles, functional shifts, trauma, infection and unilateral condylar hyper or hypoplasia (Kim et al., 2013). In the field of orthodontics, it is crucial to recognize if a patient presents with any type of craniofacial asymmetry, as it will influence the correct diagnosis and etiology of malocclusion, and ultimately the prognosis of the orthodontic treatment. The need for accurate analysis of facial asymmetry has increased concomitantly, and now includes the factors that directly contribute to asymmetry and determining its treatment (Lee et al., 2011). Despite much efforts and the need to create a classification system for skeletal asymmetry in the field of orthodontics, oral and maxillofacial surgery and plastic surgery, there still is lack of a universally accepted classification system or method for craniofacial asymmetry. Recently, Baek et al. introduced four different types of facial asymmetry using computed tomography (Baek et al., 2012), however, CBCT is not yet widely used in orthodontic practices.

Factors that contribute to the development of craniofacial asymmetry include both genetic and environmental causes. Many previous studies have identified gene variation and expression that may be associated with causes leading to facial asymmetry: Single nucleotide polymorphism (SNPs) in *ACTN3* (α -actinin 3) and *ESR1* (estrogen receptor α) with Class II malocclusion, *ENPP1* (ectonucleotide pyrophosphatase/phosphodiesterase) with mandibular prognathism, and differences in gene expression for *PITX 1* and *PITX2* between left and right masseter muscle in asymmetry (Zebrick et al., 2014; Dizak et al., 2016; Nicot et al., 2016; Nicot et al., 2014). It will be interesting and beneficial to investigate if the subclassifications of craniofacial asymmetry associate with previously identified genes relating to skeletal malocclusion and muscle asymmetry.

The contributing factor and actual underlying etiology for temporomandibular mandibular disorders (TMDs) have always been the subject of debate (McNeill, 1997). Although the relationship of occlusal factors to parafunction or TMD is still debatable (Seligman & Pullinger, 1991), a high incidence of TMD has been reported in a group of patients requiring orthodontic treatment, suggesting occlusal factors contributing to the development of TMD (Williamson, 1977; Fushima et al., 1999). Interestingly to an orthodontist's point of view, some studies have previously reported a high incidence of signs and symptoms of TMD in subjects with Class II malocclusion (Riolo et al., 1987; Pullinger et al., 1988). Furthermore, most Class II malocclusions do not limit to dental problems, but also present with skeletal ones such as a retrognathic mandible (McNamara, 1981). Fushima and Sato demonstrated that facial asymmetry due to mandibular asymmetry is a relatively common problem in patients with internal derangement of TMJ (Fushima & Sato, 1999).

The association of TMD and furthermore, gene variation with craniofacial asymmetry has not been investigated deeply until this day. It will not only be interesting but also crucial for practitioners treating or studying patients with craniofacial asymmetry presenting with TMD symptoms to acknowledge if the patients has SNPs in their genes that predispose them to the craniofacial asymmetry and TMD symptoms. By utilizing the classification system identified by Baek et al., we identified single nucleotide polymorphism (SNPs) associated craniofacial asymmetry. Furthermore, in our population with dentofacial deformity, TMD symptoms or masticatory muscle pain coexisted in over 50% of patients with facial asymmetry. This led us to investigate if there is any association between certain subclassification of asymmetry and prevalence of TMD.

CHAPTER 2:

REVIEW OF THE LITERATURE

2.1: Facial Asymmetry

It is our instinct to prefer attractive faces over unattractive ones as a human (Dion et al., 1972; Eagly et al., 1991). The quest to define facial beauty either by size or shape of isolated facial features or by the spatial relations between facial features date back to antiquity, when the Ancient Greeks believed beauty was represented by a golden ratio of 1:1.618 (Atalay, 2006). Facial asymmetry being a common phenomenon, was probably first observed by the artists of early Greek statuary who recorded what they found in nature – normal asymmetry (Lundstrom, 1961).

Facial asymmetry is commonly observed in humans, and to a certain degree, is considered normal. However, it is not easily defined as to at what point a “normal” asymmetry becomes “abnormal” and it is often determined by the clinician’s and the patient’s sense and perception of balance and imbalance (Bishara et al., 1994). Right-left differences occur everywhere in nature where two congruent but mirror image types are present, and humans frequently experience functional and morphological asymmetries (Bishara et al., 1994). One past study evaluated ancient Egyptian skulls and found that bones of the cranium showed asymmetry, with the right frontal, temporal and parietal bones being larger. The contralateral side of the facial complex exhibited an asymmetry with the left zygoma and maxilla being larger (Woo, 1931). More recently, researchers evaluated 63 postero-anterior (PA) cephalograms of “normal” children ranging from 9 to

18 years of age and found that an overall asymmetry was found in most of the children with the left side being larger. The cranial base, and mandibular regions exhibited a left side excess while the maxillary region showed a larger right side. (Vig & Hewitt, 1975). It is interesting to note, that unilateral cleft lip and/or palate, which are genetically influenced and result in a facial deformity, occurs roughly twice as often on the left side as on the right (Vagervik, 1981). On the contrary, one Japanese study reported that the right hemiface is usually wider than the left hemiface with the chin deviated to the left (Haraguchi et al., 2008). It is assumed that this preference is associated with genetic differences between the halves of the body (Lundstrom, 1961). On the vertical direction, it has been reported that the frequencies of facial laterality of 5%, 36% and 74% in the upper, middle and lower thirds of the face, respectively. Also, the lower face tends to deviate more frequently and in a greater amount than the upper or middle parts (Severt & Proffitt, 1997). Further detailed discussion on the environmental and genetic etiologies of facial asymmetry will be presented in subsequent subchapters.

It is reported that facial asymmetry is observed up to 50% in the United States (Proffit et al., 1990). Even though some degree of facial asymmetry is considered normal and generally accepted in the public eyes, significant facial asymmetry can interfere with normal function and have negative impact on esthetics (Cheong & Lo, 2011). Therefore, it is extremely important for a clinician to recognize if a patient exhibits some sort of facial asymmetry and to correctly diagnose the etiology, to produce the best treatment outcome for the patient.

2.2: Causes of Facial Asymmetry

Many different factors have identified as the etiological factors including congenital disorders, acquired diseases, and traumatic and developmental deformities (Cheong & Lo, 2011). **Table 1** summarizes the three different categories for etiology of facial asymmetry according to Cheong and Lo.

Table 1. Etiologic factors of facial asymmetry in three categories (Cheong & Lo, 2011)		
Congenital	Developmental	Acquired
Cleft lip and palate	Unknown causes	TMJ ankylosis
Hemifacial microsomia		Facial trauma
Neurofibromatosis		Childhood radiotherapy
Torticollis		Infection (i.e., neonatal bacterial infection in condyles, osteomyelitis)
Craniosynostosis		Fibrous dysplasia
Vascular disorders		Other facial tumors
Others		Unilateral condylar hyperplasia/ Idiopathic condylar resorption
		Romberg's disease
		Others

Certain genetic conditions such as multiple neurofibromatosis and hemifacial macrosomia may manifest facial asymmetry in mandibular body, ramus, and condyles as well as other external and internal facial structures (Bishara et al., 1994). Some clefts of lip

and/or palate are also genetically influenced and result in facial deformity. Some of the most well-known craniosynostoses include Apert syndrome, and Saethre-Chotzen, and Crouzon syndrome (Cohen, 1995). Craniosynostosis is a premature fusion of one or more cranial sutures in an infant, producing abnormal head shape. Intra-uterine pressure during pregnancy and at the time of delivery can place significant pressure on the fetal skull, resulting in facial asymmetry (Bishara et al., 1994). Intrauterine constraint produces deformational plagiocephaly but may also result in coronal or lambdoid synostotic plagiocephaly (Cohen, 1995). Unilateral coronal and lambdoid synostoses are known to have multiple etiologies including mutant genes, as well as constraint and other causes (Cohen, 1995). Mandibular asymmetry may be caused by infection and trauma during the growing period, leading to asymmetry and ankylosis (Souyris et al., 1983).

2.3: Genetic Influence on Facial Asymmetry and TMD

As described in the previous subchapter, many genetic conditions such as clefts of lip and/or palate, hemifacial macrosomia, and craniosynostosis contribute to facial asymmetry. In addition to genetic disorders or syndromes, it will be interesting to explore if there are certain genetic variations that cause craniofacial asymmetry in a population absent of genetic disorders.

The genetic background (genome) and environmental (non-genetic) factors are two main aspects that determine phenotype. Even though these two influences have been considered as two separate entities, genetic and environmental factors essentially interact to develop the phenotype. Traits, such as diseases, can be divided into two categories based on the genetic components' pattern of transmission (Hartsfield, 2002). The first

category is the Mendelian or simple traits that generally follows the two laws of heredity, and result from a mutation of a single gene (Abass & Hartsfield, 2008). A single gene mutation usually results in a recognizable phenotype, and environmental factors and other genes may modify the clinical expression of the disease, showing variable expressivity (Chanock & Wacholder, 2002; Abass & Hartsfield, 2008). The second category involves complex traits, which are more common than Mendelian traits. Although they tend to run in families, it does not follow a clear pattern of inheritance. The genetic determinants of such traits are difficult to identify due to the disease resulting from a set of genetic variations, or polymorphisms, that may be common within the population. The interaction of these polymorphisms in different genes with environmental factors leads to the manifestation of complex traits (Abass & Hartsfield, 2008). **Table 2** summarizes Mendelian traits and complex traits (Hartsfield, 2011; Cobourne, 2004). Facial morphology is influenced by genetic and environmental factors and their complex interactions, therefore, genetic variations from single nucleotide polymorphisms, that may be associated with facial asymmetry is an important topic to investigate.

Table 2. Mendelian and complex traits summary	
Mendelian Traits	Complex traits
<p>The diagram illustrates the Mendelian model. It shows a central vertical flow: Environment Factors (dashed arrow) points to Gene, which points to Protein, which points to Phenotype. A Modifying Gene(s) (dashed arrow) points to Protein(s), which also points to Phenotype. There are also dashed arrows from Environment Factors to Protein and Protein(s).</p>	<p>The diagram illustrates the complex trait model. It shows a network of interactions. At the top, Gene 1, Gene 2, Gene 3, and Gene 4 are shown. Below them are Protein 1, Protein 2, Protein 3, and Protein 4. Environmental Factors (EF) are shown as arrows pointing to each gene and protein. A large arrow from Environmental Factors (EF) points to Phenotype. The diagram shows a complex web of interactions between genes, proteins, and environmental factors leading to a phenotype.</p>
<p>i.e. Syndromic cleft lip/palate associated with van der Woude syndrome due to a nonsense mutation in interferon regulatory factor-6 (<i>IRF6</i>)</p>	<p>i.e. Non-syndromic clefting exhibiting varying levels of penetrance, sex differences and environmental overlays such as maternal cigarette smoking/drinking</p>

The relationship between facial asymmetry and malocclusion is noteworthy. Some studies have found that the frequencies of facial asymmetry are higher in patients with malocclusion than the population with normal occlusion. When Scanavini et al. evaluated the dental arch symmetry in natural normal occlusion compared with Class II malocclusion individuals, individuals with natural normal occlusion showed smaller degree of asymmetry than individuals with Class II patients, with the degree of asymmetry in mandibular dental arches greater than maxillary dental arches (Scanavini et al., 2012). Another study which evaluated comparative evaluation of facial harmony in Class I and II found that asymmetries are much higher in subjects with malocclusion (Mishara et al., 2014). This further questioned us if there is any SNPs or gene variation that are involved with malocclusions associate with craniofacial asymmetry. Some of the genes have been suggested to be linked to malocclusions and are presented in the **Table 3** (Fontoura et al., 2015; Zebrick et al., 2014; Dizak et al., 2016; Nicot et al., 2016; Hottenstein et al., 2014).

Table 3. Currently identified genes and SNPs associated with malocclusion		
Genes	Skeletal malocclusion	SNPs associated with malocclusion
<i>SNAI3</i>	Severe Class II with accentuated convex profile	rs4287555
<i>TWIST1</i>	Shorter ramus, larger body length, and a steep anterior cranial base orientation	rs2189000
<i>FGFR2</i>	Increased risk for classes II and III	rs2162540, rs11200014
<i>EDN1</i>	Reduced risk of Class II	rs2070699
<i>TRX5</i>	Reduced risk of Class III	rs1248046
<i>COL1A1</i>	Increased risk of Class III	rs2249492
<i>MYOH1</i>	Class III	rs10850110
<i>PITX1</i>	Facial asymmetry (posterior areas of the face)	rs1131611
<i>PITX2</i>	Facial asymmetry (posterior areas of the face)	rs2595110
<i>ACTN3</i>	Class II, TMD	rs1671064, rs1815739
<i>ESR1</i>	Class II, TMD	rs1643821, rs302318, rs3020377, rs2077647
<i>ENPP1</i>	Mandibular prognathism, TMD	rs9373000, rs6569759, rs858339, rs1409181

There have been ongoing investigations on how genetic variations associate with the development of skeletal malocclusions in the patient population undergoing orthognathic surgery at the University of Lille. This is a typical population of French patients, relatively young with a normal distribution of open, deep, Class II and Class III skeletal malocclusions. In previous studies, researchers found differences in gene expression for *PITX1* and *PITX2* between left and right masseter muscle in asymmetry (Nicot et al., 2014). *PITX2* is a gene influencing the *Nodal* Pathway that determines left-right asymmetry during embryogenesis, and has recently been reported that when facial asymmetry is part of skeletal malocclusion, there are decreases of NSP genes in masseter muscle. Their study suggested that the NSP is down regulated to help promote

development of asymmetry, and that *PITX2* expression differences also contributed to both skeletal and muscle development (Hottenstein, et al., 2014).

α-Actinins are myofibril anchor proteins that influence the contractile properties of skeletal muscles, and *ACTN3* is expressed only in fast fibers. Recent study by Zebrick et al. reported 577XX mutation in *ACTN3* is overrepresented in subjects with skeletal Class II malocclusion, suggesting a biologic influence during bone growth. On the contrary, *ACTN3* 577XX is underrepresented in subjects with deep bite malocclusion, suggesting that muscle differences contribute to variations in vertical facial dimensions (Zebrick et al., 2014).

Ectonucleotide pyrophosphatase/phosphodiesterase-1 (*ENPP1*), coded by the gene *ENPP1*, is a membrane-bound, nucleoside triphosphate pyrophosphohydrolase responsible for generating pyrophosphate by hydrolyzing nucleotides and nucleotide sugars (Evans et al., 1973; Terkeltaub et al., 1994). *ENPP1* is an important negative regulator of bone mineralization, and cultured osteoblasts with elevated *ENPP1* expression have reduced mineral formation (Mackenzie et al., 2012). Pyrophosphate exhibits an inhibition effect on hydroxyapatite crystallization and growth; and, through the expression of pyrophosphate, *ENPP1* plays an inhibitory role on hard and soft tissue mineralization (Fleisch et al., 1966; Register and Wutheier, 1985). Interestingly, Ermakov et al. recently found genetic variant that influences bigonial breadth in Eurasian population (Ermakov et al., 2010).

Recent discovery of the association between a genetic variant of *ESR1* and symptoms of TMD is also noteworthy. The estrogen receptor is a protein of the steroid receptors family, which acts through two receptors, estrogen receptor- α and estrogen

receptor- β , producing effects on the inflammatory process (McEwen & Alves, 1999). The role of estrogen via the α receptor in the pathophysiology of TMD functions through the inflammatory response, bone mineralization and nervous system (Craft, 2007). Recent studies identified strong associations between SNPs of *ESRI* and symptoms of TMD (Kim et al, 2010). Nicot et al also identified rs1643821 to be a risk factor for symptomatic worsening in post-orthognathic surgical patient population (Nicot et al, 2016).

In previous studies, significant associations between SNPs in *ACTN3* (α -actinin 3) and *ESRI* (estrogen receptor α) with Class II malocclusion and *ENPPI* (ectonucleotide pyrophosphatase/phosphodiesterase 1) with mandibular prognathism (Zebrick et al., 2014; Dizak et al., 2016; Nicot et al., 2016) have been identified. In this study, we explored associations between these genes and subclassifications of asymmetry. We also sought to determine if the subclassifications are related to the prevalence or severity of TMD before and after orthodontic treatment with jaw osteotomy.

2.4: Diagnosis and Classification of Facial Asymmetry

In orthodontics, there have been many approaches to classify skeletal asymmetry utilizing either postero-anterior (PA) cephalograms or submentovortex (SMV) radiographs; however, there is not a universally accepted method or classification system. A standardized, easy-to-interpret, and definitive classification will be useful in identifying an association between the subclasses of asymmetry and prevalence of TMD, and furthermore, an etiologic-based phenotypic classification may be useful in identifying gene single nucleotide polymorphisms (SNPs) associated with asymmetry.

Posterior-anterior (PA) cephalograms have been most widely used for the diagnosis of facial asymmetry since its introduction to the field in the 1930's (Damastra et al., 2011). Submentovertex (SMV) radiographs have also been used for the assessment of facial asymmetry, however, due to its difficulties in identifying landmarks because of superimposed structures or poor radiographic technique, it is less frequently used compared to PA cephalograms (Houston, 1983; Ahlqvist et al., 1986). The early classification system mainly focused on mandibular asymmetry, then the whole craniofacial complex was closely looked upon starting the 90's. However, these classifications are based solely on affected structure, and these schemes do not provide the necessary information required for diagnosis and surgical treatment (Baek et al., 2012).

Most recently, cone beam computed tomographic imaging has been utilized to more accurately describe and subclassify types of craniofacial asymmetry in dentofacial deformity populations (Hwang et al., 2006; Ryu et al., 2015). The classification system of Baek et al. has identified four types of asymmetry with computed tomography, which arise from different growth imbalances of the jaws, teeth, nasal septum and cranial base (Baek et al., 2012). Baek et al. classified facial symmetry into four groups: Group 1 with asymmetry caused by a shift or lateralization of the mandibular body, Group 2 with a significant difference between the left and right ramus height with menton deviation to the short side, Group 3 with atypical asymmetry including deviation of the menton to the short side, prominence of the angle/gonion on the larger side and reverse maxillary canting, Group 4 with severe maxillary canting, ramus height differences and menton deviation to the short side. This became the basis of subclassification system used in this study, which is later further explained and described in materials and methods section.

2.5: The association of Temporomandibular Joint Disorders (TMD) and Facial Asymmetry

According to the American Academy of Orofacial Pain, the term temporomandibular disorder refers to a set of clinical problems that involve the masticatory musculature, the temporomandibular joint (TMJ) and associated structures, or both, being identified as the leading cause of non-dental pain in the orofacial region and is considered a subclass of musculoskeletal disorders. The signs and symptoms that indicate any abnormality of the TMJ include limitation of mandibular movement, limitation of mouth opening, joint pain with mandibular function, constraint function, joint noises, asymptomatic radiographic changes of the TMJ and jaw locking with open mouth and closed mouth (Leite et al., 2009).

Many etiologic factors contributing to temporomandibular disease (TMDs) have been implicated including occlusal factors, parafunction (bruxism), trauma, hypermobility, stress, age, gender, personality, heredity and systematic diseases (Oral et al., 2009). Among them, occlusal factors and their association and contribution to TMD have been the subject of an intense discussion within the field of dentistry (Oral et al., 2009), along with the role of malocclusion in the development of TMD (Leite et al., 2009). An extensive systematic review concluded that there is a significant association between the presence of some occlusal factors such as skeletal open bite, unilateral crossbite, absence of five or more teeth, deep overbite and severe overjet, and the presence of TMD signs and symptoms (McNamara et al., 1995).

When there is an occlusal interference, the mandible may shift to seek a more stable position (maximum intercuspatation), accompanied with condylar displacement in

the glenoid fossa. During the growth period, it has been suggested that the functional displacement of the mandible suppresses or activates mandibular growth especially in the condylar region (McNamara, 1981). Also, the occlusal interference may cause the occlusal fulcrum to load against the TMJ as compression or distraction, causing micro trauma to the TMJ structures and inducing the internal derangement or osteoarthritis of the TMJ (Hatcher et al., 1986; Ito et al., 1986). It is reported that temporomandibular joint remodeling in the adults is an adaptive phenomenon to variations of functional load and once the adaptive capability of the system is exceeded, pathology such as degenerative joint disease will occur (Mongini, 1989). Therefore, it is possible that the tilt of the frontal occlusal plane deviates the mandibular posture including pathological changes in the TMJ in adults (Inui et al., 1999).

Interestingly, some studies reported that the presence of Class II malocclusion is an important risk indicator for TMD development (Selaimen et al., 2007; Seligman et al., 1988; Riolo et al., 1987). The posterior displacement of retrognathic mandible in Class II malocclusion has been suggested as the cause for producing TMD symptoms. In recent studies, some authors suggested that there might be a potential association between TMJ dysfunction and mandibular asymmetry. Inui et al. also concluded that facial asymmetry due to mandibular lateral deviation is an important characteristic in the etiology of internal derangement of TMJ (Inui et al., 1999). Many studies suggested that degenerative joint disease might be associated with mandibular asymmetry (Nickerson & Moystad, 1983; Katzberg et al., Goske, & Wood, 1985). Ahn et al. also concluded from their recent study that internal derangement of TMJ, especially when it is more advanced on the unilateral side, can cause mandibular asymmetry (Ahn et al., 2005)

CHAPTER 3:

AIMS OF THE INVESTIGATION

The specific aims of this study are:

- To subclassify the types of craniofacial asymmetries in pre-surgical dentofacial deformity patient population with Baek et al.'s classification method through the utilization of posterior-anterior (PA) cephalograms.
- To investigate if genes previously identified (*ACTN3*, *ENPP1*, *PITX1* and *PITX2*) to be associated with sagittal and vertical malocclusion are related to subclassifications of facial asymmetry.
- To investigate if the craniofacial asymmetry subclassifications are related to the prevalence or severity of TMD before and after orthodontic treatment with orthognathic surgery.

CHAPTER 4:

MATERIALS & METHODS

4.1: Patient Population

The subjects included in this study were of the topic of a recent publication in *American Journal of Orthodontics and Dentofacial Orthopedics* (Chung et al., 2017). 174 patients undergoing comprehensive orthodontic treatment with mandibular or mandibular and maxillary osteotomies for correction of jaw deformation malocclusion were recruited for study participation during their pre-surgical consultation at the University of Lille Department of Oral and Maxillofacial Surgery. At that time, the patients signed consent for participation according to human subject research protocols approved by the Committee for Personal Protection, University of Lille and the Institutional Review Board Committees at the University of Pittsburg and Temple University. Patients provided a saliva sample collected in Oragene Kits for genotyping. Deidentified demographic information, panorex, lateral and posterior anterior cephalograms, and clinical examination information were compiled for analysis. The patient population had a mean age of 25.7 years, majority female (76%), and a normal mixture of sagittal (66% Class II, 33% Class III) and vertical jaw deformations (65% open bite, 24% normal bite, 11% deep bite, Appendix A). Sagittal and vertical malocclusion classifications were based on both the Delaire Cephalometric Analysis of lateral cephalograms and the type of osteotomy repositioning done to correct malocclusion.

4.2: Assessment of Asymmetry

The classification system of Baek et al. was used to classify our patient's skeletal asymmetry into one of four groups as described previously: Group 1 – lateralization of mandibular body only (“mandibular body asymmetry”); Group 2 – difference in ramus heights with menton deviation to the shorter ramus side (“ramus asymmetry”); Group 3 – difference in ramus heights with menton deviation to the longer ramus side, gonion contour more prominent on larger mandibular side and reverse maxillary canting (“atypical asymmetry”); Group 4 – difference in ramus heights with menton deviation to short ramus side and severe maxillary canting (“C-shaped asymmetry”) (**Figure 1**). Since this classification system was derived from CBCT images of orthognathic patients, we developed a PA (posterior anterior) cephalometric analysis, which allowed us to perform comparable measurements using digital two-dimensional images with Dolphin morphometric software.

Six cephalometric measurements were used: occlusal plane tilt, maxillary canting (JR or JL to ZR or ZL), menton deviation (A to Me to MSR), mandibular width to midsagittal plane (AG or GA to MSR), mandibular width to menton (AG or GA to Me), and ramal height (**Table 4 and 5**). In the maxilla, occlusal plane tilt was determined by the difference between Frankfort Horizontal and the horizontal line bisecting the buccal cusp tips of UR6 and LR6, as well as the buccal cusp tips of UL6 and LL6, measured in degrees (°). If the occlusal plane tilt was greater than 2°, the subject was considered to have maxillary canting. To further verify the maxillary canting was skeletal in etiology, left and right vertical distances from jugal process and frontozygomatic suture were compared. If the difference between the left and the right side was greater than 3 mm, the

subject was considered to have maxillary canting. In the mandible, menton deviation was determined by the angle between midsagittal plane and the line connecting ANS and menton. If the angle was greater than 2° , the subject was considered to have mandibular facial asymmetry with menton deviation. To compare the left and right mandibular width, the distance between antegonial notch and midsagittal plane was compared to the contralateral side. If the difference was greater than 2 mm, it was considered to have mandibular deviation. We also measured the distance between antegonial notch and menton, and compared it with its' contralateral side. Again, if the difference was greater than 2mm, it was considered to have mandibular body asymmetry. Lastly, ramal height of left and right side was compared. If the difference between left and right side was greater than 3 mm, ramus asymmetry was diagnosed.

Subjects were diagnosed as symmetric if there was no maxillary canting ($<2^\circ$), no menton deviation ($<2^\circ$), and no significant difference in ramal height (<3 mm). Asymmetric subjects were further divided into four groups. Group 1 subjects had menton deviation greater than 2° without any maxillary canting or any significant ramal height difference. Group 2 subjects had menton deviation greater than 2° with shorter ramal height on the deviated side. Group 3 subjects displayed “atypical symmetry”, with shorter ramal height on the opposite of the deviated side, with slight maxillary canting towards or opposite of the deviated side. Group 4 subjects showed both shorter ramal height and maxillary canting towards the deviated side, displaying “C-shaped asymmetry” as described by Baek et al.

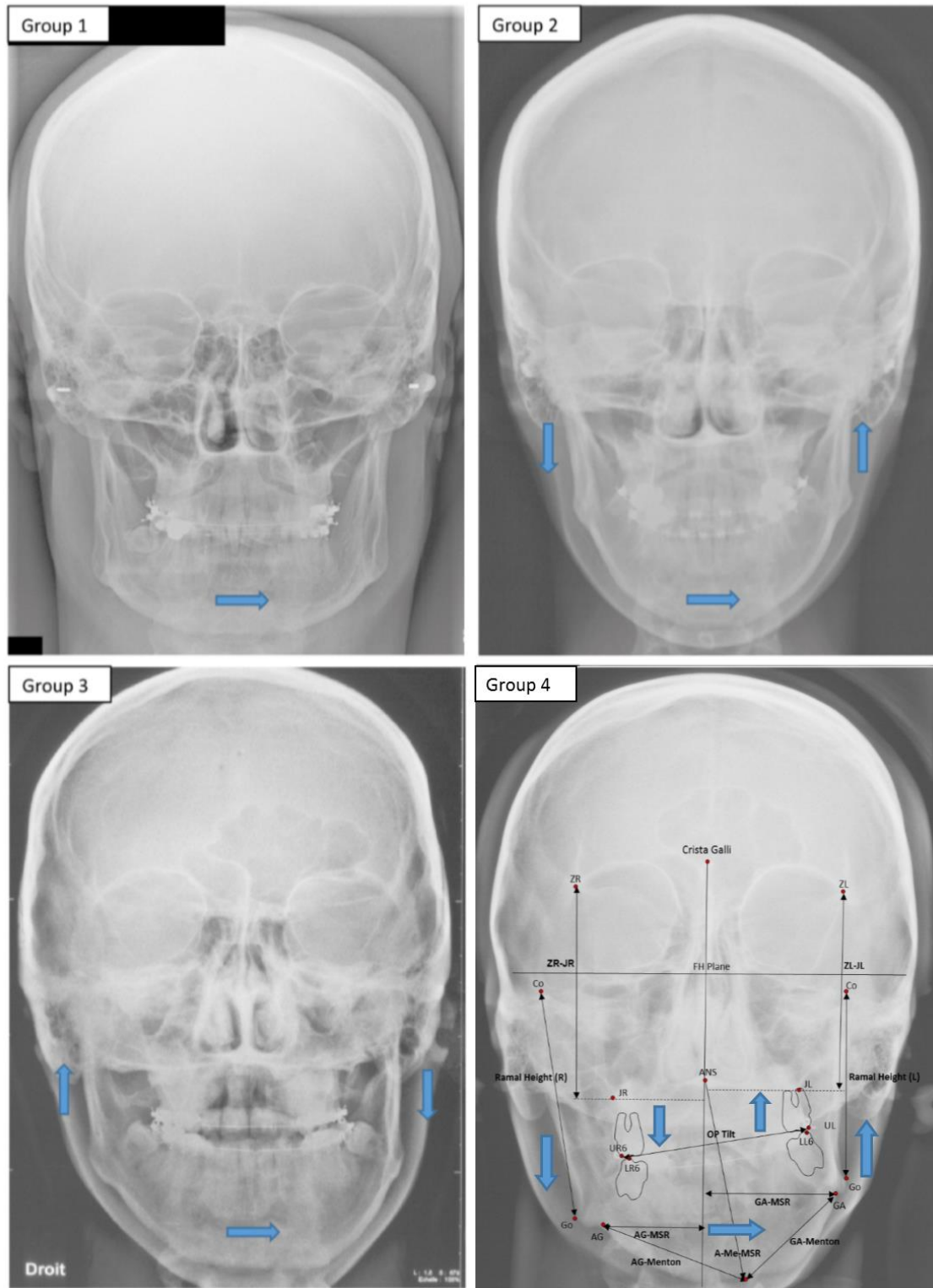


Figure 1. Prototypes for four asymmetry subtypes and illustration of PA cephalometric tracing: Group 1 with mandibular body asymmetry, Group 2 with ramus asymmetry, Group 3 with atypical asymmetry, and Group 4 with C-shaped asymmetry. Landmarks used for cephalometric analysis labelled on Group 4

Table 4. Landmarks used for PA cephalometric analysis	
Landmarks	Definitions
ANS	Anterior nasal spine
AG/GA	The highest point in the antegonial notch (left and right)
Co	Condylion; Most superior point on condylar head
Crista Galli	Most superior point at its intersection with the sphenoid
Gonion/Most Lateral Ramus	Most inferior, posterior, and lateral point at the gonial angle of the mandible
JL/JR	Bilateral points on the jugal process at the intersection of the outline of the tuberosity of the maxilla and zygomatic buttress
Me	Menton; most inferior point at symphysis
Midsagittal Plane	A plane bisecting the head and face through the crista galli, ANS, and genial tubercles in a symmetric face
Occlusal Plane	Horizontal line bisecting UR6 and LR6 as well as UL6 and LL6
UR6/UL6 LR6/LL6	Buccal cusp tip of right/left maxillary molar Buccal cusp tip of right/left mandibular molar
ZL/ZR	Medial aspect of frontozygomatic suture (Bilateral)

Table 5. Measurements used for evaluation of facial asymmetry in maxilla and mandible	
Measurements	Definitions
MAXILLA	
Occlusal Plane Tilt (°)	Difference between Frankfort Horizontal and the horizontal line bisecting UR6 and LR6 as well as UL6 and LL6
Maxillary Canting JL or JR – ZL or ZR (mm)	Difference between vertical distance from left or right jugal process between left or right frontozygomatic suture
MANDIBLE	
Menton Deviation A-Me-MSR (°)	Angle formed between midsagittal plane and line going through ANS and menton
Mandibular Width to Midsagittal Plane GA or AG-MSR (mm)	Distance between left or right antegonial notch and midsagittal plane
Mandibular Width to Menton GA or AG-Menton (mm)	Distance between left or right antegonial notch and menton
Ramal Height (R or L) (mm)	Linear distance between condylion to most lateral ramus (gonion)

4.3: Genotype Assessment

Saliva samples, one per patient, were collected at University of Lille in Oragene kits and shipped to University of Pittsburgh for DNA extraction and posterior genotyping using TaqMan chemistry and end-point analysis in an automatic sequence-detection instrument (ABI Prism 7900HT, Applied Biosystems, Foster City, CA), as described previously. Thirteen SNPs were selected for genotyping: in *ACTN3* rs1815739, rs678397 and rs678397(10,24-25); in *ENPP1* rs937300, rs6569759, rs858339 and rs4109181(25-29); in *ESR1* rs1643821, rs302318, rs3020377 and rs2077647(30-33); in *PITX1* rs1131611 and in *PITX2* rs2595110(4,34) to determine if specific allelic variants are over-represented in subjects with malocclusion sub-classifications. Genotyping was performed by Dr. Alexandre Vieira at University of Pittsburgh. Thirty-three additional anonym SNPs were genotyped to assess the presence of population substructure among the controls selected.

4.4: Assessment of TMD

TMD was assessed using the routine clinical examination done by the Maxillofacial Surgeons Drs. Nicot, Raoul and Ferri at University of Lille before surgical treatment and entered into the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). In addition, the Jaw Pain and Function (JPF) questionnaire was used to determine the presence and severity of TMD, as a subjective patient report (**Figure 2**). The JPF Questionnaire was developed as a screening tool to determine presence or absence of TMD conditions (Clark et al., 1989). It consists of 8 questions relating to jaw pain and 5 questions related to jaw function. The questionnaire has been validated to reliably

distinguish between normal and TMD subjects with up to 98% sensitivity and 100% specificity when a cut off score of 6 is used for responses (Gerstner et al., 1994). The questionnaire has been translated for use in Germanic, and we prepared a French version for use in Lille, as a standard assessment of presence and severity of TMD.

One hundred twenty-one patients have attended a one year post-treatment reevaluation appointment, where a second JPF survey was recorded (Nicot et al., 2016). In their study, Nicot et al. divided the patients into five groups for comparative purposes based upon difference in pre-surgical and one year post-treatment JPF scores. *No Change* in TMD, *Improvement* of TMD if the JPF score decreased by 3 or more; *Worsening* of TMD if the JPF score increased by 3 or more; *Cured* of TMD if the JPF score was ≥ 6 before surgery and < 6 after treatment; and *Iatrogenic* TMD if the JPF score was < 6 before surgery and > 6 after treatment. They used the terms “*cured*” or “*iatrogenic*” since a score ≥ 6 is diagnostic for presence or absence of TMD with the JPF assessment (Nicot et al., 2016). For this study, we applied the TMD classification based on JPF scores to our patients and compared them with our asymmetry classification.

Jaw symptom and oral habit questionnaire						
Name						
Date						
Examiner						
Instructions: Please check the appropriate answer to the following questions						
A	Jaw pain questions	Doesn't hurt at all	Hurts a little	Hurts a lot	Almost unbearable	Unbearable pain without relief
1	Does it hurt when you open wide or yawn ?					
2	Does it hurt when you chew or use the jaws ?					
3	Does it hurt when you are not chewing or using the jaws ?					
4	Is your pain worse on waking ?					
5	Do you have pain in front of the ears or earaches ?					
6	Do you have jaw muscle (cheek) pain?					
7	Do you have pain in the temples ?					
8	Do you have pain or soreness in the teeth ?					
B	Jaw function questions	No	Maybe a little	Quite a lot	Almost all the time	All the time without stopping
9	Do your jaw joints make noise so that it bothers you or others?					
10	Do you find it difficult to open your mouth wide ?					
11	Does your jaw ever lock closed so you cannot open it?					
12	Does your jaw ever lock open so you cannot close it?					
13	Do you have a problem with your bite being uncomfortable?					

Figure 2. Example Jaw Pain and Function Questionnaire

4.5: Statistical Analysis

For cephalometric assessment of asymmetry, an unpaired t test was used to compare if differences between sides for individual cephalometric measurements were significantly different between symmetric and asymmetric patients. An ANOVA test was used between

the four subclassifications of asymmetry to determine anatomical differences between groups. Tests for measurement error included intrarater reliability in cephalometric measurements (by repeating cephalometric tracing on 10% of the radiographs by 1 examiner [K.C.]; this resulted in an R^2 value of 0.98.

For genotype assessment, the characteristics of the population were presented with the usual rules of descriptive statistics: frequencies and percentages for categorical variables; mean and standard deviation for quantitative variables. The association between the change in JPF scores and clinical or surgical characteristics of the population was assessed by Chi^2 test for categorical variables or Fisher exact test in the case of small numbers. Quantitative variables were analyzed using Student's t-test. When the distribution of the variable did not follow a normal distribution, a nonparametric Wilcoxon test was performed. After ensuring compliance with the Hardy-Weinberg equilibrium, the association of different SNPs was also sought by a Chi-square test or Fisher exact test when small numbers were present. For each SNP, the analysis was performed by considering the three genotypes separately.

For TMD assessment, an unpaired t-test was used to determine if there were significant differences in pre-surgical JPF scores between symmetric and asymmetric patients, and an ANOVA test was performed between the four asymmetric groups. An ANOVA was also used to compare if there were significant changes in the JPF score 1 year after post-treatment between the symmetric group and the four asymmetric groups. To further evaluate, a post-hoc t-test was done to compare individual JPF scores of asymmetric groups.

CHAPTER 5:

RESULTS

5.1 Cephalometric Analysis

The patient population represented a normal demographic distribution of subjects seeking orthodontic and orthognathic surgery treatment for dentofacial deformity malocclusion from the geographic areas of Northern France and Southern Belgium. All patients treated for this condition were referred to the Oral and Maxillofacial Surgery Department at the University of Lille under the National Health Care Service of France. Fifty-two percent of the patients were diagnosed as symmetric, and 48% were diagnosed as asymmetric. Segregating the asymmetric subjects into the 4 proposed subtypes was relatively easy to accomplish with the 11 cephalometric anatomic landmarks and 6 cephalometric measurements (**Tables 4 and 5; Fig 1**). This classification system was validated by the significant differences obtained by comparing the cephalometric measurements between the symmetric and asymmetric groups, and the asymmetric subtypes (**Table 6**). Complete raw data for the cephalometric measurements are presented in Appendix B-E.

Table 6. Cephalometric measurement comparison between symmetric and asymmetric subjects and statistical significance			
Measurements	Symmetric	Asymmetric	p value*
Occlusal Plane Tilt (°)	0.87 ± 0.59	1.36 ± 1.47	0.0040
Maxillary Canting JL or JR – ZL or ZR (mm)	0.68 ± 0.44	2.70 ± 5.17	0.0003
Menton Deviation A-Me-MSR (°)	0.31 ± 0.29	4.47 ± 2.10	<0.0001
Mandibular Width to Midsagittal Plane GA or AG-MSR (mm)	1.51 ± 1.31	14.2 ± 12.8	<0.0001
Mandibular Width to Menton GA or AG-Menton (mm)	1.51 ± 2.69	21.4 ± 14.9	<0.0001
Ramal Height (mm)	1.41 ± 0.79	14.5 ± 12.1	<0.0001

*Unpaired t-test derived from mean and SD.

All 6 posteroanterior cephalometric measurements were compared between the symmetric and asymmetric patients, and across the 4 asymmetry subclassification groups. For the bilateral points, the values are the difference between the left and right sides. The posteroanterior cephalometric analysis results demonstrated significant differences in all 6 cephalometric measurements between the symmetric and asymmetric patients. All mandibular measurements— menton deviation, mandibular width, and ramal height— showed notably significant differences between the symmetric and asymmetric patients, clearly indicating mandibular asymmetry. Cephalometric measurements were also compared across the different asymmetry groups (**Figure 3**). Generally, group 4 showed greater amounts of canting and deviation when we compared cephalometric measurements. The subjects in this group had the most severe malformations, with occlusal plane tilt, maxillary canting, and mandibular deviation that were proportionally much more imbalanced than in the other asymmetry groups. Because group 4 had maxillary canting, the anterior nasal spine to menton line used to measure chin deviation

in the mandible was higher than in the other 3 groups ($p = 0.003$; **Figure 3**). The second characteristic evident with asymmetry was the worsening of the ramal height differences from group 1 to group 4.

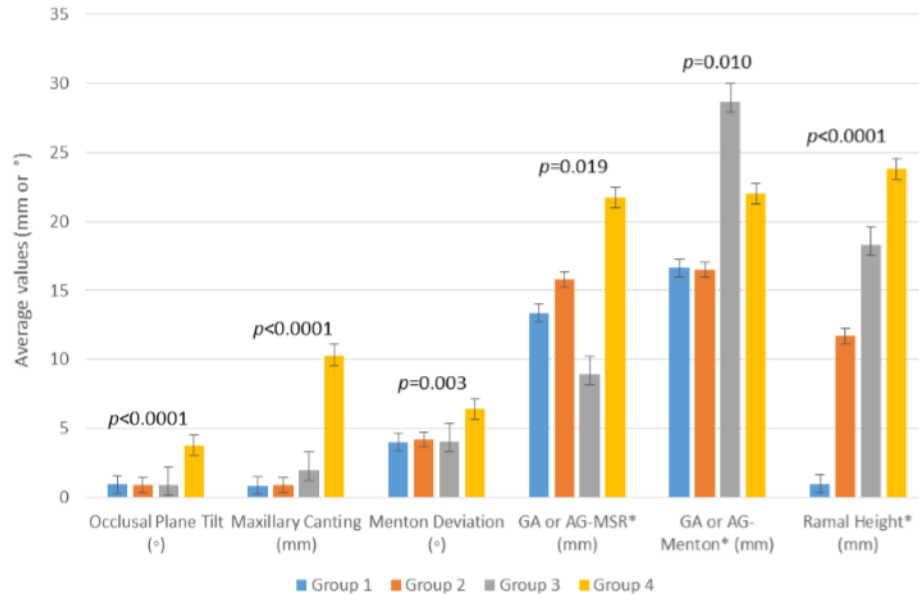


Figure 3. Histogram of cephalometric measurement comparisons by different asymmetry groups. Average values for each cephalometric measurement were compared across different asymmetry groups.

**For bilateral points, the difference between the left and right sides was used and denoted as a positive value. Average values are expressed in millimeters for maxillary canting, GA or AG-MSR, GA or AG-menton, and ramal height, and in degrees for occlusal plane tilt and menton deviation.*

5.2 Genotype Analysis

Differences in the posteroanterior cephalometric measurements indicated that the 4 subtypes may be considered as anatomically different forms of asymmetry that can be compared for differences in genotype variations. We therefore compared differences in genotype for the symmetric and asymmetric subjects and between the asymmetric subtypes (**Table 7-10**). From the 13 SNPs selected from *ACTN3*, *ENPP1*, *ESR1*, *PITX1* and *PITX2*, significant differences were detected for the *ENPP1* and *ESR1* genotypes, but

not for *ACTN3*, *PITX1*, and *PITX2*. *ENPP1* SNP rs6569759 was significantly different for genotype and alleles for group 1 compared with the other asymmetry subtypes. *ENPP1* SNP rs858339 was different for genotype between the symmetric and asymmetric groups and for group 3 compared with the other asymmetric groups. *ESR1* SNP rs1643821 was also significantly different for genotype and allele for group 4 compared with the other asymmetric groups. Subjects with group 1 asymmetry were almost 4 times more likely to carry the G allele of *ENPP1* rs6569759 (odd ratio, 3.89; 95% confidence interval, 1.02-14.78). These results further support the appropriateness of the asymmetry subclassifications, since this phenotypic organization can be used to recognize meaningful genotypic differences.

Table 7. Comparison of SNP genotypes by symmetry subclassification – *ACTN3*

Gene	SNP	Genotype	Asymm- A	Asymm- B	Asymm- C	Asymm- D	Symmetric	Symmetry vs. Asymmetry	
								p value - genotype	p value - allele
<i>ACTN3</i>	rs1671064	AA n – (%) GA GG	2 (29) 3 (42) 2 (29)	5 (18) 15 (55) 8 (27)	6 (27) 14 (64) 2 (9)	1 (12) 4 (44) 4 (44)	11 (17) 38 (58) 16 (25)	0.82	0.7
			0.9 0.67 0.78	0.23 0.14	0.45 0.3	0.45 0.3			
	rs1815739	CC TC TT	2 (29) 3 (42) 2 (29)	6 (21) 14 (50) 8 (29)	6 (27) 14 (64) 2 (9)	1 (12) 4 (44) 4 (44)	13 (20) 36 (55) 16 (25)	0.93	0.8
			0.8 0.87	0.88 0.87	0.28 0.19	0.44 0.25			
	rs678397	CC CT TT	2 (29) 3 (42) 2 (29)	6 (21) 12 (46) 8 (33)	8 (36) 12 (55) 2 (9)	2 (22) 3 (34) 4 (44)	10 (15) 32 (49) 17 (36)	0.51	0.42
			0.73 0.67	0.69 0.96	0.17 0.08	0.49 0.68			

Gene	SNP	Genotype	Asymm-A	Asymm-B	Asymm-C	Asymm-D	Symmetric	Symmetry vs. Asymmetry	
								p value - genotype	p value - allele
ENPP1	rs9373000	AA	4 (57)	17 (65)	9 (41)	6 (67)	35 (54)	0.24	0.83
		AG	2 (29)	7 (27)	10 (46)	3 (33)	28 (43)		
		GG	1 (14)	2 (8)	3 (13)	0 (0)	2 (3)		
		p - value genotype	0.33	0.21	0.15	0.62			
	p - value allele	0.23	0.46	0.13	0.19				
rs6569759	GG	5 (72)	6 (21)	5 (22.5)	2 (22)	11 (17)	0.27	0.38	
	AG	1 (14)	12 (46)	12 (55)	5 (56)	38 (58)			
	AA	1 (14)	8 (33)	5 (22.5)	2 (22)	16 (25)			
	p - value genotype	0.004	0.56	0.83	0.92				
	p - value allele	0.016	1	0.66	0.76				
rs858339	TT	1 (16)	3 (9)	0 (0)	0 (0)	20 (30)	0.002	0.55	
	AT	3 (42)	10 (36)	13 (59)	4 (44)	12 (18)			
	AA	3 (42)	15 (55)	9 (41)	5 (56)	33 (52)			
	p - value genotype	0.33	0.13	0.002	0.33				
	p - value allele	0.27	0.77	0.14	0.24				
rs1409181	CC	1 (14)	5 (12)	5 (22.5)	1 (12)	9 (15)	0.53	0.31	
	CG	4 (57)	15 (55)	13 (59)	7 (76)	36 (55)			
	GG	2 (29)	8 (33)	4 (18.5)	1 (12)	20 (30)			
	p - value genotype	0.99	0.88	0.41	0.41				
	p - value allele	0.92	0.69	0.21	0.49				

Gene	SNP	Genotype	Asymm-A	Asymm-B	Asymm-C	Asymm-D	Symmetric	Symmetry vs. Asymmetry	
								p value - genotype	p value - allele
ESR1	rs1643821	GG	1 (16)	3 (9)	3 (13)	3 (32)	4 (7)	0.23	0.3
		AG	3 (42)	14 (50)	10 (46)	5 (56)	36 (55)		
		AA	3 (42)	11 (41)	9 (41)	1 (12)	25 (38)		
		p - value genotype	0.63	0.72	0.48	0.019			
	p - value allele	0.58	0.8	0.76	0.02				
rs3020318	TT	3 (43)	12 (46)	12 (55)	1 (12)	24 (37)	0.39	0.27	
	CT	4 (57)	13 (45)	7 (32)	7 (76)	28 (43)			
	CC	0 (0)	3 (9)	3 (13)	1 (12)	13 (20)			
	p - value genotype	0.47	0.46	0.4	0.16				
	p - value allele	0.17	0.29	0.23	0.4				
rs3020377	GG	3 (43)	13 (45)	12 (55)	3 (33)	27 (41)	0.78	0.51	
	AG	4 (57)	12 (46)	6 (26.5)	6 (67)	31 (48)			
	AA	0 (0)	3 (9)	4 (18.5)	0 (0)	7 (11)			
	p - value genotype	0.65	0.8	0.23	0.28				
	p - value allele	0.22	0.51	0.73	0.91				
rs2077647	TT	2 (29)	2 (8)	3 (13)	1 (12)	7 (11)	0.63	0.53	
	CT	2 (29)	20 (71)	11 (51)	6 (67)	34 (52)			
	CC	3 (42)	6 (21)	8 (36)	2 (21)	24 (37)			
	p - value genotype	0.37	0.2	0.98	0.67				
	p - value allele	0.71	0.52	0.93	0.59				

Table 10. Comparison of SNP genotypes by symmetry subclassification – PITX1 & PITX2									
Gene	SNP	Genotype	Asymm- A	Asymm- B	Asymm- C	Asymm- D	Symmetric	Symmetry vs. Asymmetry	
								p value - genotype	p value - allele
PITX1	rs1131611	GG	4 (57)	18 (59)	14 (64)	8 (88)	48 (74)	0.67	0.74
		GT	3 (43)	8 (33)	8 (36)	1 (12)	15 (23)		
		TT	0 (0)	2 (8)	0 (0)	0 (0)	2 (3)		
			0.47	0.27	0.61				
			0.22	0.76	0.54	0.2			
PITX2	rs2595110	AA	1 (16)	10 (36)	11 (50)	4 (44)	35 (54)	0.13	0.32
		AG	5 (64)	14 (50)	11 (50)	5 (56)	23 (35)		
		GG	1 (16)	4 (14)	0 (0)	0 (0)	7 (11)		
			0.12	0.08	0.37				
			0.96	0.27	0.66	0.95			
				0.14					

5.3. Temporomandibular Disorder Analysis

Since these subjects often had TMD before treatment, we wanted to know whether there was a higher prevalence within and between asymmetry subclassifications. TMD diagnoses were positive in only 3% of the symmetric patients and high in the asymmetric patients (**Table 11**).

Table 11. Percentage of TMD diagnosis among symmetric and asymmetric classification groups						
	Symmetric	All Asymmetric	Group 1	Group 2	Group 3	Group 4
DDR*	3	78	11	29	52	23
Myalgia	4	61	11	33	44	23
Arthralgia	1	33	0	17	19	8
Headache**	0	12	0	9	7	8
DD w/o R***	0	6	0	3	11	0

*DDR = Disc displacement with reduction

**Headache attributed to TMD

***DD w/o R = Disc displacement without reduction with limited opening

Disc displacement with reduction was most common in the asymmetric patients (78%), followed by 61% with myalgia of masticatory muscles, 33% with arthralgia, and

12% with TMD-related headache. Disc displacement without reduction was the least common Axis I diagnosis at 6%. Overall, the population was young, with an average age of 26 years and without fibromyalgia or pain-related disability diagnosed in Axis II of the diagnostic criteria. When we compared genotypes with TMD diagnosis for disc displacement with reduction, myalgia, and arthralgia, and not disc displacement without reduction or TMD related headache, since these groups had few subjects, *ENPP1* rs858339 had significant associations for genotype or allele for all 3 TMD diagnoses (Table 12).

Table 12. <i>ENPP1</i> rs858339 genotypes by DC-TMD Classification						
Gene	SNP	Genotype	No TMD	DDR	Myalgia	Arthralgia
<i>ENPP1</i>	rs858339	TT	50 (67)	9 (35)	0 (0)	0 (0)
		AT	21 (28)	16 (62)	14 (61)	7 (78)
		AA	4	1 (3)	9 (39)	2 (22)
		<i>p</i> - value genotype	(5)	0.009	0.01	0.01
<i>p</i> - value allele	0.33	0.02	0.11	0.056		
			0.27			

We used the JPF questionnaire as an efficient assessment tool for patient perceptions of the presence and severity of TMD. Most symmetric subjects had few or no symptoms of TMD and an average JPF score of 1.97, which agreed with the clinical examination assessment for TMD (Table 13). Asymmetric subjects had an average JPF score of 6.87, which was significantly higher than in the symmetric patients. Furthermore, there were significant differences within the asymmetric subtypes, with group 3 having the highest average score of 9.11, and groups 1 and 4 having the lowest average scores of about 4. When we compared JPF scores between groups, a t test demonstrated significant differences between the symmetric and asymmetric patients

($p < 0.001$). Among the asymmetric patients, an ANOVA comparison also showed significant differences between subclassifications. These results indicated a more likely chance for signs or symptoms of TMD when asymmetry is part of a patient's dentofacial deformity. Complete raw data for JPF scores are presented in Appendix F.

Table 13. Pre- and post-surgical JPF scores of symmetric and asymmetric patients						
	n	Mean Pre-tx JPF Score	SD	p-value*	Mean Post-tx JPF Score	SD
Symmetric	90	1.97	2.53	<0.001	1.97	3.53
Asymmetric	84	6.87	5.43		2.34	2.90
Group 1	9	3.75	4.09	0.009		
Group 2	35	6.94	5.46			
Group 3	27	9.11	5.62			
Group 4	13	4.00	3.61			

*Comparison between symmetric and asymmetric groups with unpaired t-test. Comparison across the four groups with ANOVA.

After treatment, we followed the subjects for 1 year to determine whether TMD improved or worsened, or new conditions occurred. Overall, those without TMD remained so, with 52% of the patients having no change in JPF score (**Figure 4**). Twenty percent of all patients were cured of TMD, with posttreatment JPF scores below 6, and 17% had improvement with JPF scores decreased by 3 or more. Four percent of the patients had worsening of TMD after treatment, with JPF scores increased by 3 or more. Finally, 7% could be diagnosed with TMD with JPF scores less than 6 before surgery and greater than 6 after treatment. All groups, including the symmetric group, had decreases in posttreatment JPF scores that were statistically significant by ANOVA comparisons ($p < 0.001$). Further post hoc t tests showed significantly greater decreases in posttreatment JPF scores for asymmetry groups 2 and 3 compared with the symmetric group ($p < 0.001$).

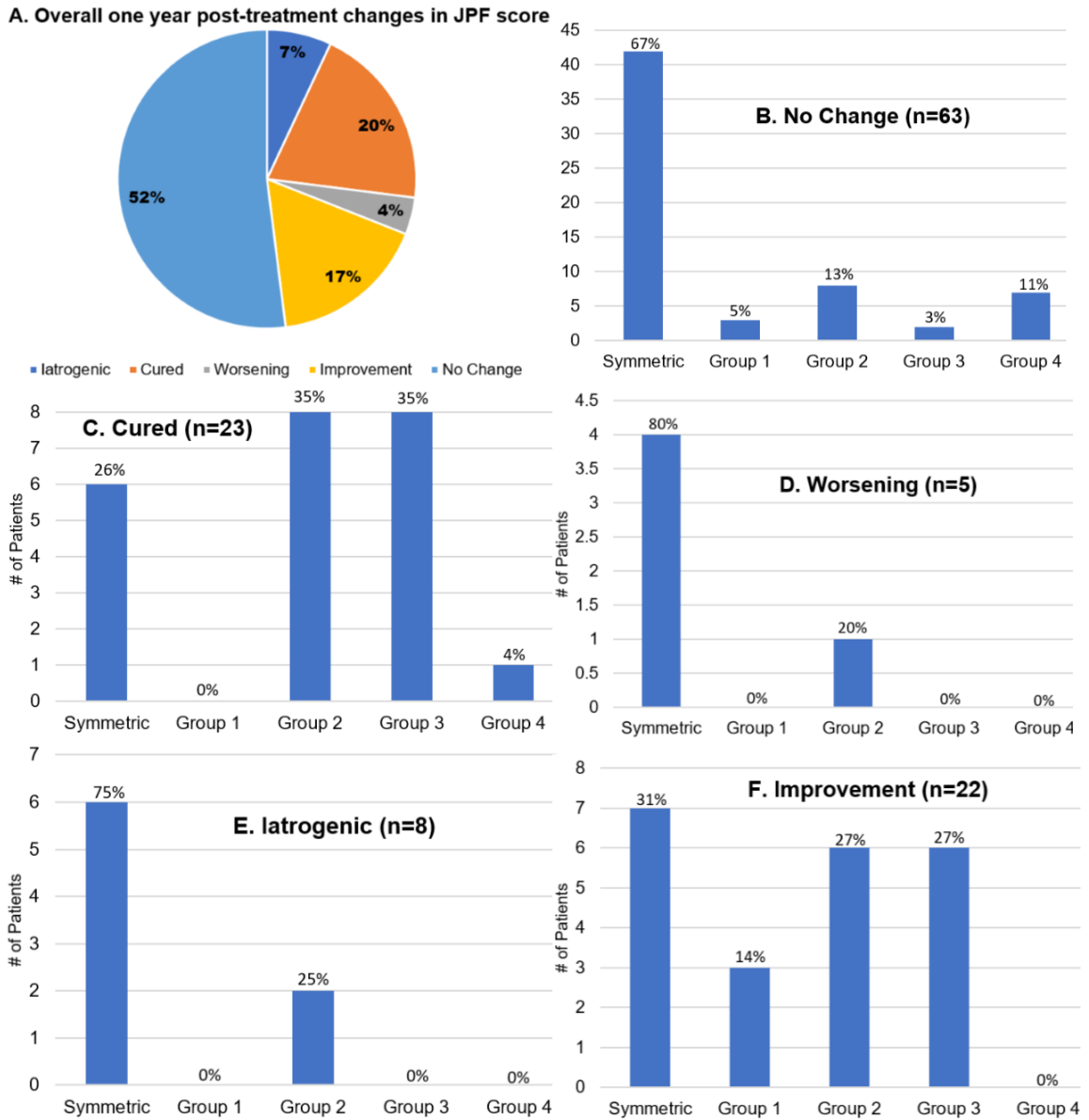


Figure 4. One-year posttreatment changes in JPF scores by asymmetry classification.

A, Distribution of patients based on differences in presurgical and 1-year postsurgical JPF scores: *no change* in TMD; *improvement* of TMD if the JPF score decreased by 3 or more; *worsening* of TMD if the JPF score increased by 3 or more; *cured* of TMD if the JPF score was >6 before surgery and <6 after treatment; and *iatrogenic* TMD if the JPF score was <6 before surgery and >6 after treatment. **B**, Distribution of symmetric and asymmetric groups in the *no change* category. **C**, Distribution of symmetric and asymmetric groups in the *cured* category. **D**, Distribution of symmetric and asymmetric groups in the *worsening* category. **E**, Distribution of symmetric and asymmetric groups in the *iatrogenic* category. **F**, Distribution of symmetric and asymmetric groups in the *improvement* category.

CHAPTER 6:

DISCUSSION

6.1 General Statements

Craniofacial asymmetry, which arises from normal developmental processes, takes many shapes and forms, given the complexity of the nervous, skeletal, muscular, and physiologic components that drive cognitive, sensory, stomatognathic, and respiratory functions (Sciote et al, 2013; Moss, 1969). A functional variation or genetic polymorphism influencing a subject or multiple components can lead to a localized skeletal asymmetry or a generalized craniofacial asymmetry depending on the cause and growth response over time (Moss, 1981). Lateral skeletal distortions are known to occur in the skull (Lundstrom, 1991); cranial base (Brodie, 1955; Russo & Smith, 2011); and midfacial structures, including the vomer, pterygoid process, piriform apertures, and maxilla⁴; and in the mandible by hemimandibular elongation or hyperplasia (Obwegeser, 1986). These ubiquitous configurations of asymmetry have made simple classifications for patterns of craniofacial growth or etiology elusive. Yet a systematic classification system is needed to more accurately plan surgical corrections (Baek et al., 2012; Kim et al, 2014) and serve as a phenotypic clustering for gene association studies (Hwang et al., 2012). Using either traditional tracings of posteroanterior cephalograms (Chew, 2006) or cluster analysis of 3-dimensional computed tomographic image analysis, a classification system with 4 groups or subtypes of craniofacial asymmetry has been formulated for Korean people (Baek et al., 2012).

Our first objective was to determine whether French people with asymmetry could also be subclassified using this diagnostic grouping. We adapted the asymmetry classification system of Baek et al. (Baek et al., 2012), which used 3-dimensional computed tomography, with a new cephalometric analysis of posteroanterior cephalograms for our patients. The diagnostic classification identified differences between groups because of the significant differences in cephalometric measurements. However, the French subjects differed from the Koreans in the percentages of distribution among the asymmetric groups. Koreans were more likely to have group 1 or group 2 asymmetry, whereas the French were more likely to have group 2 or 3 asymmetry. The French patients were almost equally matched; 52% of the subjects undergoing orthognathic surgery were symmetric, and 48% were asymmetric. To our knowledge, no similar estimates are available for Koreans, but in Singapore, the prevalence of asymmetry ranges from 8% to 50% in orthognathic surgery patients (Chew, 2006). These population differences most likely stem from 3 distinct influences. The first is the tendency for the percentage of asymmetry to differ in different combinations of Class II, Class III, and open-bite and deep-bite malocclusions (Severt & Proffit, 1997). The second is the almost certain likelihood that genetic differences in facial shape that mark race and ethnicity can also influence the distribution of asymmetry subclassifications (Nicot et al., 2014). Finally, there were differences in the type of images used in diagnosis and some differences in the morphology of asymmetry subclassification groups.

The second study objective was to determine whether gene variations already identified as contributing to sagittal and vertical malocclusions (*ACTN3*, *ENPP1*, *ESR1*, *PITX1*, and *PITX2*) might also be associated with asymmetry (**Table 7-10**). We compared

13 SNPs to determine differences for genotype and allele between symmetric and asymmetric patients and between the asymmetric groups. Two SNPs were associated with differences in both genotype and alleles when compared between asymmetry groups. SNP rs6569759 in *ENPP1* was different in group 1, and SNP rs1643821 in *ESR1* was significantly different in group 4. The SNP rs858339 in *ENPP1* was significantly different for genotype between symmetric and asymmetric subjects and between asymmetric groups for group 3. *ENPP1* has pleiotropic effects for mineralization and insulin signaling. The intronic SNP rs6569759 has previously been associated with an increased risk for type 2 diabetes, which results in insulin-mediated glucose metabolism that affects fiber type composition of skeletal muscles.⁴⁵ The SNP has also been associated with changes in bigonial width dimensions in western Eurasians (Ermakov et al., 2010). This corroborates our finding that rs6560759 was significantly different in group 1, which has asymmetry in mandibular body breadth but not in ramus height. Rs858339 is an intronic SNP previously associated with variations in bone mineral density (Sheu et al., 2008). We recently reported that rs858339 has a significant association with preoperative TMD in our patients. *ENPP1* rs858339 TT genotype was associated with pretreatment absence of TMD and the AT genotype as a pretreatment risk factor for TMD as determined by JPF scores (Brevi et al., 2015). In this study, rs858399 had strong statistical associations with the TMD diagnoses of disc displacement with reduction, masticatory muscle myalgia, and arthralgia, further confirming its role in etiology (**Table 11**). The association of *ENPP1* with TMD diagnoses or asymmetry group 3 is not yet informative as to the biologic mechanisms responsible. The conditions of arthralgia and disc displacement may be related to *ENPP1* biomineralization functions

and myalgia to insulin signaling in skeletal muscles, but more investigations are necessary to confirm these possibilities. *ESRI* polymorphisms are associated with skeletal Class II malocclusions and symptomatic osteoarthritis of the temporomandibular joint in Korean women (Lee et al., 2006). The rs1643821 intron SNP contributes to the susceptibility for osteoporotic fracture in postmenopausal Chinese women (Wang et al., 2012). Since the incidence of TMD in group 4 was relatively low, rs1643821 may be contributing to the development of this type of asymmetry through variations in bone mineral density with growth, rather than by specific problems in the temporomandibular joint. Rs3020318 is an intronic SNP without any known functional effects. However, this marker belongs to a haplotype associated with a greater cancer risk (Einarsdottir et al., 2008). There is growing evidence that the impact of genetic risk factors on breast cancer varies by hormone receptor status. Genetic variations of the estrogen metabolism pathway—particularly the genes involved in the production of estrogen through androgen conversion—influence the risk for the development of estrogen-sensitive breast cancer (Low et al., 2010).

This could also be true for craniofacial deformities that include symptomatology in the temporomandibular joint, with variations in hormonal levels (i.e., estrogen) influencing the risk for development of TMD. There is a broad consensus among orthodontists that little or no relationship exists between specific types of malocclusions and the development of TMD (Mohlin et al., 2007). Yet for many people with jaw deformations, pain in the TMJ or the masticatory muscles is a common and debilitating comorbid condition estimated to occur in over 50% of patients with facial asymmetry (Miyatake et al., 2003; Dahlberg et al., 1995). Our results affirm this finding. The JPF

scores for asymmetric subjects were significantly higher than for symmetric subjects ($p < 0.0001$). Furthermore, asymmetry groups 2 and 3 were most likely to have preoperative TMD before orthodontic treatment. To determine whether the musculoskeletal imbalances were the root cause of TMD, we followed these patients for at least 1 year after orthodontic treatment to see whether the condition resolved or persisted. Groups 2 and 3 were most likely to be cured of TMD at the 1-year follow-up, or at least they had significant symptom improvement (**Figure 6**). There is growing evidence, confirmed in different populations, that patients with dentofacial deformities have a higher prevalence of TMD (Kobayashi et al., 1999); when treated with combined orthodontic and surgical procedures, they usually have better masticatory function and improved TMD symptoms, especially for the relief of pain (Dujoncquoy et al., 2010; Abrahamsson et al., 2013). These findings led to the conclusion that orthodontic and orthognathic treatment of craniofacial asymmetry helps to cure or alleviate TMD for most patients. However, as with any clinical treatment, a small percentage of patients had significant worsening or presentation of TMD symptoms in the retention phase (7%). Most of these (80%) were subjects without asymmetry, in whom we would not have expected this to occur. Because of the small number of subjects with this result, a total of 5, many more patients must be followed for further characterization of posttreatment development of TMD. Overall, these treatments are highly effective at correcting skeletal malocclusions, producing a physiologic balance that alleviates TMD symptoms.

6.2 Potential Errors

Although posteroanterior cephalometric analysis has been the traditional approach for diagnosis of asymmetry, variability of head positioning in the cephalostat may introduce radiographic projection errors that diminish diagnostic reliability (Malkoc et al., 2005). CBCT images are considered more reliable for diagnosis of asymmetry, but there is insufficient evidence to conclude that CBCT is superior to posteroanterior cephalograms for detecting transverse facial differences (Sawchuk et al., 2016). We routinely use posteroanterior cephalograms in surgical treatment planning of patients and attempt to minimize head rotation around the vertical z-axis where most projection errors occur (Yoon et al., 2002).

A second potential error is landmark identification due to unclear radiographic representations of anatomic locations. However, the 4 patterns of asymmetry are almost always discernible by visual observation, which helps decrease uncertainty as to where landmarks are located. There have been limited reports on landmark identification on posteroanterior cephalograms (Leonardi et al., 2006), but a recent study (Ulkur et al., 2016) estimated rater reliability to be consistently high at 0.9 to 0.95, or above for most points. Our intrarater reliability had an R^2 value of 0.98 and was similar to that of Ulkur et al. Therefore, our methods may introduce some measurement error in diagnosis, but given the large differences in specific cephalometric measures between groups (**Figure 3**), it was not a major influence on the classification of subjects into asymmetry groups.

6.3 Future Directions

Although there is no specific polymorphism associated with *PITX2* and facial asymmetry, differences in gene expression were previously identified in right and left masseter muscle samples from asymmetric patients (Nicot et al., 2014). The variation in *PITX2* gene expression most likely has an association with facial asymmetry and warrants further research. One possibility to be investigated is that *PITX2* may interact with *ENPP1* to produce differences in mineral density and bone growth between facial sides.

Other posttreatment conditions, such as condylar remodeling and condylar resorption, could influence this rare TMD occurrence and will be studied in the future.

CHAPTER 7:

CONCLUSIONS

1. A new posteroanterior cephalometric analysis using 6 measurements to detect differences in facial sides has been developed to distinguish 4 main classifications of asymmetry that are common in patients with dentofacial deformity.
2. TMD prevalence is much higher in patients with asymmetry compared with patients with dentofacial deformity without asymmetry.
 - a. The most common TMD presentations were disc displacement with reduction, masticatory muscle myalgia, and arthralgia.
 - b. Two of the 4 asymmetry groups had both high positive diagnoses for TMD and subjective patient reporting of symptom.
3. SNP genotype rs6569759 in *ENPP1* was associated with asymmetry group 1, and rs858339 was associated with asymmetry group 3.
4. SNP genotype rs1643821 in *ESR1* was associated with asymmetry group 4. rs3020318 in *ESR1* was associated with PC1 and PC2, which relate to maxillary canting and menton deviation.
5. SNP genotype rs858339 in *ENPP1* was associated with disc displacement with reduction, masticatory muscle myalgia, and arthralgia.
6. Orthodontic and orthognathic treatment of asymmetry alleviates TMD symptoms for at least 1 year into retention in most patients.

BIBLIOGRAPHY

- Abass, S.K., Hartsfield, J.K. (2008). Investigation of genetic factors affecting complex traits using external apical root resorption as a model. *Seminars in Orthodontics*, 14, 115-124.
- Abrahamsson, C., Henrikson., T., Nilner, M., Sunzel, B., Bondemark, L., Ekberg, E. (2013). TMD before and after correction of dentofacial deformities by orthodontic and orthognathic treatment. *International Journal of Oral and Maxillofacial Surgery*, 42,252-8.
- Ahlqvist, J., Eliasson, S., Welander, U. (1986). The effect of projection errors on cephalometric length measurements. *European Journal of Orthodontics*, 8, 141-148.
- Ahn, S.-J., Lee, S.-P., & Nahm, D.-S. (2005). Relationship between temporomandibular joint internal derangement and facial asymmetry in women. *American Journal of Orthodontics and Dentofacial Orthopedics*, 128, 583-591.
- Atalay, B. (2006). *Math and the Mona Lisa: The Art & Science of Leonardo da Vinci*. New York, Harper Collins Publishers.
- Baek, C., Paeng, J.-Y., Lee, J., & Hong, J. (2012). Morphologic Evaluation and Classification of Facial Asymmetry Using 3-Dimensional Computed Tomography. *Journal of Oral and maxillofacial Surgery*, 70, 1161-1169.
- Bishara, S., Burkey, P., & Kharouf, J. (1994). Dental and facial asymmetries: a review. *The Angle Orthodontist*, 64(2), 89-98.
- Brevi, B., DiBlasio, A., DiBlasio, C., Piazza, F., D'Ascanio, L., Sesenna, E. (2015) Which cephalometric analysis for maxilla-mandibular surgery in patients with obstructive sleep apnea syndrome? *Acta Otorhinolaryngologica Italica*, 35,332-7.
- Brodie, AG. (1955). The behavior of the cranial base and its components as revealed by serial cephalometric roentgenogram. *The Angle Orthodontist*, 25,148-60.
- Bruce, R., & Hayward, J. (1968). Condylar hyperplasia and mandibular asymmetry: A review. *Journal of Oral Surgery*, 26, 281.
- Chanock, S., & Wacholder, S. (2002). One gene and one outcome? No way. *Trends in Molecular Medicine*, 8, 266-269.
- Chen, C., Shen, M.M. (2004). Two modes by which lefty proteins inhibit nodal signaling. *Current Biology*, 14(7), 618-624.

- Cheong, Y.-W., & Lo, L.-J. (2011). Facial Asymmetry: Etiology, Evaluation, and Management. *Chang Gung Medical Journal*, 34(4), 341-350.
- Chew, M.T. (2006). Spectrum and management of dentofacial deformities in a multiethnic Asian population. *The Angle Orthodontist*, 76,806-9.
- Chung, K., Richards, T., Nicot, R., Vieira, A.R., Cruz, C.V., Raoul, G., Ferri, J., Sciote, J. (2017) ENPP1 and ESR1 genotypes associate with subclassifications of craniofacial asymmetry and severity of TMD. *American Journal of Orthodontics and Dentofacial Orthopedics*, 152, 631-645.
- Clark, G.T., Seligman, D., Solberg, W.K., Pullinger, A.G. (1989). Guidelines for the examination and diagnosis of temporomandibular disorders. *Journal of Craniomandibular Disorders*, 3,7-14.
- Craft RM. (2007). Modulation of pain by estrogens. *Pain*, 132(1):S3-12.
- Cobourne, M.T. (2004). The complex genetics of cleft lip and palate. *European Journal of Orthodontics*, 26,7-16.
- Cohen, M.M.Jr. (1995). Perspectives on craniofacial asymmetry. V. The craniosynostoses. *International Journal of Oral and Maxillofacial Surgery*, 3, 191-194.
- Damastra, J., Fourie, Z., & Ren, Y. (2011). Evaluation and comparison of postero-anterior cephalograms and cone-beam computed technology images for detection of mandibular asymmetry. *European Journal of Orthodontics*, 1-6.
- Dhalberg, G., Petersson, A., Westesson, P.L., Eriksson, L. (1995). Disk displacement and temporomandibular joint symptoms in orthognathic surgery patients. *Oral Surgery, Oral Medicine, Oral Pathology*, 79,273-277.
- Dion, K.K., Berscheid, E., Walster, E. (1972). What is beautiful is good. *Journal of Personal Social Psychology*, 24, 285-290.
- Dizak, P.M., Burnheimer, J.M., Deeley, K.B., Brungo, A.J., Sciote, J.J., Vieira A.R. (2016). Underlying craniofacial abnormalities in malocclusion patients may be attributed to genetic variation among 10 genes. *Journal of Dental Research*, 95(A), 3953.
- Dujoncquoy, J.P., Ferri, J., Raoul, G., Kleinheinz, J. (2010). Temporomandibular joint dysfunction and orthognathic surgery: a retrospective study. *Head & Face Medicine*, 6, 2-7.
- Eagly, A.H., Ashmore, R.D., Makhijani, M.G., Longo, L.C. (1991). What is beautiful is good, but...: A meta-analytic review of research on the physical attractiveness stereotype. *Psychological Bulletin*, 110(1), 109-128.

Einarsdottir, K., Darabi., H., Li, Y., Low, Y.L., Li, Y.Q., Bonnard, C. (2008). ESR1 and EFG genetic variation in relation to breast cancer risk and survival. *Breast Cancer Research*, 10, R15.

Ermakov, S., Rosenbaum, M.G., Malkin, I., Livshits, G. Family-based study of association between ENPP1 genetic variants and craniofacial morphology. *Annals of Human Biology*, 37,754-66.

Evans, W, Hood, D, Gurd, J. (1973). Purification and properties of a mouse liver plasma membrane glycoprotein hydrolysing nucleotide pyrophosphate and phosphodiester bonds. *Biochemical Journal*, 135(4):819–826.

Fleisch, H, Straumann, F, Schenk, R, Bisaz, S, Allgöwer, M. (1966). Effect of condensed phosphates on calcification of chick embryo femurs in tissue culture. *American Journal of Physiology*, 211(3):821-825.

Fontoura, C.S.G., Miller, S.F., Wehby, G.L., Amendt, B.A., Holton, N.E., Southard, T.E., Allareddy, V., Uribe, L.M. (2015). Candidate gene analyses of skeletal variation in malocclusion. *Journal of Dental Research*, 94(7), 913-920.

Fushima, K., Inui, M., & Sato, S. (1999). Dental asymmetry in temporomandibular disorders. *Journal of Oral Rehabilitation*, 26, 752-756.

Gerstner, G.E., Clark, G.T., Goule, J.P. (1994). Validity of a brief questionnaire in screening asymptomatic subjects from subjects with tension-type headaches or temporomandibular disorders. *Community Dentistry and Oral Epidemiology*, 22, 235-242.

Hartsfield J.K. (2002). Development of the vertical dimension: Nature and nurture. *Seminars in Orthodontics*, 8, 113.

Hartsfield, J.K. (2011). Factors beyond the control of the clinician: Understanding the genetics underlying orthodontic treatment. In: McNamara, JA & Kapila, SD (Eds), *Understanding Genetics*. Monograph (pp.155-190). The University of Michigan: Ann Arbor, MA.

Hartsfield, J.K. (2011). The benefits of obtaining the opinion of a medical geneticist regarding orthodontic patients. In: Krishnan, V. & Davidovitch (Ed.), *Interactive Orthodontics*. Oxford: Blackwell.

Haraguchi, S., Iguchi, Y., Takada, K. (2008). Asymmetry of the face in orthodontic patients. *Angle Orthodontists*, 78(3), 421-426.

Hatcher, D.C., Faulkner, M.G., Hay, A. (1986). Development of mechanical and mathematic models to study temporomandibular joint loading. *Journal of Prosthetic Dentistry*, 55(3), 377-384.

Hinds, E., Reid, L., & Burch, R. (1960). Classification and management of mandibular asymmetry. *American Journal of Surgery*, 100, 825-834.

Hottenstein, M.K., Horton, M.J., Sciote, J.J., Nicot, R., Raoul, G., Ferri, J. (2015). Differential Expression of PITX2 in adults with facial asymmetry. *Journal of Dental Research*, 94(A), 2114583.

Houston, W.J.B. (1983). The analysis of errors in orthodontic measurements for posteroanterior radiographic cephalometry. *Angle Orthodontist*, 67, 137-142.

Hwang, H.-S., Hwang, C. -H., Lee, K., -H., Kang, B. -C. (2006). Maxillofacial 3-dimensional image analysis for the diagnosis of facial asymmetry. *American Journal of Orthodontics and Dentofacial Orthopedics*, 130, 779-785.

Hwang, H.-S., Youn, I.-S., Lee, K.-H., & Lim, H.-J. (2007). Classification of facial asymmetry by cluster analysis. *American Journal of Orthodontics and Dentofacial Orthopedics*, 132(3), 279.e1-6.

Hwang, T., Atluri G., Xie, M., Dey, S., Hong, C., Kumar, V., Kuang, R. (2012). Co-clustering phenome-genome for phenotype classification and disease gene discovery. *Nucleic Acids Research*, 40, 146.

Inui, M., Fushima, K., & Sato, S. (1999). Facial Asymmetry in temporomandibular joint disorders. *Journal of Oral Rehabilitation* (26), 402-406.

Ito, T., Gibbs, C. H., Marguelles-Bonnet, R., Lupkiewicz, S.M., Young, H.M., Lundeen, H.C., Mahan, P.E. (1986). Loading on the temporomandibular joints with five occlusal conditions. *Cranio-mandibular Function and Dysfunction*, 56(4), 478-483.

Katzberg, R., Tallents, R.H., Hayakawa, K., Miller, T., Goske, M.J., Wood, B.P. (1985). Internal derangements of the temporomandibular joint: findings in the pediatric group. *Radiology*, 154, 125-127.

Kim BS, Kim YK, Yun PY, Lee I, Bae J. (2010). The effects of estrogen receptor α polymorphism on the prevalence of symptomatic temporomandibular disorders. *Journal of Oral and Maxillofacial Surgery*, 68:2975-2979.

Kim, J.-Y., Jung, H.-D., Jung, Y.-S., & Hwang, C.-J. (2014). A simple classification of facial asymmetry by TML system. *Journal of Cranio-Maxillo-Facial Surgery*, 42(4), 313-320.

Kim, S.-J., Lee, K.-J., Lee, S.-H., & Baik, H.-S. (2013). Morphologic relationship between the cranial base and the mandible in patients with facial asymmetry and mandibular prognathism. *American Journal of Orthodontics and Dentofacial Orthopedics*, 144(3), 330-340.

- Lee, B.-R., Kang, D.-K., Son, W.-S., Park, S.-B., Kim, S.-S., Kim, Y.-I. (2011). The relationship between condyle position, morphology and chin deviation in skeletal Class III patients with facial asymmetry using cone-beam CT. *Korean Journal of Orthodontics*, 41, 87-96.
- Lee, D.F., Kin, T.W., Kang, S.C., Kim, S.T. (2006). Estrogen receptor gene polymorphism and craniofacial morphology in female TMJ osteoarthritis patients. *International Journal of Oral and Maxillofacial Surgery*, 35,165-9.
- Leite, R.A., Rodrigues, J.F., Sakima, M.T., Sakima, T. (2013). Relationship between temporomandibular disorders and orthodontic treatment: A literature review. *Dental Press Journal of orthodontics*, 18(1), 150-157.
- Leonardi, R., Annunziata, A., Caltabiano, M. (2006). Landmark identification error in posteroanterior cephalometric radiography. A systematic review. *The Angle Orthodontist*, 78,761-5.
- Low, Y.L., Li, Y., Humphreys, K., Thalamuthu, A., Darabi, H., Wedren, S. (2010). Multi-variant pathway association analysis reveals the importance of genetic determinants of estrogen metabolism in breast and endometrial cancer susceptibility. *PLoS Genetics*, 6,e1001012.
- Lundstrom, A. (1961). Some Asymmetries of the Dental Arches, Jaws, and Skull, and their etiological significant. *American Journal of Orthodontics and Dentofacial Orthopedics*, 47,81-106.
- Mackenzie, N., Huesa, C., Rutsch, F., MacRae, V. (2012). New insights into *ENPP1* function: lessons from clinical and animal studies. *Bones*, 51:961-968.
- McEwen BS, Alves SE. (1999). Estrogen actions in the central nervous system. *Endocrine Reviews*, 20:279-307.
- McNamara, J.A. (1981). Components of Class II malocclusion in children 9-10 years of age. *The Angle Orthodontist*, 51, 177.
- McNamara Jr., J.A., Seligman, D.A., Okeson, J.P. (1995). Occlusion, orthodontic treatment, and temporomandibular disorders: a review. *Journal of orofacial Pain*, 9(1), 73-90.
- McNeill, C. (1997). Management of temporomandibular disorders: concepts and controversies. *Journal of Prosthetic Dentistry*, 77(5), 510-22.
- Mercier, J.M., Perrin, J.P., Longis, J., Arzul, L., Corre, P.(2014). Facial asymmetries and their skeletal component. *The Revue de stomatologie Chirurgie Maxillo-faciale et de Chirurgie Orale*, 115(4), 219-228.

- Mishra, H. Shivaprakash, G., Maurya, R.K.(2014) Assessment of facial asymmetry in various malocclusion: A comparative analysis. *Journal of Indian Orthodontic Society*, 48(4), 537-545.
- Miyatake, E., Miyawaki, S., Morishige, Y., Nishiyama, A., Sasaki, A., Takano-Yamamoto, T. (2003). Class III malocclusion with severe facial asymmetry, unilateral crossbite, and temporomandibular disorders. *American Journal of Orthodontics and Dentofacial Orthopedics*, 124,435-45.
- Mongoni, F. (1989). Articular remodeling in the adult. *Craniomandibular and TMJ Orthopedics* (p. 29). Berlin: Quintessence Publishing Inc.
- Moss, ML. (1981). Genetics, epigenetics, and causation. *American Journal of Orthodontics and Dentofacial Orthopedics*, 80,366-75.
- Moss, ML. (1969). The Primary role of the functional matrices in facial growth. *American Journal of orthodontics and dentofacial orthopedics*, 55,566-77.
- Nickerson Jr., J.W., Moystad, A. (1983). Observations on individuals with radiographic bilateral condylar remodeling: a clinical study. *Journal of Craniomandibular and Sleep Practice*, 5, 135-141.
- Nicot, R., Hottenstein, M., Raoul, G., Ferri, J., Horton, M., Tobias, J.W., Barton, E., Gele P, Sciote, J.J. (2014). Nodal pathway genes are down-regulated in facial asymmetry. *Journal of Craniofacial Surgery*, 25(6), e458-e555.
- Nicot, R., Vieira, A.R., Raoul, G., Delmotte, C., Ferri, J., Sciote, J.J. (2016). Role of ENPP1 and ESR1 genotypes in the TMD of patients with dentofacial deformities. *Journal of Craniomaxillofacial Surgery*, in press.
- Obwegeser, H. L., & Makek, M. S. (1986). Hemimandibular Hyperplasia-Hemimandibular Elongation. *Journal of Maxillofacial Surgery* (14), 183-208.
- Oral, K., Bal Kucuk, B., Ebeoglu, B., Dincer, S. (2009). Etiology of temporomandibular disorder pain. *Agri*, 21(3), 89-94.
- Proffit, W.R., Phillips, C., Dann, C. (1990). Who Seeks Surgical-Orthodontic Treatment? *International Journal of Adult Orthodontics and Orthognathic Surgery*, 5, 81-89.
- Rambo, L. (2015). Temporomandibular joint disorders and nasal septum deviation in dentofacial deformity patients. Unpublished master's thesis, Temple University. Philadelphia, PA.
- Register,T, Wuthier, R. (1985). Effect of pyrophosphate and two diphosphonates on ⁴⁵Ca and ³²Pi uptake and mineralization by matrix vesicle-enriched fractions and by hydroxyapatite. *Bone*, 6(5):307-312.

- Reyneke, J. P., Tsakiris, P., & Kienle, F. (1997). A simple classification for surgical treatment planning of maxillomandibular asymmetry. *British Journal of Oral and Maxillofacial Surgery*, 35, 349-351.
- Riolo, M.L., Brandt, D., TenHave, T.R. (1987). Associations between occlusal characteristics and signs and symptoms of TMJ dysfunction in children and young adults. *American Journal of Orthodontics and Dentofacial Orthopedics*, 92(6), 467-477.
- Rowe, N. (1960). Aetiology, clinical features, and treatment of mandibular deformity. *British Dental Journal* (108), 64-96.
- Russo, P.P., Smith, R.L. (2011). Asymmetry of human skull base during growth. *International Journal of Morphology*, 29,1028-32.
- Ryu, H. -S., An, K. -Y., Kang, K. -H.(2015). Cone-beam computed tomography based evaluation of rotational patterns of dentofacial structures in skeletal Class III deformity with mandibular asymmetry. *Korean Journal of Orthodontics*, 45(4), 153-163.
- Sawchuk, D., Currie, K., Vich, M.L., Palomo, J.M., Flores-Mir, C. (2016). Diagnostic methods for assessing maxillary skeletal and dental transverse deficiencies: a systematic review. *Korean Journal of Orthodontics*, 46,331-45.
- Scanavini, P.E., Paranhos, L.R., Torres, F.C., Vasconcelos, M.H.F., Joias, R.P., Scanavini, M.A.(2012). Evaluation of the dental arch asymmetry in natural normal occlusion and Class II malocclusion individuals. *Dental Press Journal of Orthodontics*, 17(1), 125-137.
- Schier, A.F.(2003). Nodal signaling in vertebrate development. *Annual Review of Cell and Developmental Biology Journal*, 14,618-624.
- Sciote, JJ., Raoul G., Ferri J., Close J., Horton MJ., Rowlerson A. (2013). Masseter function and skeletal malocclusion. *Revue de Stomatologie et de Chirurgie Maxillo-faciale*, 114,79-85.
- Selaimne, C.M., Jeronymo, J.C., Brihante, D.P., Lima, E.M., Grossi, P.K. Grossi, M.L. (2007). Occlusal risk factors for temporomandibular disorders. *Angle Orthodontist*, 77(3), 471-477.
- Seligman, D.A., Pullinger, A.G. (1991). The role of functional occlusal relationships in temporomandibular disorders: a review. *Journal of Craniomandibular Disorders*. 5(4), 265-279.
- Seligman, D.A., Pullinger, A.G., Solberg, W.K. (1988). The prevalence of dental attrition and its association with factors of age, gender, occlusion, and TMJ symptomatology. *Journal of Dental Research*, 67(10), 1323-1333.

- Severt, T. R., & Proffitt, W. R. (1997). The prevalence of facial asymmetry in the dentofacial deformities population at the University of North Carolina. *International Journal of Adult Orthodontics and Orthognathic Surgery*, 12(3), 171-176.
- Sheu, Y., Yerges L.A., Kammerer, C.M., Cauley, J.A., Bunker, C.H. (2008). Association of common ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene variants with bone mineral density. Unpublished master's thesis, University of Pittsburgh. Pittsburgh, PA.
- Shih, J.P., Gross, M.K., Kioussi, C. (2007). Expression pattern of the homeobox transcription factor PITX2 during muscle development. *Gene Expression Patterns*, 7, 441-451.
- Shin, S.M., Kim, Y.M., Kim, N.R., Choi, Y.S., Park, S.B., Kim, Y.I. (2016). Statistical shape analysis-based determination of optimal midsagittal reference plane for evaluation of facial asymmetry. *American Journal of Orthodontics and Dentofacial Orthopedics*, 150(20), 252-260.
- Souyris, F., Moncarz, V., Rey, P. (1983). Facial asymmetry of developmental etiology. *Oral Surgery*, 56, 113-124. Terkeltaub, R, Rosenbach, M, Fong, F, Goding, J. (1994). Causal link between nucleotide pyrophosphohydrolase overactivity and increased intracellular inorganic pyrophosphate generation demonstrated by transfection of cultured fibroblasts and osteoblasts with plasma cell membrane glycoprotein-1. Relevance to calcium pyrophosphate dihydrate deposition disease. *Arthritis & Rheumatism*, 37:934–941.
- Ulkur, F., Ozdemir, F., Germec-Cakan, D., Kaspar, E.C. (2016). Landmark errors on posteroanterior cephalograms. *American Journal of Orthodontics and Dentofacial Orthopedics*, 150,324-31.
- Vagervik K. (1981). Orthodontic management of unilateral cleft lip and palate. *Cleft Palate Journal*, 18, 256.
- Vig, P.S., Hewitt, A.B. (1931). Asymmetry of the human facial skeleton. *Angle Orthodontist*. 22, 324-352.
- Wang, C., Zhang, Z., Zhang, H., He, J.W., Gu, J.W., Hu, W.W. (2012). Susceptibility genes for osteoporotic fracture in postmenopausal Chinese women. *Journal of Bone and Mineral research*, 202(27),2582-91.
- Williamson, E.H. (1977). Temporomandibular dysfunction in pretreatment adolescent patients. *American Journal of Orthodontics*, 71, 429-433.
- Woo, T.L. (1931). On the Asymmetry of the Human Skull. *Biometrika*. 22,324-352.

Yoon, Y.J., Kim, D.H., Yu, D.S., Kim, H.J., Choi, E.H., Kim, K.W. (2002). Effects of head rotation on posteroanterior cephalometric radiographs. *The Angle Orthodontist*, 71,36-42.

Zebrick, B., Teeramongkolgul T., Nicot, R., Horton, M.J., Raoul, G., Ferri, J., Vieira, A.R., Sciote, J.J. (2014). ACTN3 R577X genotypes associate with Class II and deep bite malocclusions. *American Journal of Orthodontics and Dentofacial Orthopedics*, 146(5), 603-611.

APPENDICES

APPENDIX A: SUMMARY OF SAGITTAL/VERTICAL MALOCCLUSION DIAGNOSIS OF SUBJECTS

Subject	Sagittal	Vertical	Subject	Sagittal	Vertical	Subject	Sagittal	Vertical
001	Class II	Open	083	Class III	Open	173	Class II	Open
002	Class II	Normal	084	Class III	Open	177	Class II	Open
003	Class II	Open	086	Class III	Open	178	Class II	Deep
004	Class II	Open	089	Class II	Normal	182	Class II	Open
005	Class III	Open	090	Class II	Open	183	Class II	Open
007	Class III	Normal	091	Class III	Open	184	Class II	Open
009	Class III	Normal	092	Class II	Open	187	Class II	Open
010	Class I	Deep	093	Class II	Open	188	Class II	Deep
012	Class II	Normal	101	Class II	Normal	189	Class II	Open
015	Class II	Open	102	Class II	Normal	190	Class II	Open
020	Class II	Open	104	Class II	Open	195	Class III	Open
021	Class II	Open	106	Class II	Open	197	Class II	Open
031	Class II	Open	110	Class II	Open	199	Class II	Open
032	Class III	Open	111	Class III	Normal	201	Class II	Deep
034	Class II	Open	112	Class II	Normal			
035	Class II	Open	113	Class III	Open			
038	Class II	Open	114	Class III	Open			
040	Class II	Open	120	Class III	Open			
041	Class II	Open	121	Class II	Deep			
042	Class III	Normal	122	Class III	Normal			
044	Class II	Deep	123	Class II	Normal			
048	Class III	Normal	124	Class III	Open			
050	Class II	Open	128	Class II	Normal			
051	Class II	Normal	130	Class II	Deep			
054	Class III	Normal	136	Class II	Open			
056	Class II	Open	139	Class III	Open			
057	Class III	Open	144	Class II	Open			
058	Class II	Normal	145	Class III	Deep			
061	Class III	Normal	149	Class II	Deep			
062	Class II	Open	151	Class II	Deep			
064	Class III	Open	152	Class III	Open			
065	Class II	Normal	154	Class III	Open			
068	Class II	Normal	160	Class III	Open			
075	Class II	Open	165	Class III	Open			
082	Class III	Open	167	Class II	Open			

**APPENDIX B: SUMMARY OF SUBJECTS & PA CEPH RAW DATA
COLLECTION (ASYMMETRIC PATIENTS - MAXILLARY MEASUREMENTS)**

Subjects	Deviation	Classification	OP Tilt (°)	J-Z R (mm)	J-Z L (mm)
1	R	B	0.7	276.5	277.6
2	R	B	1.6	224.3	225.6
3	L	B	-0.4	241.7	242.8
4	R	D	3.4	288.6	293.5
5	L	D	-3.9	293.7	283.6
7	L	B	0.6	274	275.5
9	L	B	-0.9	269.1	268.3
10	L	C _{NOMAXCANT}	-0.5	276.7	278.6
12	L	A	0.1	242.4	243.7
15	R	A	-1.3	259.7	259.3
20	L	B	-1.1	252.8	251.8
21	R	A	1.3	270.7	270.3
31	R	B _{CONDRESORP}	1.9	253.3	254.1
32	L	C _{NOMAXCANT}	-0.6	266.7	265.8
34	R	C _{NOMAXCANT}	1	250.2	250.5
35	R	B	1.2	247.3	246.2
38	R	C _{NOMAXCANT}	1.7	244.8	243.7
40	L	C _{NOMAXCANT}	-1.9	249.8	251.1
41	L	D	-3.2	261.9	238.5
42	R	C _{NOMAXCANT}	-0.1	250.8	251.7
44	L	C _{NOMAXCANT}	1.1	227.8	229
48	L	B	0	243.7	243.8
50	L	B	0.4	230.1	230.7
51	R	D	3.5	226.6	230.1
54	L	B	-0.5	278.3	278.1
56	R	B	-0.5	263.7	264.4
57	R	D	2.4	219.1	238.1
58	L	B	-0.7	229.9	230.8
61	L	B	-1.2	250.4	249.5
62	L	B	0.5	247.5	247.8
64	L	B	-1.2	245.4	245
65	L	B	0.5	274.7	273.6
68	L	B	-1.2	253.2	251.4
75	R	B	0	240.4	241
82	L	B	0.2	253.9	254.7
83	L	D	-4.6	264.1	259.7
84	L	C _{NOMAXCANT}	0.4	259.4	259.1
86	L	D	-2.2	279.7	274.1

APPENDIX B (continued): SUMMARY OF SUBJECTS & PA CEPH RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MAXILLARY MEASUREMENTS)

Subjects	Deviation	Classification	OP Tilt (°)	J-Z R (mm)	J-Z L (mm)
89	R	B	1.3	226	224.1
90	R	C _{NOMAXCANT}	0	271.7	271
91	R	C _{NOMAXCANT}	0	286.2	288.1
92	R	C _{NOMAXCANT}	1.1	278.7	279.7
93	L	B	-1.2	280.5	279.3
101	R	C _{NOMAXCANT}	-0.1	243.3	241.9
102	L	B	-1.5	255.2	255.1
104	R	C _{NOMAXCANT}	0.9	301.2	300.9
106	L	D	-2.5	254.3	262
110	L	A	-0.7	293.5	295.4
111	R	C _{NOMAXCANT}	-0.4	260.9	259.5
112	R	A	0.2	223.9	223
113	R	C	3	287.3	293.9
114	L	B	-0.4	268.9	267.7
120	L	B	-1.4	289.3	289
121	L	C _{NOMAXCANT}	-1.8	271.4	270
122	L	C _{NOMAXCANT}	-0.8	231.3	230.8
123	R	C _{NOMAXCANT}	1.7	246.1	246.1
124	L	A	-2	259.7	259.8
128	L	C _{NOMAXCANT}	1.4	248.3	250
130	L	B	-0.1	214.5	216.4
136	R	B	1.1	262.2	262
139	R	C _{NOMAXCANT}	1	254.9	255.3
144	L	D	2.2	217.9	212.6
145	R	C	2.3	233.7	254.7
149	R	C _{NOMAXCANT}	1.5	296	297.7
151	R	A	1.6	241.2	242.4
152	R	B	-0.6	292.9	291.6
154	R	D	5.1	237	242.9
160	L	C _{NOMAXCANT}	0.5	272.1	273.7
165	L	D	-3.1	258.7	252.8
167	L	D	-2.2	283.6	276.3
173	L	C _{NOMAXCANT}	-0.1	289.2	287.4
177	R	B	-1.4	229.8	228.2
178	R	C _{NOMAXCANT}	-0.8	276.4	275.2
182	R	C _{NOMAXCANT}	-0.4	218.9	218.7
183	L	A	0.6	253.8	254.7
184	R	C _{NOMAXCANT}	-0.1	270.4	271.6
187	L	B	0.9	283.7	284.3
188	L	B	-1.5	262.2	260.6

APPENDIX B (continued): SUMMARY OF SUBJECTS & PA CEPH RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MAXILLARY MEASUREMENTS)

Subjects	Deviation	Classification	OP Tilt (°)	J-Z R (mm)	J-Z L (mm)
189	R	A	0.9	235.2	234.6
190	L	B	-1.8	258.8	259.5
195	L	B	-1.1	233.3	234.5
197	R	B	1.4	219.1	219.1
199	L	D	-10.7	291.2	260.3
201	L	B	-0.6	253.9	254.6

**APPENDIX C: PA RAW DATA COLLECTION (ASYMMETRIC PATIENTS -
MANDIBULAR MEASUREMENTS)**

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
1	-4	176.2	174.2	189.9	215.5	288.8	307.1
2	-3.5	175	164.3	170.2	187.5	246.5	275.7
3	5.4	153.6	185.2	181.9	171.4	222.8	216.4
4	-3.5	176.5	173.2	220.7	228.8	273.7	297
5	2.8	172.7	181	230.7	224.8	296.1	260.5
7	2.7	182.1	189.8	208.2	188.7	321.7	314.5
9	5	156.6	205.8	235.2	186.4	257.4	238.4
10	4.8	172.4	176.3	224	193.2	281	285.6
12	5.2	170.9	196.9	201.9	183.2	278.8	279.9
15	-2.9	176	167.2	232.9	245.5	181.2	180.4
20	3.1	170.1	195.8	200.8	195.2	261.9	235.6
21	-3.9	181.4	177.6	204	226.3	283.4	281.6
31	-3	180.8	176.2	194.1	201.8	233.1	241
32	3.2	178.6	187.2	227	196.4	301	322.9
34	-4.4	175.8	172.4	178.6	218.4	265.4	246.4
35	-4.5	194.3	178.2	228	238.6	173.7	181.9
38	-4.9	196.4	194.8	204.6	240.1	222.5	207
40	2.3	165.1	174.1	187.3	175.3	229.3	238.6
41	8.8	161.1	187	217	182.4	223.1	214.7
42	-4.1	188.2	187.1	192.5	222.2	245.1	230.8
44	7.7	141.5	176.8	184.2	151.8	245.3	265.9
48	2.8	165.8	178.8	190.2	179.5	264.7	258.5
50	3.2	153.4	169	180.8	177	223.1	214.2
51	-3.4	175.5	171.8	206.5	209.3	271.3	291.9
54	4.3	174	180.3	211.7	176.2	255.2	247.4
56	-4.6	197.1	196.5	225.3	250.5	265.9	273
57	-4.9	204.7	195.5	214.1	269.9	277.2	286.9
58	3.3	171.6	179.2	195.2	173.1	300	288.4
61	6.7	145.8	180.5	207.8	197.8	250.7	229.7
62	4.5	164.5	183.4	232.2	226.8	218.8	204.7
64	2.1	181.2	186.5	219.7	213.6	270.8	263.5
65	3	192.4	193.8	215.7	197.3	254.7	249.5
68	2.7	179.5	185.6	207.1	196.4	232.8	222.5
75	-3.7	187.4	168.4	200.9	207.6	247.8	258
82	3.7	141.8	161.9	184.1	170.2	293.1	286.3
83	3.6	197.5	206.4	270.6	258.8	270.1	296.5
84	3.6	193.6	208.6	247.2	225.4	251.4	258.9

APPENDIX C (continued): PA RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MANDIBULAR MEASUREMENTS)

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
86	8.4	172.1	188.3	250.5	200.8	286.4	244.2
89	-5.1	175.8	144.9	165.9	170	238.7	256.5
90	-2.5	183.4	182.3	179.7	205.4	301.5	286
91	-3	184.1	183.5	221.4	242.4	248.8	238.6
92	-2.8	182.2	165.5	235.4	243.9	252.2	239.4
93	4.4	190.7	206.8	264.7	235.7	301.7	284.2
101	-3	187.6	165.7	188.1	194.5	275.7	267.9
102	4.6	167.4	176.6	200.2	176.6	211.3	206
104	-3.8	185.4	161.3	195.2	211.5	320.5	305.2
106	5.1	170.9	202.3	246.2	236.3	278.4	256.8
110	3.4	180.6	196	214.9	199.8	321.3	321.6
111	-2.4	187.4	186.2	213.4	244.4	285	269.9
112	-3.5	175.3	171.7	174.1	198	216.6	216.3
113	-3	201.6	183.3	218.4	222.2	335.4	326.4
114	5	173.5	190.7	245.3	229.2	293.6	286.2
120	3	197.4	213.2	268.2	247.2	346.9	338.7
121	3.5	188.4	193.9	220.7	196.8	263.6	275.9
122	6.3	143.8	165	171.1	146.9	273.7	290.6
123	-4.5	167.3	160.8	173.6	206.1	231.5	218.2
124	3.8	167.1	189.1	200.3	189.8	267.8	268.5
128	3.7	173.2	176.5	206.7	186	235.6	245.4
130	4.8	155.2	177.7	170.6	166.4	238.2	229.2
136	-3.8	180	177.9	201.4	215.9	243.9	249.7
139	-10.6	179.1	169.3	162.9	263.4	317.8	255.7
144	6.3	167.2	199.3	229.1	217	252.7	231.5
145	-3	186.2	175.7	182.6	202.1	278.5	264.7
149	-4.6	210.7	208.8	241	278.8	280.3	259.3
151	-2.5	187.7	182.1	189.2	207.6	289.8	287.7
152	-9	224.6	182.9	228.3	262.2	286.8	307
154	-12.8	212	147.1	194.6	208.3	204.3	225.9
160	6.8	175.4	185.2	214	159.1	273	330.2
165	7.7	157.4	200.7	219.9	201	262.9	252.9
167	4.4	173.9	177	228.3	209.4	269.3	260.2
173	2.8	186.7	186.1	258.2	218.3	255.3	282.6
177	-5.1	162.3	153.7	179.7	201.8	221.8	228.7
178	-3.1	177.4	173.6	192.1	211.4	273	261.1
182	-3.1	171.3	165.5	183.3	209.2	206.6	187.4

APPENDIX C (continued): PA RAW DATA COLLECTION (ASYMMETRIC PATIENTS - MANDIBULAR MEASUREMENTS)

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
183	6.8	162.7	189.8	223.9	212.3	234.3	236.1
184	-2.2	177.1	177.5	212.8	242.3	247.9	216.8
187	3.3	189.9	189.4	238.7	213.2	311.6	293.9
188	7.9	157.9	193.7	217.9	193.3	266.3	260.4
189	-4.2	171.4	163.3	204	220.6	223.5	223.5
190	3.3	165.3	173.3	219	202.1	255.5	249.6
195	2.7	174.3	189.8	222.1	216.2	229.8	214.3
197	-4.3	170.5	151.8	163.5	174.7	250.3	262.5
199	11.9	141.5	173.7	206	162.2	303.3	243.5
201	4.2	172.1	185.6	207.8	190.2	245.5	231

**APPENDIX D: PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS –
MAXILLARY MEASUREMENTS)**

Subjects	Occlusal Tilt (°)	J-Z R (mm)	J-Z L (mm)
8	NO PA		
11	0.7	225.9	226.6
14	-1.2	256.5	257.4
16	-2	253.6	254.3
17	-0.4	256.4	257.4
18	-0.8	264	264.9
24	-1.5	223.7	225
25	NO PA		
26	NO PA		
27	0.7	269.1	268.1
28	-0.5	242	240.8
29	0.9	266.7	265.2
30	-1.6	287.3	286.1
33	1.2	250.6	249.7
36	1	242.9	243
39	0.7	237.2	237.1
43	0	262	261
45	1.2	238.7	238.5
46	0	258.7	259.4
47	-1	262.7	262.3
49	1.4	230.5	230.2
52	-2	275.2	274.7
55	-0.3	240.2	241.6
59	0.4	229.4	231.1
63	1.1	280	279.4
66	-0.1	254.3	255
67	1.8	307.7	307.6
69	-1	258.6	257.9
70	-0.3	242.8	243.3
71	0.1	280	281.9
72	0.5	230.2	231
73	0.6	258.7	258
76	-1	233.1	233.6
79	-0.1	231.3	230.4
80	0.3	262.2	262.8
81	0.4	246.1	246.7
85	0.1	266.7	266.2
87	-0.4	239.5	239.1
88	-0.1	255.8	256.2

APPENDIX D (continued): PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MAXILLARY MEASUREMENTS)

Subjects	Occlusal Tilt (°)	J-Z R (mm)	J-Z L (mm)
94	-0.4	254.5	255.5
97	0.7	240.6	240.8
103	-1.1	262.1	262.7
107	-1.3	259.4	259.9
108	2.1	296.5	297
116	1.4	231.8	232.5
117	1.3	240.2	240.9
118	0.6	283.3	283.3
119	-0.2	247.8	248.2
126	2	249.3	249.3
127	-0.5	245.5	244
129	-0.5	302	301.6
131	1	248.8	248.8
132	0.1	239	239.3
133	1	245.7	247
135	0	289.8	289.8
138	1	288.6	289.5
140	0.7	224.6	224.7
141	-1.8	249.4	250.6
143	0.3	251.6	251.5
146	1.3	311.5	310.6
147	-1.2	233.8	232.9
148	-1.7	284.6	285
150	1.3	227.3	227.9
153	1.8	288	286.4
155	-0.7	277.8	277.6
159	0.2	278.8	278.1
161	1.7	279.7	280.2
162	-0.7	274.7	274.9
163	0.6	277.2	276.8
164	0.2	247.1	246.2
166	0.6	215.8	216.4
168	0	294	292.9
169	1.4	268.3	268
170	-2	279.1	280.3
171	0.4	266.2	265.7
172	-0.5	244.5	245.3
174	0.1	281.6	281.2
175	-1.9	296.6	296.9

APPENDIX D (continued): PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MAXILLARY MEASUREMENTS)

Subjects	Occlusal Tilt (°)	J-Z R (mm)	J-Z L (mm)
176	-0.6	276.8	275.5
179	-1.1	249	248.7
180	2	242.9	243.4
181	-0.7	289.5	289.4
186	1.1	295.3	295.6
191	-0.3	270.1	271.1
192	-0.6	237.6	236.2
193	1.1	265.8	266.1
194	1.2	240.9	241.9
196	0.3	243	244.3
198	-0.7	264.9	263.8
200	1.4	260.4	260.8

**APPENDIX E: PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS –
MANDIBULAR MEASUREMENTS)**

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
8	0	171.5	173	177.5	179.6	271	271
11	-0.1	179.3	179.5	230.1	229.1	280.5	282.8
14	-0.2	163.3	160.5	190.8	188.9	230	229.9
16	-0.1	164.3	161.7	206.7	208.3	274.8	272.1
17	-0.1	177.8	179.7	231.5	233.2	257.7	255.9
18	-0.1	160.6	158.3	183.2	184.8	208.2	208.4
24	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-
26	-0.3	179.9	178.2	194.3	192.8	243.8	243
27	-0.1	157.2	158.1	147.9	173.4	222.8	224.1
28	0.6	189.3	189.5	211.7	211	269.7	271.1
29	-0.2	202.2	203.5	215.7	214.9	331.2	329.1
30	-0.4	188.2	188.8	201.6	203.5	276.9	276.5
33	-0.1	164.3	166.7	191.4	193.9	255	253.3
36	0.7	163.4	166.8	188	186.4	255.2	258.2
39	-0.5	161.3	161.8	160.7	163.9	265	265.6
43	0	184.2	184.7	220.2	219.4	245	246.8
45	-0.1	178.6	179.7	212.3	214.4	229.4	227.7
46	-0.3	166.6	167.9	168	171	289.8	290
47	-0.1	179.1	179.7	212.2	211.6	270.8	271.2
49	-0.1	186.4	185.2	229.8	229.8	258.6	260.2
52	0.6	158.8	159.6	168.2	167.7	265	263.2
55	0.4	167.8	167.9	183.2	181.4	261.1	263
59	-0.1	191.6	192.8	217	217.4	312.7	314.8
63	0.5	175.2	177.8	199.4	200.7	280.2	279.8
66	-0.9	202.1	199.9	224.7	224.6	321.5	323.5
67	-0.4	179.8	178.1	186.2	187.2	275.3	273.2
69	-0.1	187.9	188.3	191.3	192.3	291.2	289.1
70	0.2	197.3	197.9	215.7	214	319.3	321.6
71	-0.3	169.6	170.7	218	219.2	231.4	234.1
72	-1.2	176.9	179.9	216.7	218.7	269.2	267.7
73	0.2	179.2	178.2	183.2	183.7	300.5	302.1
76	0.9	166.4	167.7	196.1	195.4	185.3	183.7
79	-0.7	186.6	187.8	229.5	230.1	256.9	257.9
80	0.5	166.2	168.8	213.7	212.2	207.4	207.3
81	-0.5	169	166.3	169.8	171.7	310.5	312.1
85	0.5	160.5	162.1	175.2	173.7	281.5	279.1

APPENDIX E (continued): PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MANDIBULAR MEASUREMENTS)

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
87	0.3	173	175.7	177.2	175.3	297.3	299.9
88	-0.1	164.9	166.8	201.2	200.4	251.6	250.3
94	-1	182	181.2	191.8	189	240	242.4
97	-0.4	178.2	180.1	187.9	187.6	235.5	236.8
103	-0.3	174.2	172.7	226.7	224.8	274.7	274.4
107	0	186.7	183.7	215.7	214.2	295.3	294
108	0.4	169.3	169.7	206.1	206.6	243.4	243
116	0.1	175.3	174	176.2	174.3	276.4	273.8
117	-0.2	184.4	185	232.2	232.7	279.5	277.1
118	0.1	169.8	169.2	173.7	174.3	300	302
119	0.5	194	196.9	207.8	207.6	264.3	262.1
126	-0.1	164.6	163.3	164.4	164.3	286.4	287.8
127	0.8	172	174.7	249.2	248.9	244.8	245.2
129	-0.1	156.1	153.9	165.8	166.2	279	279.8
131	0.3	177.9	179.7	192	190	300.9	299.3
132	0.2	175.5	174.6	200.8	200.7	221.8	224.3
133	0.1	199.6	197.8	276.4	276	274.6	273.4
135	-0.1	181.9	181.7	195.6	193.8	339.4	340
138	0	171.8	174.4	175.3	173.3	252.4	253.6
140	-0.2	176.7	179	184.7	186.5	241	243.7
141	-0.4	190.8	191.5	197.2	198.1	272.9	272.1
143	0.5	191	190.1	225	225.8	296	296.7
146	0.2	175.4	173	204.7	202.9	230.4	227.9
147	-0.2	182.8	193.4	214.2	212	238.8	237.3
148	0.5	189.5	190.9	193.9	191.8	221.3	220.8
150	0.2	175.8	176.7	220.7	220.1	289.2	287.8
153	0.1	187.6	185.7	195.5	197.5	283.9	284.7
155	0.1	188.9	189.7	230.6	229.4	282.6	284
159	-0.5	180.5	180.1	193.6	194.8	294.3	297
161	-0.4	181.3	178.6	206.2	206.2	300.5	298.9
162	-0.5	184.2	182.2	199.2	199.3	275.6	274.2
163	-0.1	167.7	166.6	175.2	173.2	260.3	263
164	0	157.8	157.7	205.4	203.3	266.2	265.2
166	-0.1	189.1	187.3	214.6	213	322.3	321.1
168	0	175.4	175	214.3	216.5	251.2	249.4
169	0.1	169.1	170.5	208.8	210.9	267.1	265.6
170	1.6	178.9	176.5	225.8	226.6	274.8	272.6

APPENDIX E (continued): PA RAW DATA COLLECTIONS (SYMMETRIC PATIENTS – MANDIBULAR MEASUREMENTS)

Sub jects	A-Me- MSR (°)	GA- MSR (mm)	AG- MSR (mm)	GA- Menton (mm)	AG- Menton (mm)	Ramal Height R (mm)	Ramal Height L (mm)
171	0.8	156.5	156.1	171.2	171.4	245.3	244.6
172	0.1	184.9	184.3	222.3	220.9	299.2	298.6
174	-0.5	206.9	208.1	256.2	256.3	302	303.6
175	-0.4	173.9	174.8	194	192.8	320.4	320.8
176	0.2	170.3	171.9	173.4	175.9	315	313.5
179	-0.5	173	170	172.3	172.8	276.4	277.7
180	-0.4	197	198.7	207.1	208	324.7	326.6
181	0.1	194	192.9	237.9	235.6	297.7	298.3
186	-0.2	180.7	180.3	200.5	201.2	300.9	299.6
191	-0.1	190	189.4	218.7	221.2	298.2	295.8
192	-0.2	192.1	190.4	222.1	221.6	300.3	301.9
193	0.6	174.3	174.9	202.4	204.2	301.6	300.4
194	-0.5	151.2	150.7	172.3	171	261.5	262.3
196	0.2	161.1	160	171.8	171.9	265.4	264.5
198	0.3	190.5	192.9	200	199.1	331.4	330.9
200	0.3	185.3	183.3	185.0	202.2	324.5	323.4

APPENDIX F: PRE- AND POST-TX JPF SCORE DATA COLLECTION

Patient	Post-tx JPF	Before tx JPF	Asymmetry Classification	Variation score (0=No change; 1=Amelioration; 2 Aggravation; 3=Cured; 4=Iatrogenic TMD)
1	0	0	b	0
2	0	0	b	0
3	6	2	b	4
4	0	2	d	0
5	0	0	d	0
6	0	0	Symmetric	0
9	0	0	b	0
10	0	0	C _{nomaxcant}	0
11	0	9	Symmetric	3
12	0	5	a	1
13	1	5	Symmetric	1
14	1	4	Symmetric	1
15	2	5	a	1
16	0	3	Symmetric	3
17	3	1	Symmetric	0
18	0	2	Symmetric	0
19	2	4	tilted	0
20	0	3	b	1
21	1	3	a	0
22	1	0	Symmetric	0
23	2	1	Symmetric	0
24	0	2	Symmetric	0
25	2	1	Symmetric	0
26	1	2	Symmetric	0
27	5	17	Symmetric	3
28	2	0	Symmetric	2
29	0	0	Symmetric	0
30	3	3	Symmetric	0
31	8	6	b cond resp	0
32	1	1	C _{nomaxcant}	0
33	0	0	Symmetric	0
34	6	14	cnomaxcant	1
35	3	5	b	0
36	1	2	Symmetric	0
37	0	4	Midface deficiency	1
38	0	14	C _{nomaxcant}	3

APPENDIX F (continued): PRE- AND POST-TX JPF SCORE DATA COLLECTION

Patient	Post-tx JPF	Before tx JPF	Asymmetry Classification	Variation score (0=No change; 1=Amelioration; 2 Aggravation; 3=Cured; 4=Iatrogenic TMD)
39	0	1	Symmetric	0
40	2	6	c _{nomaxcant}	3
41	1	3	d	0
42	1	7	c _{nomaxcant}	3
43	0	1	Symmetric	0
44	8	13	c _{nomaxcant}	1
45	0	1	Symmetric	0
46	4	2	Symmetric	0
47	0	0	Symmetric	0
48	0	5	b	1
49	0	9	Symmetric	3
50	9	18	b	1
51	0	1	d	0
52	0	0	Symmetric	0
53	0	0	Symmetric	0
54	10	9	b	0
55	3	0	Symmetric	2
56	8	12	b	1
57	2	4	d	0
58	0	2	b	0
59	0	0	Symmetric	0
60	0	0	Symmetric	0
61	1	10	b	3
62	3	10	b	3
63	0	2	Symmetric	0
64	0	14	b	3
65	1	2	b	0
66	0	6	Symmetric	3
67	0	0	Symmetric	0
68	0	12	b	3
69	0	2	Symmetric	0
70	0	0	Symmetric	0
71	0	0	Symmetric	0
72	12	1	Symmetric	4
73	3	2	No	0
74	8	1	Symmetric	4
75	4	19	b	3

APPENDIX F (continued): PRE- AND POST-TX JPF SCORE DATA COLLECTION

Patient	Post-tx JPF	Before tx JPF	Asymmetry Classification	Variation score (0=No change; 1=Amelioration; 2 Aggravation; 3=Cured; 4=Iatrogenic TMD)
76	1	1	Symmetric	0
77	0	3	Symmetric	1
78	0	6	Symmetric	3
79	1	5	Symmetric	1
80	1	3	Symmetric	0
81	7	3	Symmetric	4
82	2	13	b	3
83	2	0	d	0
84	0	6	c _{nomaxcant}	3
85	1	2	Symmetric	0
86	2	3	d	0
87	1	6	Symmetric	3
88	0	4	Symmetric	1
89	2	12	b	3
90	0	5	c _{nomaxcant}	1
91	5	15	c	3
92	0	16	c _{nomaxcant}	3
93	7	14	b	1
94	0	0	Symmetric	0
95	0	2	Symmetric	0
96	18	7	Symmetric	4
97	4	3	Symmetric	0
98	0	0	Symmetric	0
99	0	1	Symmetric	0
100	1	5	Symmetric	1
101	0	5	c _{nomaxcant}	1
102	0	9	b	3
103	14	3	Symmetric	4
104	1	7	c	3
105	6	7	Symmetric	0
106	3	7	d	3
107	0	0	Symmetric	0
108	0	1	Symmetric	0
109	0	0	Symmetric	0
110	2	0	a	0
111	5	8	c _{nomaxcant}	3
112	0	0	a	0

APPENDIX F (continued): PRE- AND POST-TX JPF SCORE DATA COLLECTION

Patient	Post-tx JPF	Before tx JPF	Asymmetry Classification	Variation score (0=No change; 1=Amelioration; 2 Aggravation; 3=Cured; 4=Iatrogenic TMD)
113	0	12	c	3
114	5	0	b	2
115	5	0	Symmetric	2
116	1	1	Symmetric	0
117	3	0	Symmetric	2
118	3	0	Symmetric	2
119	8	1	Symmetric	4
120	8	3	b	4