THE INFLUENCE OF GENOTYPE AND PERIORAL MUSCULATURE ON MAXILLARY AND MANDIBULAR DEVELOPMENT

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Growth and development of the maxilla and mandible are important in diagnosing and treatment planning orthodontic cases. This study reports significant influences on jaw position in 42 adult patients of the University of Pittsburgh School of Dental Medicine. OBJECTIVE: of the study was to determine whether there is an association between skeletal jaw position and perioral musculature, and if genotype can predict skeletal growth. METHODS: a prospective study on 42 patients over one year was performed. Protocols were followed to ensure HIPAA compliance. The average age of subjects was 28.5 years and included 22 females and 20 males. Lip strength measured by the IOPI system was compared to radiographic cephalometric measurements. Allelic and genotypic frequencies from polymorphisms rs678397 and rs1815739 of gene *ACTN3*, and rs10850110 of gene *MYO1H*, were compared to each variable. Chi–square and Fisher exact calculations determined significance of associations. RESULTS: showed significant differences between rs10850110 genotype and Steiner classification (P = 0.04); between rs678397 genotype and allele frequency and SNA angle (P = 0.01; P = 0.003); between rs1815739 allele frequency and SNA angle (P = 0.01); between rs678397 allele frequency and ANB angle (P = 0.049); between rs678397 genotype and allele frequency and lip strength in females (P = 0.045; P = 0.02); and between rs678397 allele frequency and overall lip strength (P = 0.049), after being corrected for sex.

CONCLUSIONS: The genotype for rs10850110 is associated with a Class III skeletal relationship, and the genotype for polymorphisms rs678397 and rs1815739 are associated with both weak lips and a skeletal Class II phenotype, due to a a protrusive maxilla.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Over the last century, many have attempted to understand the nature of craniofacial growth and development and to determine their influences. If the etiology of growth is known, and contributing factors can be found, it may be possible to influence development. In the Orthodontic field,specifically, understanding the process of growth is important in diagnosing and treating malocclusion. Clinicians and researchers often search for all-encompassing explanations of development, and they have produced several theories to explain the process. Major theories include the genetic theory, the sutural theory, the cartilaginous theory, the functional matrix theory, and the servosystem theory (Phulari 2011).

1.1 THEORIES OF GROWTH AND DEVELOPMENT

Genetic theory was introduced by Allan G. Brodie in 1941. Brodie postulated that growth of the skull was controlled only by genetic factors, and that phenotypic expression was a direct result of an organism's genotype. Sicher developed the sutural theory in 1947, suggesting that craniofacial growth was largely determined by the sutures. As sutures separate, the forces exerted would cause the skull to increase in size. The cartilaginous theory, or nasal septum theory, was suggested by Scott in the 1950s. In this theory, genotype was responsible for the growth of cartilage, which would eventually be replaced by bone and result in craniofacial growth. The nasomaxillary complex was thought to grow mainly due to development of primary cartilage in the nasal septum. Moss's functional matrix theory, introduced in 1962, explained that while neither bone nor cartilage played a significant role in growth, soft tissue did. Moss suggested that genotype alone does not provide enough information to regulate

skeletal development, and epigenetic factors must play a role. In the 1980s, Petrovic et al. began to describe a servosystem theory, or cybernetic model, which combined concepts from several of the previous theories. The servosystem theory relied on feedback loops and growth mechanisms, as well as communication between systems of the body (Phulari 2011).

The merits and shortcomings of each theory have been considered over time. These analyses suggest growth and development are complex processes that cannot be explained by a single determinant, and may be a combination of the above theories. However, if epigenetic factors exist that can, to some extent, be controlled, perhaps alteration of craniofacial growth is possible.

1.2 THE FUNCTIONAL MATRIX HYPOTHESIS

One theory heavily dependent on epigenetic control is the functional matrix hypothesis of Melvin Moss. "The origin, development and maintenance of all skeletal units are secondary, compensatory and mechanically obligatory responses to temporally and operationally prior demands of related functional matrices," wrote Moss (1962). Although he believed that genotype played an important role in skeletal growth, other factors such as muscles, organs, nerves, and even functioning spaces could have an effect (Moss & Young 1960). The functional matrix hypothesis establishes functional cranial components consisting of a periosteal matrix, which carries out a specific function, and a microskeletal unit, which supports the periosteal matrix (Moss 1962).

The temporalis muscle and the lateral pterygoid muscle are two periosteal matrices described by Moss. The temporalis works with its microskeletal unit, the coronoid process of the mandible, to elevate the mandible. As a result of changes in the function of the temporalis, the coronoid process grows, remodeling posteriorly over time. The lateral pterygoid functions to protrude the mandible with its microskeletal unit, the condylar process of the mandible. Changes in the function of the lateral pterygoid result in posterior and superior remodeling of the condylar processes (Moss 1962).

The functional matrix hypothesis has been applied to the effects of masticatory muscles

on hard tissue, and has been studied extensively in various ways, including measuring bite force (Oyen et al. 1991), muscle cross section sizes (Weijs & Hillen 1984), and electromyography activity (Miller & Vargervik 1983). For example, patients with higher electromyography activity in the masticatory muscles tended to have a wider maxilla and a shorter lower anterior facial height than those with lower activity (Ingervall & Thilander 1974; Miralles et al. 1981).

1.3 DENTAL DEVELOPMENT

Soft tissue can also affect dental development. It is generally accepted that forces from the tongue, lips, and cheeks help guide the erupting teeth into their final positions (Tomes 1873). For example, perioral forces can exhibit an effect on incisor inclination (Ballard 1965; Posen 1972; Lowe & Takada 1984; Jung et al. 2003). Upper lip closing force is able to affect the angulation of the upper incisors, while muscle atrophy due to disuse has been labeled an important factor in the development of malocclusion (Jung et al. 2003). More upright or retroclined maxillary central incisors are associated with increased orbicularis oris activity (Lowe & Takada 1984). Similarly, proclination of the maxillary incisors is associated with a tongue thrust habit (Dixit & Shetty 2013). Increased forces from tongue pressure have also been associated with lip incompetency, a mouth-breathing habit, hyperactive mentalis muscle activity, an open bite, and lisping (Dixit & Shetty 2013). Knowledge of soft tissue effects on the dentition can aid in treating patients orthodontically at an early age.

1.4 ORTHODONTIC APPLIANCES

Historically, several orthodontic appliances have been important treatment tools in addition to traditional brackets and wire. A myofunctional appliance is defined as a functional appliance that "harnesses the natural forces and transmits it to the teeth and alveolar bone in a pre-determined direction," and is said to become "active by muscle force" (Alam 2011).

A few important myofunctional appliances used in Orthodontics are the Frankel appliance, a lip bumper, and a tongue crib.

1.4.1 FRANKEL APPLIANCE

A Frankel appliance consists of vestibular shields and labial pads which oppose restrictive muscle forces, theoretically allowing forward and transverse development of the maxilla and maxillary dentition (McNamara & Huge 1985). The vestibular shields extend from the lowest point of the mandibular vestibule to the highest point of the maxillary vestibule, counteracting lateral forces on the maxilla and buccal surfaces of the dentition. If the shields are positioned close to the mandible, but stand away from the maxilla, it is thought that maxillary alveolar growth may be encouraged, while mandibular growth is restricted. Labial pads are thought to allow forward movement of the incisors without opposing pressure from the lips (McNamara & Huge 1985). It has been found that vestibular shields can increase maxillary transverse dimensions (Popovic 1981), and that maxillary intermolar width, mandibular intercanine width, and mandibular intermolar width increase significantly with use of the Frankel appliance (Hamilton et al. 1987).

1.4.2 LIP BUMPER

Another appliance, the lip bumper, is similar to the Frankel and removes labial pressure from the mandibular dentition, alone. By altering the relationship between opposing soft tissue forces of the tongue and lips, dentoalveolar widening and remodeling can occur (Werner et al. 1994). With a lip bumper, arch width increases, incisors procline, and molars distalize (Werner et al. 1994).

1.4.3 TONGUE CRIB

The tongue crib can be used to inhibit forces from the tongue in a tongue thrust habit. Tongue thrust habits are often associated with flared incisors and open bites. Placement of a crib is thought to modify tongue posture, once again altering the balance between opposing

tongue and lip forces. If tongue cribs can help break the tongue thrust habit, it is suggested the treatment stability may be improved (Huang et al. 1990).

If the balance of soft tissue forces can affect dental development, and microskeletal units require a periosteal matrix for development, this raises the question of whether dentoalveolar development can be affected by orthodontic appliances. In Orthodontics, the positions of the maxilla and mandible play an important role in diagnosis and treatment planning. A patient with a well-positioned maxilla and mandible is usually considered to be skeletally Class I in profile, while a protrusive maxilla combined with a retrusive mandible gives the patient a convex, or Class II, appearance. If a protrusive mandible is combined with a retrusive maxilla, this results in a concave profile, or Class III, appearance. Often, skeletal discrepancies are minimal and can be camouflaged by movement of the dentition alone, however, some discrepancies are more severe and must be treated in adulthood with orthognathic surgery. If soft tissue measurements could be used to predict skeletal growth and alter it while the patient is still growing, surgery may not be necessary. There is a need for more effective nonsurgical methods to reduce costs and treatment time and to improve patient acceptance of treatment.

1.5 GENETICS

1.5.1 MALOCCLUSION GENETICS

If associations between perioral musculature and jaw position are significant, it will be beneficial to know which specific genotypes are associated with abnormal musculature and malocclusion. Perioral force and muscle activity during chewing increase with age in growing children (Posen 1972; Ingervall & Thilander 1974), so finding genotypes linked to muscle strength or jaw position may be more reliable than simply examining the soft tissue alone. Craniofacial and dental morphology are influenced by genetic mechanisms, so determining how malocclusion is inherited will improve patient treatment.

Mossey defines heritability as "the proportion of phenotypic variance attributable to the

genotype" (1999a). Because phenotype is often determined by both genetics and environment, knowing how much the genotype contributes to malocclusion helps us understand how well we can predict development. Malocclusion exhibits a pattern of multifactorial inheritance, meaning that multiple genes in different locations, in combination with environmental factors, determine the phenotype (Mossey 1999a). The traditional Mendelian pattern of inheritance is not typically present in family pedigress of multifactorial inheritance. Malocclusion is a continuous trait, so a range of phenotype expression is possible (Mossey 1999a). Significant hereditary variations have been found between fraternal and identical twins in the regions of the anterior cranial base, length of the mandibular body, lower facial height, and total facial height (Horowitz et al. 1960). Hunter found that vertical measurements have a greater genetic component of variability than do anteroposterior measurements (1965).

Genetic heterogeneity is present between different types of malocclusion. Interestingly, a Class II division 2 malocclusion is a completely separate entity from a Class II division 1 malocclusion (Mossey 1999b). Class II division 1 malocclusions are often characterized by flared upper incisors and significant overjet. Class II division 2 malocclusions often display a combination of a Class II skeletal relationship, deep overbite, retroclined maxillary incisors, a high lip line, and an active mentalis muscle (Mossey 1999b). A number of studies have tried to determine the contribution of genetics to the Class III malocclusion. It is thought to have polygenic inheritance (Litton et al. 1970), though the infamous mandibular prognathism in the Hungarian/Austrian monarchy, referred to as the Hapsburg jaw, was determined to be an autosomal dominant trait (Strohmayer 1937). Although many studies have tried to pinpoint the heritability of malocclusion and the specific genes involved, many questions still exist. Two genes that been previously associated with skeletal malocclusion are *MYO1H* and *ACTN3*.

1.5.2 *MYO1H*

Myosins are motor proteins that interact with actin filaments and are dependent on adenosine triphosphate hydrolysis to produce mechanical force (Rowlerson et al. 2005). The *MYO1H* gene encodes a Class I myosin protein that is involved in vesicle transport and cell

motility (Sciote et al. 2013). In gene *MYO1H*, the G allele of marker rs10850110 tends to be overrepresented in subjects with mandibular prognathism (Tassopoulou-Fishell et al. 2012; Sciote et al. 2013; Cruz et al. 2017). Overrepresentation of the G allele at marker rs10850110 has also been associated with more qualitative factors of the Class III phenotype, including Class III molar relationship and negative overjet (Cruz et al. 2017).

1.5.3 *ACTN3*

Alpha–actinins (α–actinins) are myofibril anchor proteins that influence the contraction of skeletal muscles (North et al. 1992). Both α–actinin–2 and α–actinin–3 crosslink actin filaments to dense bodies in the Z disk of the sarcomere during skeletal muscle contraction (Zebrick et al. 2014). While α–actinin–2 is found in both slow-contracting type I and fast-contracting type II muscle fibers, α–actinin–3 is found only in fast-contracting type II fibers (Zebrick et al. 2014). Alpha–actinin–2 is encoded by the *ACTN2* gene, which has been mapped to the long arm of chromosome 1, and alpha–actinin–3, encoded by the *ACTN3* gene, is found on chromosome 11 (Beggs et al. 1992). R557X, a common nonsense mutation in the *ACTN3* gene, occurs when a stop codon is produced at residue 577 (North et al. 1992). The stop codon is only generated when the genotype is homozygous XX, yet it causes the absence of α–actinin–3 in about 18% of Europeans (Zebrick et al. 2014). This lack of α–actinin–3 "diminishes fast contractile ability, enhances endurance performance, and reduces bone mass or mineral density" (Zebrick et al. 2014). Along with affecting fiber type, it may also have an influence on muscle fiber diameter (Swoap et al. 2000; Vincent et al. 2007).

Interestingly, higher frequencies of the 577R allele are found in elite sprinters. Yang et al. reported in 2003 that none of the Olympian sprinters in their study, and very few sprint athletes, had the XX genotype that encodes a stop codon. However, endurance Olympians had the highest percentage of the XX genotype. In another study, α–actinin–3 deficiency was induced in knockout mice, and resulted in improved endurance (Macarthur et al. 2007). It is thought that the deficiency reduces the activity of glycogen phosphorylase and results in a shift in metabolism to more oxidative pathways, allowing endurance to increase. This enhancement has not been consistently found in human studies, but there are differences

between humans and mice in muscle metabolism and contractile properties that may cause the effects to be greater in mice(Berman & North 2010).

The effects of α–actinin—3 on the cranial muscles are another subject of interest. Although limb muscle tends to be highly influenced by training, exercise and environmental factors, cranial muscles show less responsiveness to activity (Miyamoto et al. 1996; Mew 2004; Ahmetov et al. 2012). Despite this lack of response, α–actinin–3 absence results in smaller diameters of fast-contracting type II fibers in the masseter muscles. Its absence is associated with skeletal Class II malocclusions, while its presence is associated with deep bite malocclusions (Zebrick et al. 2014).

Specifically, single nucleotide polymorphisms rs1815739 and rs678397 in the *ACTN3* gene were found by Zebrick et al. (2014) to show statistically significant associations with Class II malocclusions. Polymorphism rs1815739 is a cytosine to thymine transition that converts an arginine to a stop codon at residue 577. Three genotypes are produced: CC, the normal genotype, TC, the heterozygote, and TT, the genotype producing no α-actinin-3. Polymorphism rs678397 is also a cytosine to thymine transition, and it is located in an *ACTN3* gene intron, which has been associated with significant differences in sagittal and vertical malocclusion classifications. It is hypothesized to have some effect on the function of α–actinin–3. It, too, produces three genotypes: CC, TC, and TT (Zebrick et al. 2014). Overall, genotypes reducing the production of α–actinin–3 are underrepresented in deepbite malocclusions, and overrepresented in Class II patients (Zebrick et al. 2014).

1.6 AIMS AND HYPOTHESIS

The aims of this study are: 1) to determine whether there is an association between skeletal jaw position and labial musculature, and 2) to determine if genotype can be used to predict skeletal growth in the maxilla or mandible. Because soft tissue has been shown to influence the final position of the erupting dentition, and Moss's functional matrix theory supports that microskeletal unit development is affected by its periosteal matrix, it seems plausible that the perioral musculature could alter growth of the jaws in a pre-pubertal

patient. Often, patients with protrusive maxillae have a lack of lip seal, so it is reasonable that more protrusive jaws might be associated with weaker lips. Since the *MYO1H* gene has been linked to mandibular prognathism, we would expect there to be an association between rs10850110 and a Class III phenotype, due to a protrusive mandible. Because the *ACTN3* gene has been linked to Class II malocclusion, we would expect there to be an association between both rs1815739 and rs678397 and a Class II phenotype, due to a protrusive maxilla.

2.0 MATERIALS AND METHODS

For this study, we recruited 42 patients that presented to the Orthodontic department at the University of Pittsburgh School of Dental Medicine in Pittsburgh, Pennsylvania from January 2017 to January 2018. Written consent was obtained from each subject before recruitment, and this study was approved by the University of Pittsburgh Institutional Review Board. Subjects were required to be over 18 years of age, have had no previous orthodontic treatment, and have provided a DNA sample, a measurement of lip strength, and a lateral cephalogram radiograph.

2.1 DNA ACQUISITION AND ANALYSIS

Since September 2006, patients seeking treatment at the University of Pittsburgh School of Dental Medicine have been invited to be a part of the Dental Registry and DNA Repository (DRDR). In this study, subjects consented to contribute to the DRDR, and participants were asked to spit, providing unstimulated saliva samples. Saliva was collected and stored in Oragene DNA Self-Collection kits (DNA Genotek Inc.) for processing. They were de-identified and assigned a DRDR number so analysis could be performed blindly. We extracted genomic DNA from saliva samples without centrifugation, according to published protocols (Trevilatto & Line 2000). One sample was lost in processing, so only 41 samples remained for DNA analysis. Spectrophotometry (NanoDrop) was used to determine DNA concentration for each sample, and the samples were diluted to 2 ng DNA/μL with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Genotyping was completed with Taqman chemistry. In this method, Taq polymerase degrades probes that anneal to the DNA, creating a detectable

florescence at that site. Each reaction mixture contained 10 μL TaqMan universal polymerase chain reaction master mix, 0.5 μL TaqMan SNP kit (probe/primer mix), 2 μL DNA, and 7.5 μL DNase-free water in a final volume of 20 μL. Three single nucleotide polymorphism (SNP) probes were selected in candidate genes *MYO1H* and *ACTN3* based on associations confirmed in previous studies (Tassopoulou-Fishell et al. 2012; Zebrick et al. 2014; Cruz et al. 2017). Two negative controls using sterile water as the template were used instead of DNA in the reaction plate. We used end-point analysis, detecting florescence using an Applied Biosystems Quant Studio 6 Flex Real-Time PCR System, and determining genotype with SDS Plate Utility v2.2 software. Amplification conditions were 95°C for 10 minutes, 40 cycles at 92°C for 15 seconds, and 60°C for 40 seconds.

2.2 LIP STRENGTH MEASUREMENT

Lip strength was measured via the Iowa Oral Performance Instrument (IOPI), Model 2.3, for all 42 patients. The system uses a pressure sensor that consists of an air-filled bulb connecting to a force-measuring device. While most published studies using this system have measured tongue force, research on lip strength is more scarce. Patients were asked to close their teeth together, and the bulb was placed inside each patient's right cheek, lateral to the corner of the mouth. They were then asked to press the bulb against their teeth by pursing their lips as hard as they could for about two seconds. Pressure in the bulb is dependent on the strength of orbicularis oris, the circumferential muscle that closes and purses the lips (IOPI Medical 2013) and originates from the maxilla and the mandible. The peak pressure, corresponding with the magnitude of the strength of the lips (IOPI Medical 2013), was recorded in kilopascals (kPa). The maximum pressure was recorded three times for each subject with one minute of rest between each measurement, and the highest of the three values was used for analysis.

2.3 RADIOGRAPHIC ANALYSIS

Maxillary and mandibular positions were determined through digital tracings of lateral cephalograms (Dolphin, Chatsworth, CA). Tracings were completed by one observer (S.E.H.), and intra-examiner agreement was assessed by a second cephalometric tracing done by the same observer at the end of data collection. The mean variation in angle measurement was 0.42°. Tracings were evaluated with the Steiner analysis, using sella, nasion, and A and B points, to determine the positions of the maxilla and the mandible, along with the patients skeletal classification. SNA was recorded as the position of the maxilla, SNB was recorded as the position of the mandible, and ANB was recorded as the discrepancy between the jaws. The ANB value was used to classify the subjects as Steiner Class I, Class II, or Class III in Dolphin. All values were compared with their respective norms provided by Dolphin.

The SNA norm was set at 82°, so values above or equal to it were considered to have a protrusive maxilla tendency, and values below it were considered to have a retrusive maxilla tendency. The SNB norm was set at 80.9°, so values above or equal to it were considered to have a protrusive mandible tendency, and values below it were considered to have a retrusive mandible tendency. The ANB norm was set at 1.6°, so values above or equal to it were considered to have a Class II discrepancy tendency, and values below it were considered to have a Class III discrepancy tendency.

2.4 STATISTICAL ANALYSIS

Although 42 samples were used for lip strength measurements and radiographic analysis, only 41 underwent DNA analysis. Statistics for lip strength measurements and radiographic analysis were analyzed out of 42 samples in order to retain as many data points from the small sample size as possible. However, statistics involving genotype were analyzed with 41 samples. The distribution of sex and ethnicity is shown in Table 2.1. Out of the original 42 samples, the average age was 28.5 years; 22 were female and 20 were male; and 23 were

white, 8 black, 7 Asian, 2 Hispanic, and 2 Indian.

Table 2.1: Distribution of Sex and Ethnicity

	White	Black	Asian	Hispanic	Indian	Total
Male	13	3	3	1	2	20
Female	10	5	4	1	0	22
Total	23	8	7	2	2	42

Lip strength was compared to continuous skeletal measurements (SNA angle, SNB angle, and ANB angle) with simple linear regression. Chi-square or Fisher exact calculations were used to determine associations between genetic markers rs678397, rs1815739, and rs10850110, and Steiner classification, SNA angle, SNB angle, ANB angle, sex, ethnicity, and lip strength. The number of copies of each allele and each genotype per marker were compared for each variable, and an α (i.e., P value) of less than 0.05 was considered statistically significant. For each calculation requiring the Fisher exact test (i.e., fewer than five observed values), the determined significance was supported by chi-square significance, so only chi-square values were recorded.

3.0 RESULTS

3.1 JAW POSITION VS. LIP STRENGTH

In Figure 3.1, we show SNA angle as a function of lip strength, where the solid line is a fit to the values with linear regression. This suggests a significant negative correlation ($P = 0.03$; Table 3.1). Figure 3.2 shows SNB angle as a function of lip strength, and Figure 3.3 shows ANB angle as a function of lip strength. The dispersion of values in both figures suggest no significant association between lip strength and SNB angle or ANB angle (Table 3.1). Although the relationships with lip strength are insignificant, the fitted lines display trends toward negative associations with SNB and ANB.

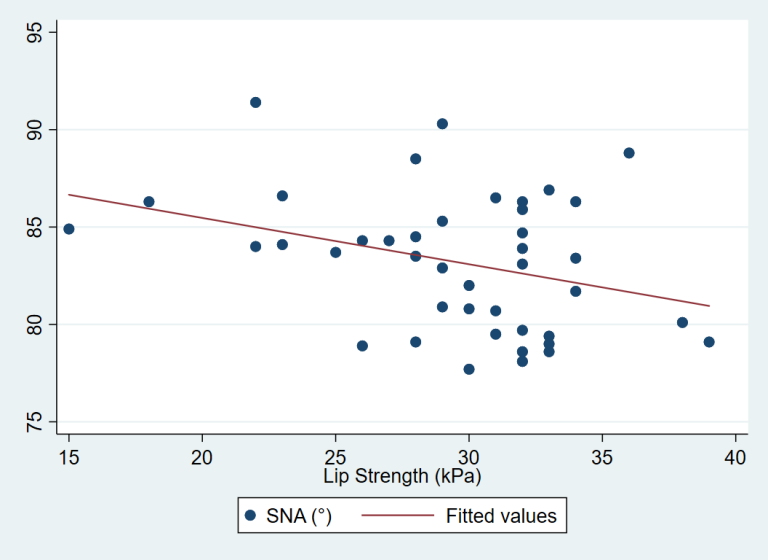

Figure 3.1: SNA vs. Lip Strength.

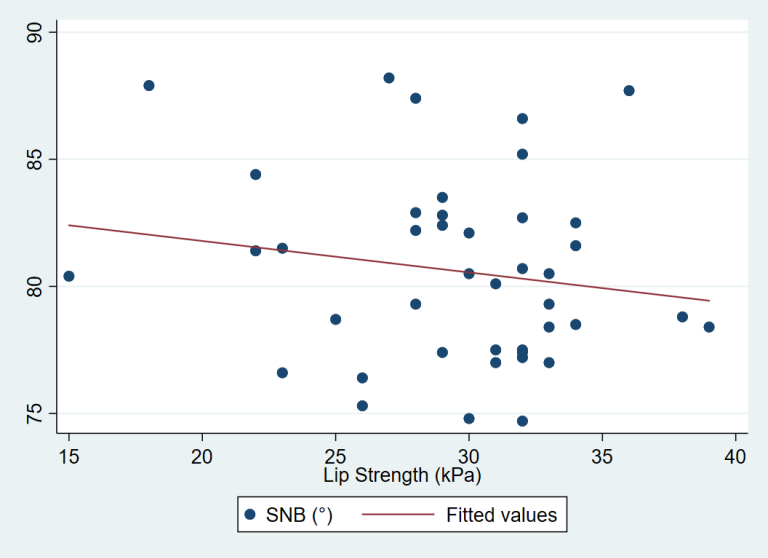

Figure 3.2: SNB vs. Lip Strength.

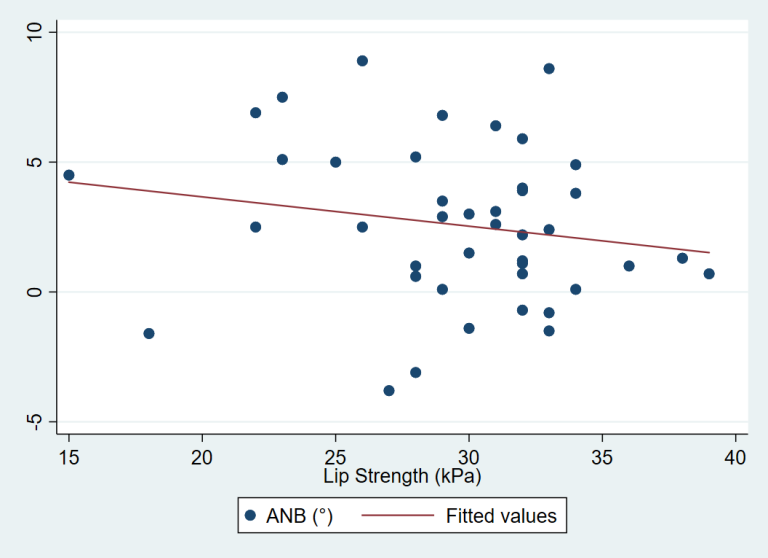

Figure 3.3: ANB vs. Lip Strength.

Table 3.1: Cephalometric Measurements vs. Lip Strength

	P Value
SNA	0.03*
SNB	0.30
ANB	0.25

P values according to simple linear regression.
* P = <0.05.

3.2 STEINER CLASSIFICATION VS. GENOTYPE

In Table 3.2, we list the genotypic breakdown for each skeletal classification, according to the Steiner analysis. In gene *ACTN3*, SNP rs678397 showed an almost significant difference in genotype in Steiner Class II patients (P = 0.09). A greater percentage of Class II patients (57.1%) had a homozygous CC genotype (Table A.1). In gene *MYO1H*, SNP rs10850110 genotypic frequency significantly differed between Class I and Class III patients (P = 0.04). A greater percentage of Class III patients (80%) exhibited the homozygous GG genotype (Table A.1).

Table 3.2: Steiner Classification vs. Genotype

	Class I vs. Class II		**Class I vs. Class III**	
	Genotype	Alleles	Genotype	Alleles
rs678397 (C/T)				
χ^2	4.82	2.01	3.28	1.38
P value	0.09	0.16	0.19	0.24
Odds ratio (95% CI)		0.46 (0.15–1.36)		2.29 (0.57–9.25)
rs1815739 (T/C)				
χ^2	1.92	0.67	3.22	1.83
P value	0.38	0.41	0.20	0.18
Odds ratio (95% CI)		1.55 (0.54–4.47)		0.42 (0.12–1.49)
rs10850110 (A/G)				
χ^2	1.72	0.32	6.27	0.45
P value	0.42	0.57	0.04*	0.50
Odds ratio (95% CI)		0.67 (0.16–2.75)		0.53 (0.08–3.45)

P values according to chi-square analysis.
* P = <0.05.

3.3 SNA ANGLE VS. GENOTYPE

In Table 3.3, we list the results of statistical tests comparing genotype and SNA angle. There were significant differences in genotypic and allelic frequencies for the *ACTN3* markers in patients that had a more protrusive maxilla compared to those with a more retrusive

maxilla. Genotypic and allelic variations for rs678397 were significantly different between the groups (P = 0.01; P = 0.003). A greater percentage (52.4%) of subjects with a high SNA angle had a homozygous CC genotype, while subjects with a low SNA angle tended to have heterozygous (57.1%) or homozygous TT (35.7%) genotypes. The presence of a C allele at rs678397 increased the patient's risk of having a high SNA by 4.5 times. A higher percentage of patients with an SNA angle greater than or equal to 82° (71.4%) had a C allele (Table A.2).

Genotypic variation for rs1815739 showed an almost significant difference between SNA angle groups (P = 0.07). A greater percentage (56%) of subjects with a high SNA angle had the CC genotype (Table A.2). Allelic variation between the two groups was found to be significantly different (P = 0.01), and the presence of a T allele made a patient 0.31 times as likely to have a high SNA.

No significant associations were found between *MYO1H* marker rs10850110 and SNA angle.

Table 3.3: SNA Angle vs. Genotype

	SNA $\geq$ 82° vs. $<$ 82°	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	8.56	8.75
P value	0.01*	0.003*
Odds ratio (95% CI)		4.5 (1.6–12.5)
rs1815739 (T/C)		
χ^2	5.27	6.06
P value	0.07	0.01*
Odds ratio (95% CI)		0.31 (0.118–0.8)
rs10850110 (A/G)		
χ^2	0.25	0.32
P value	0.88	0.57
Odds ratio (95% CI)		0.69 (0.19–2.51)

P values according to chi-square analysis.
* P = <0.05.

3.4 SNB ANGLE VS. GENOTYPE

Table 3.4 shows that no significant differences were found between genotypic or allelic frequency and SNB angle at any of the markers.

Table 3.4: SNB Angle vs. Genotype

	SNB $\geq$ 80.9° vs. $<$ 80.9°	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	0.44	0.18
P value	0.80	0.68
Odds ratio (95% CI)		1.23 (0.47–3.21)
rs1815739 (T/C)		
χ^2	0.35	0.24
P value	0.84	0.62
Odds ratio (95% CI)		0.79 (0.32–2.0)
rs10850110 (A/G)		
χ^2	2.51	1.34
P value	0.28	0.25
Odds ratio (95% CI)		0.44 (0.11–1.81)

P values according to chi-square analysis.
* P = <0.05.

3.5 ANB ANGLE VS. GENOTYPE

Table 3.5 shows that there was no significant difference between genotypic frequency and ANB angle at any of the markers. There was a significant overrepresentation of the C allele in rs678397 (P = 0.049), as well as an almost significant overrepresentation of the C allele in rs1815739 (P = 0.08), in patients with a higher ANB angle. A greater percentage of patients with a large ANB angle had C alleles at rs678397 (66.7%) and rs1815739 (71.7%) (Table A.4). No significant associations were found between rs10850110 and ANB angle.

Table 3.5: ANB Angle vs. Genotype

	ANB $\geq$ 1.6° vs. $<$ 1.6°	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	4.25	3.89
P value	0.12	0.049*
Odds ratio (95% CI)		2.67 (0.995–7.15)
rs1815739 (T/C)		
χ^2	2.93	2.99
P value	0.23	0.08
Odds ratio (95% CI)		0.44 (0.18–1.12)
rs10850110 (A/G)		
χ^2	0.25	0.32
P value	0.88	0.57
Odds ratio (95% CI)		0.69 (0.19–2.51)

P values according to chi-square analysis.
* P = <0.05.

3.6 SEX VS. GENOTYPE

Table 3.6 shows no significant differences between genotypic or allelic frequency and sex for any of the markers. An almost significant difference exists between allele frequency and sex for rs678397 (P = 0.08), with a greater percentage of males exhibiting T alleles (52.8%) and females exhibiting C alleles (67.6%) (Table A.5).

Table 3.6: Sex vs. Genotype

	Male vs. Female	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	2.84	2.98
P value	0.24	0.08
Odds ratio (95% CI)		0.43 (0.16–1.13)
rs1815739 (T/C)		
χ^2	2.53	1.67
P value	0.28	0.20
Odds ratio (95% CI)		1.84 (0.73–4.67)
rs10850110 (A/G)		
χ^2	3.29	1.16
P value	0.19	0.28
Odds ratio (95% CI)		0.49 (0.13–1.83)

P values according to chi-square analysis.
* P = <0.05.

3.7 ETHNICITY VS. GENOTYPE

Table 3.7 shows no significant differences between genotypic or allelic frequency in white patients compared to black patients for any of the markers. An almost significant difference in allelic frequency was present between whites and blacks for marker rs1815739 (P = 0.07). A higher percentage of T alleles were present in whites (36.4%) than in blacks (12.5%) (Table A.6).

Table 3.7: Ethnicity vs. Genotype

	White vs. Black	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	2.78	1.79
P value	0.25	0.18
Odds ratio (95% CI)		0.38 (0.09–1.61)
rs1815739 (T/C)		
χ^2	3.87	3.18
P value	0.14	0.07
Odds ratio (95% CI)		4 (0.80–19.89)
rs10850110 (A/G)		
χ^2	3.57	0.00
P value	0.17	1.00
Odds ratio (95% CI)		1 (0.17–5.77)

P values according to chi-square analysis.
* P = <0.05.

3.8 LIP STRENGTH VS. GENOTYPE

The mean lip strength of the subjects in this study was 30 kPa. Subjects with a lip strength greater than or equal to 30 kPa were considered "strong" and subjects with a lip strength less than 30 kPa were considered "weak." Table 3.8 lists the results of chi–square analysis comparing genotype and lip strength. No significant differences were found between genotypic frequency in patients with strong lips compared to patients with weak lips for rs678397 or rs1815739 (P = 0.17; P = 0.22). However, an almost significant difference was found in allelic frequency for rs678397 between patients with strong lips and patients with weak lips (P = 0.06). While 70% of all alleles were C alleles in the weak lip group, only 47.5% were C alleles in the strong lip group (Table A.7).

Table 3.8: Lip Strength vs. Genotype

	Lip Strength $\geq$ 30 kPa vs. $<$ 30 kPa	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	3.51	3.54
P value	0.17	0.06
Odds ratio (95% CI)		0.39 (0.14–1.05)
rs1815739 (T/C)		
χ^2	3.03	1.20
P value	0.22	0.27
Odds ratio (95% CI)		1.69 (0.66–4.34)
rs10850110 (A/G)		
χ^2	2.51	1.34
P value	0.29	0.25
Odds ratio (95% CI)		2.27 (0.55–9.38)

P values according to chi-square analysis.
* P = <0.05.

A two-sample t-test revealed a significant difference in lip strength between males and females (P = 0.008). Therefore, average lip strength for females was calculated to be 28 kPa, and average lip strength for males was 31 kPa. Females with lip strength greater than or equal to 28 kPa were considered to have strong lips, and females with lip strength less than 28 kPa were considered to have weak lips. Males with lip strength greater than or equal to 31 kPa were placed in the strong lips group, and males with lip strength less than 31 kPa were placed in the weak lips group. Table 3.9 lists the results of chi square analysis of genotype and lip strength by sex. For rs678397, a significant difference was found between genotype and lip strength in females (P = 0.045), as well as between allelic frequency and lip strength in females (P = 0.02). Females with a C allele were 0.14 times as likely to have strong lips than weak lips. A greater percentage of females with strong lips had a higher frequency of T alleles (50%), while only 12.5% of those with weak lips had T alleles at rs678397 (Table A.8). For rs1815739, an almost significant difference was found between allelic frequency and lip strength in females (P = 0.06). In female patients with strong lips, 40.9% of the alleles were T alleles, while only 12.5% of alleles in females with weak lips were T alleles (Table A.8). No significant differences were found between rs10850110 and lip strength in females.

In males, no significant differences were found between genotypic or allelic frequency in subjects with strong lips compared to subjects with weak lips for any of the markers.

Table 3.9: Lip Strength by Sex vs. Genotype

	Female Strong vs. Weak		**Male Strong vs. Weak**	
	Genotype	Alleles	Genotype	Alleles
rs678397 (C/T)				
χ^2	6.19	5.44	1.03	0.06
P value	0.045*	0.02*	0.60	0.81
Odds ratio (95% CI)		0.14 (0.03–0.82)		0.85 (0.21–3.39)
rs1815739 (T/C)				
χ^2	3.98	3.64	1.16	0.01
P value	0.14	0.06	0.56	1.00
Odds ratio (95% CI)		4.85 (0.88–26.74)		0.94 (0.27–3.32)
rs10850110 (A/G)				
χ^2	3.10	0.89	0.61	0.54
P value	0.21	0.35	0.44	0.46
Odds ratio (95% CI)		2.33 (0.39–14.04)		2.37 (0.22–25.14)

P values according to chi-square analysis.
* P = <0.05.

Table 3.10 shows that the overall allelic frequency for rs678397 significantly differed between subjects with strong lips and those with weak lips (P = 0.049). Presence of a C allele made a subject 0.36 times as likely to have strong lips. Patients with weak lips had a greater percentage of C alleles (71.4%) than those with strong lips (47.6%) (Table A.9). No significant differences were found between genotypic or allelic frequencies for rs1815739 or rs10850110.

Table 3.10: Corrected Lip Strength vs. Genotype

	Strong vs. Weak Lips	
	Genotype	Alleles
rs678397 (C/T)		
χ^2	3.65	3.89
P value	0.16	0.049*
Odds ratio (95% CI)		0.36 (0.13–1.01)
rs1815739 (T/C)		
χ^2	2.35	1.52
P value	0.31	0.22
Odds ratio (95% CI)		1.83 (0.70–4.77)
rs10850110 (A/G)		
χ^2	2.51	1.34
P value	0.29	0.25
Odds ratio (95% CI)		2.27 (0.55–9.38)

P values according to chi-square analysis.
* P = <0.05.

4.0 DISCUSSION

The results of this study support findings from previous research regarding select polymorphisms and their associations with jaw development (Tassopoulou-Fishell et al. 2012; Sciote et al. 2013; Zebrick et al. 2014; Cruz et al. 2017). In our study, *ACTN3* polymorphisms rs678397 and rs1815739 were associated with larger SNA and ANB angles, and rs678397 was almost significantly associated with a Class II skeletal phenotype. *MYO1H* SNP at rs10850110 was associated with a Class III skeletal phenotype, although it was not associated with a protrusive mandible. Our hypothesis that jaw position would be associated with lip strength was supported by our findings for the maxilla. Weak lips were significantly associated with a higher SNA angle, or a more protrusive maxilla. Furthermore, in females, weak lips were associated with an overrepresentation of the C allele in rs678397 and rs1815739. Although lip strength was not significantly associated with SNB angle, there was a trend toward a negative association, and future research with a larger sample size is needed.

Our results suggest that an overrepresentation of C alleles for markers in gene *ACTN3* may be related to weaker lips, as well as more protrusive maxillae, larger jaw discrepancies, and a Class II skeletal relationship. We also found that a marker in gene *MYO1H* may be related to a Class III skeletal relationship. Therefore, it is possible that both genotype and soft tissue have an effect on skeletal development of the jaws.

An underrepresentation of T alleles for rs1815739 in deep bite patients, and an overrepresentation in Class II patients, have been established by existing research (Zebrick et al. 2014). In this study, we did not examine the parameters of the vertical dimension of the occlusion, but we did find an association between a Class II phenotype, due to a protrusive maxilla, and an overrepresentation of C alleles. Although Zebrick et al. found that the

skeletal Class II malocclusion was associated with the homozygous TT genotype, and our results seem contradictory, there are notable differences between the populations in their study and ours.

In the study by Zebrick et al., subjects were organized into three groups of skeletal malocclusion. In our study, we not only looked at skeletal malocclusion, but also grouped the patients into high and low measurements for SNA, SNB, and ANB. The patients in Zebrick's study were Class II and Class III orthognathic cases from the University of Lille in France, but the control group was from Pittsburgh, Pennsylvania (Zebrick et al. 2014). All of the patients were of European descent. In our study, the necessity for orthognathic surgery was not examined, so it is possible that some orthognathic cases were present in our sample, but they tend to be more scarce in the general population. Also, our entire sample consisted of patients presenting for treatment at the University of Pittsburgh, and patient ethnicity was representative of the city of Pittsburgh. Allele frequencies between the populations in Pittsburgh, Pennsylvania may be different than the population in France.

It is also possible that the discrepancy is due to the type of Class II malocclusion present. A Class II skeletal discrepancy can be due to a protrusive maxilla, a retrusive mandible, or a combination of the two. There are also two divisions of dental Class II patients. Class II division 1 patients often present with flared incisors and significant overjet, while Class II division 2 patients tend to have retroclined upper incisors and mild overjet. We did not record which type of Class II malocclusion was present, nor did we examine vertical measurements. The number of patients with a protrusive maxilla or the number of Class II division 1 patients in this study may have been different than the amount of patients with a retrusive mandible or Class II division 2 patients. It is possible that only Class II relationships with a protrusive maxilla, a division 1 malocclusion, or an open bite may have been associated with the C allele, while retrusive mandibles, division 2 malocclusions, or deep bite patients were not. Perhaps our data could be combined with that of (Zebrick et al. 2014), and Class II patients with a protrusive maxilla and a C allele could also have had deep bites, strong masseters, and weak lips, while Class II patients with a T allele could have weak masseters, but strong lips.

The homozygous CC genotype is needed for production of α–actinin–3. Alpha–actinin–3

plays a role in the production of fast, powerful type II muscle fiber contraction, and the CC genotype is often found in deep bite patients, as well as sprint and power athletes. Absence of α–actinin–3 is produced by a mutation creating a stop codon at residue 577, and is linked to an overrepresentation of T alleles. The TT genotype has been associated with smaller type II muscle fiber diameters in the masseter muscles, decreased muscle power, and decreased bone mineral density (Zebrick et al. 2014).

Although the CC genotype has been associated with faster and more powerful muscle contractions, in this study, the C allele was associated with weaker lip strength. Masseter muscles are used for mastication and need to provide, quick, strong movements in order to operate effectively, however, orbicularis oris does not provide the same function. Orbicularis oris is a facial expression muscle, primarily used in speech. Regulation of lip movement involves a coordinated, sustained muscle contraction (Burrows et al. 2014) that may not necessarily be influenced by an absence of α–actinin–3. In this case, greater amounts of α–actinin–2 may be preferred for endurance strength. It is also possible that although fast-twitch fiber diameter may be decreased, the number of fibers may be increased (Zebrick et al. 2014).

The relationship between lip strength and position of the maxilla was more significant in females than males. Previously, Yang et al. (2003) suggested that the effect of *ACTN3* on muscle performance was influenced by the sex of the subject. In the study, none of the female sprint athletes had a homozygous TT genotype that would cause α–actinin–3 deficiency, and 57% were heterozygous. Conversely, 29% of female endurance athletes had a homozygous CC genotype. In males, this difference was not found. Yang et al. (2003) suggests that the influence of α–actinin–3 on muscle power may be lower in males because the androgen hormone response to strength training may contribute to performance.

The relationship between the GG genotype at rs10850110 and a Class III skeletal phenotype has also been suggested by previous studies (Tassopoulou-Fishell et al. 2012; Sciote et al. 2013; Cruz et al. 2017). Myosin 1 is a motor protein that produces mechanical force (Cruz et al. 2017), and the G allele marker at rs10850110 is associated with mandibular prognathism (Tassopoulou-Fishell et al. 2012; Sciote et al. 2013; Cruz et al. 2017), as well as the Class III phenotype (Cruz et al. 2017). Because myosins are involved in cell motility,

phagocytosis, and vesicle transport (Rowlerson et al. 2005), it is possible that jaw development may not be strictly dependent on skeletal growth, but that muscular force may be involved.

No association was found between rs10850110 and SNB angle, or mandibular prognathism, in this study. It is possible that there is no relationship, although studies with larger sample sizes have found more significant results (Tassopoulou-Fishell et al. 2012; Sciote et al. 2013; Cruz et al. 2017). Because the sample size in this study was small, and the number of patients with a protrusive mandible was even smaller than the number of patients with a retrusive mandible, this relationship may warrant further research.

Lip strength was not associated with rs10850110, which contradicted our hypothesis. An association was previously discovered between Myosin 1 and mandibular prognathism (Tassopoulou-Fishell et al. 2012), so it is possible that our sample size may not have allowed for detection of a significant result. Another possiblity is that a muscle other than orbicularis oris may have more of an effect on the mandible. A method for testing the strength of muscles closer to the lower lip and chin, such the mentalis, may provide a different result.

The associations discovered in this study between genotype, lip strength, and skeletal development support the idea of Moss's functional matrix hypothesis in 1962. Moss understood that genetic factors played a role in skeletal growth, but proposed that growth was also linked to the underlying muscular matrix (Moss & Young 1960). The sustained forces of facial expression and speech from orbicularis oris may contribute the position of the maxilla. A stronger orbicularis oris may place pressure on the maxilla, limiting the amount of forward growth possible, and weaker perioral musculature may allow the maxilla to continue to grow forward with less resistance. Another possible explanation is that the maxilla influences the strength of the lip, and a more protrusive maxilla results in weaker labial musculature. If the maxilla is so protrusive that the lips cannot adequately seal around it, less force will be produced.

If growth of the maxilla is associated with strength of orbicularis oris, there may be an opportunity to alter its position before patients have completed growth. Normally, altering anteroposterior growth of the jaws is difficult to impossible, depending on the age of the patient and how much skeletal growth is completed. If genotypic information from a

saliva sample allows us to predict skeletal growth, intervention in cases of future skeletal discrepancies is possible.

In the future, knowing if a patient is genetically predisposed to skeletal discrepancies in jaw position may result in improved diagnosis and treatment planning. Patients whose genotypes are associated with a retrusive maxilla may benefit from the use of myofunctional appliances to resist lip pressure. A Frankel appliance with maxillary labial pads, for example, can be used to treat Class II malocclusion, and the maxilla becomes more retrognathic with treatment (Nielsen 1984). Patients susceptible to a protrusive maxilla might be able to reduce the risk through lip strength exercises. Therapy has been shown to improve maximum lip force and lip force endurance in school aged children with myotonic dystrophy type 1 (Sjogreen et al. 2010). The IOPI device used to measure lip strength in this study can be used for exercise therapy, and has been used to improve orofacial muscle strenth in dysphagia patients (Byeon 2016). In general, risk of unfavorable growth may be avoided and reduce the number of patients needing re-treatment if jaw position can be predicted.

Further studies should be conducted in order to confirm the association between the position of the maxilla and lip strength, as well as to determine if another muscle has more of an effect on the position of the mandible. Future research will require a larger sample size with a larger number patients with a Class III phenotype and protrusive mandible to look for associations that may have been missed in our study. Other analyses besides the cephalometric Steiner analysis should be evaluated to classify jaw position and discrepancy. Multiple radiographic analyses, as well a as clinical measurements, such as molar relationship and overjet, may be evaluated to confirm the results of this study. Finally, a CBCT analysis would allow a comparison of muscle volume with skeletal measurements.

5.0 CONCLUSIONS

This study provides evidence that genotype and soft tissue are significantly associated with skeletal phenotype. There was a significant association between the position of the maxilla and the strength of the labial musculature, and associations were found between markers in genes *MYO1H* and *ACTN3* and skeletal measures. SNP rs10850110 of *MYO1H* is associated with a Class III skeletal relationship, and SNPs rs678397 and rs1815739 of *ACTN3* are associated with lip strength, maxillary position, and skeletal classification. These associations may be important in future diagnosis and treatment planning of orthodontic cases to predict skeletal discrepancies and optimize growth modification.

APPENDIX

RAW DATA

Table A.1: Genotype Frequencies per Steiner Classification

Gene	Genotype	Class I	Class II	Class III
ACTN3	rs678397 (C/T)	n (%)	n (%)	n (%)
	CC	3 (20)	8 (57.1)	1 (16.7)
	CT	10 (66.7)	4 (28.6)	2 (33.3)
	TT	2 (13.3)	2 (14.3)	3 (50)
	C allele	16 (53.3)	20 (71.4)	4 (33.3)
	T allele	14 (46.7)	8 (28.6)	8 (66.7)
	rs1815739 (T/C)			
	TT	2 (11.1)	2 (13.3)	3 (42.9)
	TC	9 (50)	4 (26.7)	2 (28.6)
	CC	7 (38.9)	9 (60)	2 (28.6)
	T allele	13 (36.1)	8 (26.7)	8 (57.1)
	C allele	23 (63.9)	22 (73.3)	6 (42.9)
MYO1H	rs10850110 (A/G)			
	AA	0 (0)	1 (6.7)	1 (20)
	AG	4 (23.5)	3 (20)	0 (0)
	GG	13 (76.5)	11 (73.3)	4 (80)
	A allele	4 (11.8)	5 (16.7)	2 (20)
	G allele	30 (88.2)	25 (83.3)	8 (80)

Number and percentage of subjects with genotype or allele in each Steiner classification group.

Table A.2: Genotype Frequencies per SNA Group

Gene	Genotype	SNA $\geq 82°$	SNA $< 82°$
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	11 (52.4)	1 (7.1)
	CT	8 (38.1)	8 (57.1)
	TT	2 (9.5)	5 (35.7)
	C allele	30 (71.4)	10(35.7)
	T allele	12 (28.6)	18 (64.2)
	rs1815739 (T/C)		
	TT	2 (8)	5 (33.3)
	TC	9 (36)	6 (40)
	CC	14 (56)	4 (26.7)
	T allele	13 (26)	16 (53.3)
	C allele	37 (74)	14 (46.7)
MYO1H	rs10850110 (A/G)		
	AA	1 (4.3)	1 (7.1)
	AG	4 (17.4)	3 (21.4)
	GG	18 (78.3)	10 (71.4)
	A allele	6 (13)	5 (17.9)
	G allele	40 (87)	23 (82.1)

Number and percentage of subjects with genotype or allele in each SNA angle group.

Table A.3: Genotype Frequencies per SNB Group

Gene	Genotype	SNB $\geq 80.9°$	SNB $< 80.9°$
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	6 (40)	6 (30)
	CT	6 (40)	10 (50)
	TT	3 (20)	4 (20)
	C allele	18 (60)	22 (55)
	T allele	12 (40)	18(45)
	rs1815739 (T/C)		
	TT	3 (16.7)	4 (18.2)
	TC	6 (33.3)	9 (40.9)
	CC	9 (50)	9 (40.9)
	T allele	12 (33.3)	17 (38.6)
	C allele	24 (66.7)	27 (61.4)
MYO1H	rs10850110 (A/G)		
	AA	0 (0)	2 (9.5)
	AG	3 (18.8)	4 (19)
	GG	13 (81.3)	15 (71.4)
	A allele	3 (9.4)	8 (19)
	G allele	29 (90.6)	34 (81)

Number and percentage of subjects with genotype or allele in each SNB angle group.

Table A.4: Genotype Frequencies per ANB Group

Gene	Genotype	ANB $\geq 1.6°$	ANB $< 1.6°$
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	10 (47.6)	2 (14.3)
	CT	8 (38.1)	8 (57.1)
	TT	3 (14.3)	4 (28.6)
	C allele	28 (66.7)	12 (42.9)
	T allele	14 (33.3)	16 (57.1)
	rs1815739 (T/C)		
	TT	3 (13)	4 (23.5)
	TC	7 (30.4)	8 (47.1)
	CC	13 (56.5)	5 (29.4)
	T allele	13 (28.3)	16 (47.1)
	C allele	33 (71.7)	18 (52.9)
MYO1H	rs10850110 (A/G)		
	AA	1 (4.3)	1 (7.1)
	AG	4 (17.4)	3 (21.4)
	GG	18 (78.3)	10 (71.4)
	A allele	6(13)	5 (17.9)
	G allele	40 (87)	23 (82.1)

Number and percentage of subjects with genotype or allele in each ANB angle group.

Table A.5: Genotype Frequencies per Sex

Gene	Genotype	Male	Female
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	4 (22.2)	8 (47.1)
	CT	9 (50)	7 (41.2)
	TT	5 (27.8)	2 (11.8)
	C allele	17 (47.2)	23 (67.6)
	T allele	19 (52.8)	11 (32.4)
	rs1815739 (T/C)		
	TT	4 (19)	3 (15.8)
	TC	10 (47.6)	5 (26.3)
	CC	7 (33.3)	11 (57.9)
	T allele	18 (42.9)	11 (28.9)
	C allele	24 (57.1)	27 (71.1)
MYO1H	rs10850110 (A/G)		
	AA	0 (0)	2 (11.1)
	AG	4 (21.1)	3 (16.7)
	GG	15 (78.9)	13 (72.2)
	A allele	4 (10.5)	7 (19.4)
	G allele	34 (89.5)	29 (80.6)

Number and percentage of subjects with genotype or allele of each sex.

Table A.6: Genotype Frequencies per Ethnicity

Gene	Genotype	White	Black	Asian	Hispanic	Indian
ACTN3	rs678397 (C/T)	n (%)	n (%)	n (%)	n (%)	n (%)
	CC	6 (33.3)	4 (57.1)	1 (16.7)	1 (50)	0 (0)
	CT	9 (50)	3 (42.9)	4 (66.7)	0 (0)	0 (0)
	TT	3 (16.7)	0 (0)	1 (16.7)	1 (50)	2 (100)
	C allele	21 (58.3)	11(78.6)	6 (50)	2 (50)	0 (0)
	T allele	15 (41.7)	3 (21.4)	6 (50)	2 (50)	4 (100)
	rs1815739 (T/C)					
	TT	3 (13.6)	0 (0)	1 (16.7)	1 (50)	2 (100)
	TC	10 (45.5)	2 (25)	3 (50)	0 (0)	0 (0)
	CC	9 (40.9)	6 (75)	2 (33.3)	1 (50)	0 (0)
	T allele	16 (36.4)	2 (12.5)	5 (41.7)	2 (50)	4 (100)
	C allele	28 (63.6)	14 (87.5)	7 (58.3)	2 (50)	0 (0)
MYO1H	rs10850110 (A/G)					
	AA	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)
	AG	1 (5)	2 (25)	3 (60)	1 (50)	0 (0)
	GG	17 (85)	6 (75)	2 (40)	1 (50)	2 (100)
	A allele	5 (12.5)	2 (12.5)	3 (30)	1 (25)	0 (0)
	G allele	35 (87.5)	14 (87.5)	7 (70)	3 (75)	4 (100)

Number and percentage of subjects with genotype or allele of each ethnicity.

Table A.7: Genotype Frequencies per Lip Strength

Gene	Genotype	Strength $\geq$ 30 kPa	Strength $<$ 30 kPa
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	5 (25)	7 (46.7)
	CT	9 (45)	7 (46.7)
	TT	6 (30)	1 (6.7)
	C allele	19 (47.5)	21 (70)
	T allele	21 (52.5)	9 (30)
	rs1815739 (T/C)		
	TT	6 (26.1)	1 (5.9)
	TC	7 (30.4)	8 (47.1)
	CC	10 (43.5)	8 (47.1)
	T allele	19 (41.3)	10 (29.4)
	C allele	27 (58.7)	24 (70.6)
MYO1H	rs10850110 (A/G)		
	AA	2 (9.5)	0 (0)
	AG	4 (19)	3 (18.8)
	GG	15 (71.4)	13 (81.3)
	A allele	8 (19)	3 (9.4)
	G allele	34 (81)	29 (90.6)

Number and percentage of subjects with genotype or allele in each lip strength group. The mean lip strength in this sample was 30 kPa.

Table A.8: Genotype Frequencies per Lip Strength by Sex

Gene	Genotype	Female $\geq$ 28 kPa	Female $<$ 28 kPa	Male $\geq$ 31 kPa	Male $<$ 31 kPa
ACTN3	rs678397 (C/T)	n (%)	n (%)	n (%)	n (%)
	CC	2 (22.2)	6 (75)	3 (25)	1 (16.7)
	CT	5 (55.6)	2 (25)	5 (41.7)	4 (66.7)
	TT	2 (22.2)	0 (0)	4 (33.3)	1 (16.7)
	C allele	9 (50)	14 (87.5)	11 (45.8)	6 (50)
	T allele	9 (50)	2 (12.5)	13 (54.2)	6 (50)
	rs1815739 (T/C)				
	TT	3 (27.3)	0 (0)	3 (23.1)	1 (12.5)
	TC	3 (23.0)	2 (25)	5 (38.5)	5 (62.5)
	CC	5 (45.5)	6 (75)	5 (38.5)	2 (25)
	T allele	9 (40.9)	2 (12.5)	11 (42.3)	7 (43.8)
	C allele	13 (59.1)	14 (87.5)	15 (57.7)	9 (56.3)
MYO1H	rs10850110 (A/G)				
	AA	2 (20)	0 (0)	0 (0)	0 (0)
	AG	1 (10)	2 (25)	3 (27.3)	1 (12.5)
	GG	7 (70)	6 (75)	8 (72.7)	7 (87.5)
	A allele	5 (25)	2 (12.5)	3 (13.6)	1 (6.3)
	G allele	15 (75)	14 (87.5)	19 (86.4)	15 (93.8)

Number and percentage of subjects with genotype or allele in each lip strength group for each sex. The mean lip strength in females was 28 kPa, and the mean lip strength in males was 31 kPa.

Table A.9: Genotype Frequencies per Corrected Lip Strength

Gene	Genotype	Strong Lips	Weak Lips
ACTN3	rs678397 (C/T)	n (%)	n (%)
	CC	5 (23.8)	7 (50)
	CT	10 (47.6)	6 (42.9)
	TT	6 (28.6)	1 (7.1)
	C allele	20 (47.6)	20 (71.4)
	T allele	22 (52.4)	8 (28.6)
	rs1815739 (T/C)		
	TT	6 (25)	1 (6.3)
	TC	8 (33.3)	7 (43.8)
	CC	10 (41.7)	8 (50)
	T allele	20 (41.7)	9 (28.1)
	C allele	28 (58.3)	23 (71.9)
MYO1H	rs10850110 (A/G)		
	AA	2 (9.5)	0 (0)
	AG	4 (19)	3 (18.8)
	GG	15 (71.4)	13 (81.3)
	A allele	8 (19)	3 (9.4)
	G allele	34 (81)	29 (90.6)

Number and percentage of subjects with genotype or allele in each lip strength group. The strong lips group consists of females with measurements $\geq$28 kPa and males with measurements $\geq$31 kPa. The weak lips group consists of females with measurements $<$28 kPa and males with measurements $<$31 kPa.

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