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THE ASSOCIATION OF ACTN3 rs1815739 POLYMORPHISM WITH MALOCCLUSION PHENOTYPES

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BEYAN

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II. CONTENTS

III. ABBREVIATIONS

- ACTN2: α-actinin-2 gene
- ACTN3: α-actinin-3 gene
- AFH: anterior face height
- AP: Anterioposterior
- ATP: adenosine triphosphate
- BMD: bone mineral density
- DMD: Duchenne muscular dystrophy
- FSHD: facioscapulohumeral muscular dystrophy
- GHR: growth hormone receptor
- HED: hypohidrotic ectodermal dysplasia
- LBM: lean body mass
- NHP: natural head position
- NHANES: national health and nutrition examination survey
- SGP: stress-generated potentials
- SNPs: Single Nucleotide Polymorphisms
- TNF: tumor necrosis factor
- TSALD: tooth size arch length discrepancies
- VME: vertical maxillary excess

IV. FIGURES LIST

V. TABLES LIST

ACTN3 rs1815739 polimorfizminin maloklüzyon fenotipleri ile ilişkisi

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1. ÖZET

Amaç: Alfa aktin-3, kas yapısındaki iskelet ve regülatör yapısında bulunan proteindir. Alfa-Aktin 3 kromozom 11'de bulunan ACTN3 gen bölgesi tarafından kodlanır. Maloklüzyon, hem fenotipik ifadesinde hem de genetik etiyolojisinde karmaşık bir özelliktir ve birkaç gen fenotip ile ilişkilidir. Maloklüzyonlu hastalarda ACTN3 rs1815739 polimorfizmini analiz etmeyi amaçladık.

Gereç ve yöntem: Çalışmamıza 49 hasta katılmıştır. Çalışmaya gönüllü katılan hastalara kendilerinden imzalı bilgilendirme formları alınmıştır. DNA izolasyon swap vasıtası ile bukkal mukoza hücreleri temin edilecek, elde edilen örnekler Temel Tıp Bilimleri Tıbbi Biyoloji ve Genetik laboratuvarına iletileceklerdir. DNA izolasyonunda ticari kit kullanılacaktır (Van Allen Way, Carlsbad, CA, USA). Tüm polimorfizmlerin genotiplemesi gerçek zamanlı PCR (RT-PCR) metodu ile belirlenmiştir. Fisher'in kesin testi, genotipler ve fenotipler arasındaki ilişkilerin istatistiksel önemini test etmek için kullanılmıştır.

Bulgular ve Sonuç: Çalışmamıza katılan 49 bireyde ACTN3 (rs1815739) polimorfizminin RR,RX,XX genotiplerinin sayı ve yüzdeleri sırasıyla 39 (%79.6), 4(%8.2), 6(%12.2) olduğu bulunmuştur. RR genotipleri, normal açıları olan hasta sıklığı, üst ve düşük alt kesici eğim fenotiplerinde azalma, ancak düşük sınıf I fenotip sıklığı ile ilişkiliydi. RX genotipleri, daha yüksek yüksek açılı frekans, artan üst kesici alet eğimi, azalan düşük kesici alet eğim fenotipleri ve sınıf I fenotipleriyle ilişkiliydi. XX genotipleri, daha yüksek sınıf I fenotipleri ve azalmış düşük kesici diş eğim fenotipleri ile ilişkilendirildi. Çalışma grubunda genotip- fenotip olarak istatistiksel olarak anlamlı bir fark bulunmamıştır.

Anahtar kelimeler: ACTN3, polimorfizm, maloklüzyon

The Association of ACTN3 rs1815739 polymorphism with malocclusion phenotypes

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2. SUMMARY

Objective: Alpha actin-3 is the protein that is present in the structure of the skeleton and regulator in muscle structure. Alpha-Actin is encoded by the ACTN3 gene region located on chromosome 11. Malocclusion is a complex trait in both its phenotypic expression and its genetic etiology, and several genes are associated with the phenotype. We aimed to analyze the ACTN3 rs1815739 polymorphism in patients with malocclusion.

Material and methods: 49 patients participated in our study. Signed informative forms were obtained from the volunteers. DNA isolation were done from buccal epithelial cell . Genotyping of all polymorphisms was determined by real-time PCR (RT-PCR) method. Fisher's exact test was used to test for the statistical significance of the associations between the genotypes and the phenotypes.

Results: The numbers and percentages of the RR, RX, XX genotypes of ACTN3 (rs1815739) polymorphism were found to be 39 (%79.6), 4(%8.2), 6(%12.2) respectively. RR genotypes was associated with higher frequency of patients with normal angles, increased upper and decreased lower incisor inclination phenotypes but with lower frequency of Class I phenotypes. RX genotypes were associated with higher frequency of high angles, increased upper incisor inclination, decreased lower incisor inclination phenotypes and Class I phenotypes. XX genotypes were associated with higher frequency of Class I phenotypes and decreased lower incisor inclination phenotypes. None of the above association were statistically significant.

Conclusion: This study shows the possible relationship between upper incisor and lower incisors inclination and skeletal sagital relationship phenotypes and the RR, RX and XX genotypes.

Key words: ACTN3, Polimorphism, Malocclusion

3. INTRODUCTION AND AIMS

Dental and skeletal malocclusions especially bone, teeth, skeletal muscles and other soft tissues is affected by transcription and growth factors combined and additional environmental factors. Our knowledge of the etiology of many of the resulting malocclusion is very limited. The aim of our study is to investigate the potential relationship between three-dimensional malocclusion phenotypes and genes that may play a role in craniofacial development. For this purpose, the ACTN3 rs1815739 polymorphism, which is expressed in muscle cells and its related to muscle structure and has important functions in providing appropriate muscle movements, is examined in adults with malocclusions to determine its clinical effect.

The effect of muscle tissues on the bone structure of the face and therefore malocclusions has been a subject that has been included in textbooks for years (Graber 1963). In studies conducted in the last decade, it has been shown that facial muscles are quite effective on facial morphology (Brotto and Johnson 2014; Saccomanno et al. 2012). By means of attachment, muscle forces are transmitted to the skeletal structure of the face, thereby causing the skeletal structures to undergo structural changes under repetitive forces (Sciote et al. 2013). Therefore, gene variants related to muscle composition and activity have also been studied and have been associated with different craniofacial skeletal phenotypes (Klingenberg et al. 2004). ACTN3 (alpha-actinin-3) is one of these genes. Proteins expressed by these genes are closely related to muscle function. The presence of ACTN3 was found to be associated with strong and rapid contraction in type II muscle fibers (Yang N. et al. 2003). The rs1815739 polymorphism (aka R577X) of the ACTN3 gene has been associated with Class II deep-bite malocclusion (Zebrick et al. 2014).

Despite the increasing number of reports in the literature, no study has attempted to discuss or analyze the distributions of ACTN3 rs1815739 polymorphism in adult individuals with malocclusion in patients treated at Marmara University, Faculty of Dentistry, Department of Orthodontics.Therefore, our study will be useful as self-assessment for practitioners in our department, as it will have positive impact in obtaining ideal orthodontic outcomes.

4. LITERATURE REVIEW

4.1. Malocclusion

Malocclusion is a condition where there is a departure from the normal relation of the teeth to other teeth in the same arch and to teeth in the opposing arch. Keeping in mind there are many variations possible within a state of normality. (White et al., 1976). In another definition, malocclusion is defined as disarrangement of teeth and jaws that may lead to distorted facial appearance, limited masticatory function, increased risk for dental trauma, and compromise quality of life.(Claudino & Traebert, 2013)

The etiology of malocclusion is not clear, and it should be considered as a condition that does not represent a single entity and is indeed developmental. Despite the fact that in specific cases, some factors and pathologies can be defined as being the cause of a malocclusion, in the majority, the etiology of malocclusion is less clear. In each individual, there is a quite close interaction between environment and genetics during the development and growth of both the dentition and jaws.

Orthodontic treatment offers the correction of abnormalities in the face caused by dental or skeletal discrepancies to achieve functional, aesthetic and psychosocial improvements. Abnormalities of the craniofacial complex affect the quality of life and have negative social and psychological effects on an individual. (Zhou et al., 2014)

The classification by Angle is the most commonly accepted to quantify and evaluate malocclusions. Angle suggested that the first upper molars were the key to occlusion and that upper and lower molars had to be correlated in a way that the mesio-buccal segment of the upper molar occludes in the buccal groove of the lower molar. (Proffit et al., 2007)

In a study conducted in central Anatolia on a sample comprised of 2329 teenagers (1125 boys and 1204 girls), aged between 12 and 17 years (mean age: 14.6 yrs) found out the prevalence of malocclusion in relation to gender was about 10.1% of the subjects had normal occlusions, 34.9% of the subjects had Class I

malocclusions, 40.0% had Class II Division 1 malocclusions, 4.7% had Class II Division 2 malocclusions and 10.3% had Class III malocclusions. Over 53.5% had normal overbites, and 18.3%, 14.4%, 5.6%, and 8.2% had increased, reduced, edge-to-edge or anterior open bite values, respectively. Overjet relationship was normal in 58.9%, increased in 25.1%, reversed in 10.4%, and edge-to-edge in 5.6%. A posterior crossbite registered in 9.5% and scissors bite in 0.3%. Anterior crowding was present in 65.2% of the sample and midline diastema in 7.0%. No clear gender differences were noted, except for normal overbite (most frequent in girls, P<.001) and increased overbite (most frequent in boys, P<.05). Class II Division 1 malocclusion is the most prevalent occlusal pattern among the Central Anatolian adolescents and the high values (25.1% and 18.3%) of increased overjet and overbite reflected the high prevalence of Class II malocclusion. Occlusal anteroposterior relationships were assessed using the Angle classification. Other variables examined were overjet, overbite, crowding, midline diastema, posterior crossbite, and scissors bite. (Gelgör et al., 2007)

In another study by Sayin and Turkkahraman to evaluate malocclusion and crowding in 1356 patients in turkey. They concluded that Class I malocclusion was found in 875 patients, which represented 64% of the sample. The frequency of Class II, division 1 and Class II, division 2 malocclusions were 19% and 5%, respectively. Class III malocclusion was present in 12% of the patients.(Sayin & Türkkahraman, 2004)

4.1.1. Class I malocclusion

4.1.1.1. Definition

Class I malocclusion is the most prevalent form of malocclusion, even more common than normal occlusion. Individuals with Class I malocclusion have normal

molar relationships, but their teeth are not correctly positioned in the line of occlusion due to malposed teeth, rotations, spacing, overbites, open bites, posterior cross bites, or even anterior cross bites. (Buschang, 2014)

Two basic measures have been developed to quantify the malalignment that characterizes Class I malocclusion: tooth size arch length discrepancies (TSALD) and the irregularity index. Importantly, these two indices are not measuring the same attributes. Incisor irregularity only explains 25–36% of the variation in TSALD. Because the mandibular incisors have similar mesiodistal and facio- lingual dimensions, incisor rotation can have a substantial impact on incisor irregularity without impacting TSALD. Similarly, displacements of teeth that maintain the space needed for correction will affect irregularity but not TSALD. Due to these differences, incisor irregularity and changes in irregularity over time are usually greater than TSALD and changes in TSALD. TSALD provides a better measure of crowding than incisor irregularity. (E. F. Harris et al., 1987); (H. Peck & Peck, 1972)

4.1.1.2. Prevalence

Class I malocclusion is the most prevalent form of malocclusion, even more prevalent than normal occlusion, and there is no clear consensus concerning its etiology. A study showed that crowding in the upper and lower dental arches in a sample of Turkish population was the most frequent of all anomalies recorded with ranges of 70.0% and 47.3%, respectively. (Buschang, 2014); (Celikoglu, 2015)

An NCHS nationwide survey indicated that approximately 50% of Caucasians and 70% of African Americans 6–11 years of age have Class I buccal segment relationships. The data from the Third National Health and Nutrition Examination Survey (NHANES III), conducted between 1988 and 1991, showed that 45.5% of children of 8–11 years possess irregular lower incisors. (Kelly et al., 1973); (Proffit et al., 1998)

Generalized spacing occurs during the primary dentition, and it appears that maxillary spacing and some degree of mandibular crowding is actually the norm. A large sample of 184 North American white children evaluated in 1965 showed 2–3 mm of maxillary spacing and 1–1.5 mm of mandibular spacing. Based on 526 Iowan children 4–6 years of age evaluated in 2003, the primary maxillary teeth of boys and girls exhibited 2.7 and 1.9 mm of extra space, and the primary mandible teeth exhibited 0.1 and 1.4 mm of space deficiency, respectively. Approximately 58% of the boys and 76% of the girls had some degree of TSALD in the lower jaw 32% of the boys and 42% of the girls had TSALD >2.0 mm. A historical cohort of children from the Iowa Growth Study whose records were collected 50 years earlier showed twice as much maxillary spacing than their present-day counterparts, and 4–4.7 mm more spacing in the mandible. Thus, there appears to have been a secular trend toward increased space deficiencies in both jaws. (Foster et al., 1970); (Warren et al., 2003)

4.1.1.3. Etiology

Space loss or contact displacements cause anterior mandibular malalignment due to teeth erupting into abnormal positions. The anterior teeth move out of alignment when contacts are broken, and they usually move mesially, along with the posterior teeth, until a new equilibrium is established. (Araújo & Buschang, 2016)

Point-to-point interdental contacts is one of the most important causes for anterior teeth malalignment. It simply stands to reason that broader surface contacts are more stable than point-to- point contacts. Simulated arches with teeth that have concave/convex contact surfaces have been shown to be considerably more stable than arches that have point-to- point contacts. (Ihlow et al., 2004)

Another risk factor for crowding is patients with narrower arch forms. Differences in mandibular arch shape explain why there is slightly, but significantly, more (0.8mm) post-retention irregularity in extraction patients than non-extraction patients. The difference was due to the fact that patients who need extractions had

narrower arch forms. Moreover, adjacent teeth that have smaller interdental angles are at greater risk of post-treatment crowding than teeth with larger interdental angles. (Myser et al., 2013)

Transseptal fibers play an important role in the development of crowding. The fibers extend from the cementum of one tooth, over the interdental bone, to the cementum of the adjacent tooth. It acts as a biological splint, linking all of the teeth together, maintaining interdental contacts. In order to maintain tooth contacts, the transseptal fibers serve an important role in moving teeth. Moss and Picton showed that the transseptal fibers were responsible for moving the teeth mesially after space was created. If the occlusal surfaces of the teeth are ground out of occlusion, and their interproximal surfaces are ground to create interdental space, the teeth move mesially to re-establish contact. The mesial drift of the teeth is due to the contractile mechanism of the transseptal ligament. (Cate, 2008); (Moss & Picton, 1982)

Agenter et al. showed that the crown dimensions (mesiodistal and buccolingual) of subjects with malocclusion are consistently larger than in subjects with normal occlusion. Importantly, the statistically significant associations reported by these studies have been consistently low, usually explaining less than 10% of the variation. (Agenter et al., 2009); (Doris et al., 1981)

Early space loss and associated mesial tooth movements provide another explanation for crowding. Early loss of leeway space before the permanent premolars emerge can substantially increase the risk of crowding. It is generally agreed that greater space will be lost with the premature loss of the second deciduous molar than with the premature loss of the other deciduous teeth. (Hinrichsen, 1962)

There also appears that crowding is associated with the sequence of teeth eruption. This occurs because the posterior eruption will determine the way in which leeway space will be used. (Moorrees et al., 1969). Also, when teeth are put in positions of disequilibrium, they will move, and crowding often results. This is why increasing mandibular arch length during the mixed dentition in order to gain the space necessary to relieve crowding results in unacceptable levels of relapse (Little et al., 1990)

Anteriorly directed forces on the teeth can cause contacts to slip and thereby contribute to the malalignment. Due to their axial inclination, the posterior teeth tip forward during occlusal loading, producing an anterior component of biting force. When biting down on the posterior teeth, the anterior component of force decreases progressively as it moves through the more anterior teeth and across to the other side of the arch. (Southard et al., 1989)

4.1.2. Class II malocclusion

4.1.2.1. Definition

According to angle classification, when the mesiobuccal cusp of the lower first permanent molar occludes distal to the class I position is class II malocclusion. When the upper central incisors are proclined and there is increased overjet it considered as Class II division 1 and the condition when class II molar relationship is present with retroclined upper central incisors, upper lateral incisors may be proclined or normally inclined with minimal or increased overjet is known as class II division 2

Distinguishing Class II division 1 from division 2, both characterized by distal occlusion of the teeth in both lateral halves of the lower dentition, indicated by the mesio-distal relations of the first permanent molars, but with retrusion instead of protrusion of the upper incisors in Class II division 2 (E. H. Angle, 1907). From a skeletal perspective, according to Ricketts Class II division 2 subjects are characterized as having brachyfacial patterns with strong musculature. The lower facial height and mandibular arc are under the normal range, that is why the teeth are deep in the basal bone. (Graber, 1984)

Class IIs present with functional deficits. Their masticatory performance (i.e. ability to break down foods) has been reported to be only 60% of normal. Chewed particle sizes of untreated Class IIs are approximately 15% larger than those of subjects

with normal occlusion (English et al., 2002); (Henrikson et al., n.d.). Their inability to break down foods has been directly related to the decreased areas of interdental contact and near contact associated with Class II malocclusion. This explains why Class IIs apply less energy during mastication than Class Is and why Class IIs often experience problems chewing foods. The functional deficiencies are most pronounced in hyperdivergent Class IIs, who have smaller masticatory muscles and weaker bite forces than hypodivergent Class IIs. (Hisano & Soma, 1999); (B Ingervall & Minder, 1997); (Owens et al., 2002)

The convex profiles and retrusive chins that characterize Class IIs are among the least favoured among dental professionals. In fact, excessively convex profiles have been consistently shown to be aesthetically less pleasing than straight profiles. Both dental professionals and lay people believe that changing a patient's profile to be straighter, less retruded, significantly increases attractiveness. It is not just an AP skeletal aesthetic problem. Excessive anterior lower face height is perceived as unattractive by orthodontists and lay people. (Czarnecki et al., 1993); (Maple et al., 2005); (Michiels & Sather, 1994); (Naini et al., 2012a)

4.1.2.2. Prevalence

Large-scale epidemiological surveys conducted in the 1970s reported that bilateral Class II molar relationships decreased from approximately 20.4% in children 6–11 years of age to 14.5% among youths 12–17 years. The prevalence of Class II malocclusion was 3.8 times higher among white than black children, and 2.6 times higher among white than black youths (Kelly et al., 1973). Data collected by the NHANES III showed that the prevalence of Class II malocclusion (defined as overjet >5mm) decreased from 22.5% among 8–11- year-olds to 15.6% among 12–17-year olds, to 13.4% among adults. (Proffit et al., 1998)

Available epidemiological data indicates that approximately 21.5% of children 6–11 and 15% of youths 12–17 years of age might be expected to have bilateral Class II malocclusions. (Araújo & Buschang, 2016)

While these epidemiological surveys did not evaluate Class II skeletal relationships, others have established a correspondence between Class II skeletal and Class II dental relationships. Of the 2000 Class II cases evaluated by Beresford, approximately 74% had corresponding dental and skeletal relationships. Similarly, Milacic and Markovic reported that approximately 75% of the 585 Class IIs they evaluated had corresponding skeletal discrepancies. (JS, 1969); (Milacic & Markovic, 1983)

Assuming that 75% of dental Class IIs have Class II skeletal relationships, approximately 16.1% of children 6–11 and 11.3% of youths 12–17 years of age might be expected to exhibit skeletal discrepancies. (Araújo & Buschang, 2016)

4.1.2.3. Etiology

Class II malocclusions can be found to have variants in one or more of the following regions:

(1) Maxillo-mandibular relationship (mandibular retrognathism, midface protrusion or both)
(2) The cranial base (increased length of the anterior cranial base will contribute to the midface protrusion, while lengthening of the posterior cranial base will tend to position the temporomandibular articulation more retrusively)
(3) Vertical dysplasia (anterior upper face height often greater than normal)
(4) Steep occlusal plane (a reflection of vertical skeletal dysplasia)

(Shaughnessy & Shire, 1988)

Longitudinal studies indicated that Class II dentoskeletal characteristics could appear during the primary dentition. Although catch up growth can occur in some individuals, such discrepancies, in general, do not self-correct due to differences in growth magnitudes and directions between individuals with Class II and Class I malocclusions.(Baccetti et al., 1997); (Stahl et al., 2008)

The habit of sucking found to be in close and positive relation to Class II, division 1 malocclusion .(Hellman, 1922)

Several syndromes have Class II malocclusions as a significant finding. Of these syndromes, Treacher Collins, hemifacial microsomia, achondroplasia, and mobius syndrome are a few of the more widely known, while intra-arch problems also have an environmental component. Looking at the importance of environmental vs. inherited factors in the etiology of malocclusions, it was suggested that urbanization (and evolution) influence malocclusions, making them more severe. The evolutionary factors involved are a decrease in the size of the jaws, size and number of teeth. We have no control over these evolutionary factors whereas the environmental factors can often be eliminated through preventive or interceptive treatment at the appropriate time. (Shaughnessy & Shire, 1988)

4.1.3. Class III malocclusion

4.1.3.1. Definition

Class III malocclusion is defined based on the relationships of the teeth; the entire mandibular dentition occludes mesial to the maxillary dentition and there is an anterior crossbite. Angle initially portrayed Class III malocclusion as having “all the lower teeth occluding mesial to normal the width of one bicuspid, or even more in extreme cases.” He also noted that the lower incisors and canines were inclined lingually and that the skeletal component pertained to the protrusion of the lower jaw.

The skeletal components of Class III malocclusion are what makes it among the most difficult to treat. (Angle E. H., 1899)

Class III malocclusion features affect multiple craniofacial structures, appear early in development, worsen with age, and are present in most Class III individuals regardless of ethnicity. (Jacobson et al., 1974); (Miyajima et al., 1997)

Profiles of individuals with Class III malocclusion, especially those with protrusive mandibles, are considered to be among the most unattractive. Mandibular prognathic profiles were also among the least preferred among the Turkish population. In fact, the mandibular prognathic hypodivergent profile is least preferred by laypeople, orthodontists, and surgeons, even more so than the extreme retrognathic hyperdivergent profile. (Mantzikos, 1998); (Türkkahraman & Gökalp, 2004)

The masticatory function of subjects with Class III malocclusion is often compromised. Individuals with Class III malocclusion are three times more likely to report difficulties chewing firm meats than individuals with normal occlusion. They are not able to break down foods as well as individuals with any other form of occlusion. (English et al., 2002)

The inability of Class IIIs to efficiently break down foods is due primarily to reduced (by approximately 50%) areas of occlusal contact and near contact. Since the number of occlusal contacts and near contacts is closely associated with muscle and bite forces, Class IIIs might be expected to have weaker masticatory muscles. Prognathic surgery patients have been shown to have reduced mechanical advantage of their masticatory muscles. (BAKKE et al., 1990); (Ellis et al., 1996)

4.1.3.2. Prevalence

The prevalence of Class III malocclusion varies widely between populations, with the highest occurrences reported among Chinese and Malaysians. A comprehensive worldwide review found that groups living in southwest Asia exhibit

the highest prevalence (15.8%) of Class III malocclusion, followed by Middle Eastern groups (10.2%), Europeans (4.9%), and Africans (4.6%). (Hardy et al., 2012)

The prevalence of the Class III malocclusion varies greatly among and within different population groups. It shows a relatively low incidence in the Caucasian population, where its incidence has been found to be much higher in Asian. (Emrich et al., 1965); (Foster & Day, 1974); (Haynes, 1970)

Class III malocclusion tended to be slightly more prevalent among males than females; the difference was small (0.2%) but consistent both during childhood and adolescence. (Proffit et al., 1998)

It has been reported to be 1-5% for Caucasians, 9-22.4% for Asians, and 9% for Latins. Moreover, the third National examination survey presented the incidence of Class III malocclusion as 0.8% in white, 2% in black, and 1.6% in the Mexican-American population. (Burgersdijk et al., 1991); (Marthaler et al., 1996); (Proffit et al., 2007)

4.1.3.3. Etiology

The etiology of Class III malocclusion includes non- genetic growth disturbances, as well as genetic factors. Anything that prevents the midface from being displaced anteriorly could produce Class III skeletal and dental relationships. It has been well established that cleft lip/palate surgery produces Class III malocclusion due to midfacial retrusion. children who had unilateral cleft lip and palate closures at 3 and 12 months, had shorter and more posteriorly positioned maxillas than untreated controls. (Dogan et al., 2006)

Habitual anterior posturing of the mandible can also produce Class III malocclusion. Patients who present with anterior crossbite and Class III malocclusion often posture their mandibles forward into centric occlusion. Lower airway disturbances considered to be the major etiological factors explaining Class III

malocclusion. Enlarged tonsils cause children to protrude the tongue and, in turn, the mandible. (E. Angle, 1907)

Subjects with maxillary retrusion could have a genetic predisposition for synostosis of the sutures. Mutations of the fibroblast growth factor signalling molecules have been found in patients diagnosed with Crouzon, Pfeiffer, and Jackson–Weiss craniosynostosis syndrome. These same individuals could also have synostoses of the cirumaxillary and intermaxillary sutures. Premature synostosis of the premaxillary- maxillary, nasal-frontal, and maxillary-palatine sutures has been reported in mouse models of Pfeiffer and Aperts syndromes. (Eswarakumar et al., 2005); (Purushothaman et al., 2011)

Genetic factors also play a dominant role in the development of the chondrocranium. Growth of the synchondroses, like other primary cartilages, is mostly under genetic control. A cross-sectional study of untreated subjects 6–16 years of age showed that the cranial base angle of Class IIIs was approximately 7° smaller than the cranial base angle of Class Is a smaller cranial base angle effectively repositions the maxilla and mandible more posteriorly and anteriorly, respectively. (Reyes et al., 2006)

The nasal septum is also considered to be a growth center that plays a vital role in early midfacial growth displacements. In their classic work, Wexler and Sarnat showed that removal of the nasal septum limited midfacial growth of rabbits. (Wexler & Sarnat, 1961)

Genes that encode specific growth factors or other signalling molecules are involved in condylar growth under mechanical strain. These genes, which include Indian hedgehog homolog (IHH), parathyroid-hormone like hormone (PTHLH), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF), and variations in their levels of expression play an essential role in the etiology of Class III malocclusion. (Xue et al., 2010)

The Class III malocclusion associated with mandibular height and prognathism has been described with the genes ADAMTS1, ARHGAP21, GHR, Matrilin-1,

EPB41, TGFB3, LTBP2, MYO1H, and KAT6B, implying that molecular pathways involved in the development of bone (TGFB3, LTBP, KAT6B) and cartilage (GHR, Matrilin1) may be implicated in mandibular size discrepancy. (Nishio & Huynh, 2016)

4.1.4. High angle vertical discrepancy

4.1.4.1. Definition

Hyperdivergent retrognathic patients are among the most difficult for orthodontists to treat because their malocclusion is multi-faceted and complex. Hyperdivergent retrognathic patients were initially categorized as having vertical dysplasia and have since been called by a variety of names. Most investigators have referred them as skeletal open bites. Schudy was the first to characterize them as hyperdivergent, which more accurately reflects their skeletal phenotype. (Buschang et al., 2013)

Hyperdivergent subjects exhibit both esthetic and functional problems. Orthodontists and laypeople perceive excessive mandibular height(measured from lower lip to menton) as being unattractive. (Naini et al., 2012b)

Most studies that evaluated anterior maxillary height have reported no statistically significant differences between hyperdivergent subjects and normal controls, although a few have found deficits. Posterior maxillary height also does not appear to be affected. Hyperdivergence does not appear to affect the palatal plane angle. Studies consistently show increased anterior and posterior dentoalveolar heights among hyperdivergent subjects. Thus, the primary maxillary problems of hyperdivergent subjects are dentoalveolar rather than skeletal. (Buschang et al., 2013)

Table 4.1.4.1: Terms Adopted to Describe the Various Types of vertical discrepancy

Author	Terms
Wylie and Johnson	High vertical dysplasia
Schudy	Hyperdivergent/hypodivergent
Richardson	Open-bite/deep-bite
Bjork	Forward/backward rotation
Linder-Aronson	Adenoid face
Schendel et al.	Long face syndrome
Opdebeeck and Bell	Short face syndrome
Karlsen	Low-/high-angle face
Betzenberger et al.	High-angle malocclusion

The mandible shows substantially more pronounced and a higher number of differences between untreated hyperdivergent and control subjects than the maxilla. Hyperdivergent subjects have greater anterior face height. While posterior facial height shows no consistent group differences, ramus height has most commonly been reported as being smaller among hyperdivergent subjects. The gonial angle is consistently larger than normal among hyperdivergent subjects. Most studies have also reported retrognathic mandibles and steeper mandibular plane angles among hyperdivergent subjects. While anterior dentoalveolar heights do not appear to be affected, the posterior dentoalveolar height of subjects classified on skeletal criteria tends to be excessive. (Buschang et al., 2013)

The alveolar ridges, especially the mandibular, are smaller among untreated hyperdivergent than hypodivergent subjects. Hyperdivergent subjects have a higher and thinner mandibular symphysis and thinner anterior maxilla than normal and

hypodivergent subjects. Also, hyperdivergent subjects have thinner cortical bone, both in the maxilla and mandible. (Beckmann et al., 1998a); (Horner et al., 2012)

In another study, individuals with the long-face pattern are characterized by long anterior facial dimensions (increased chin-nose distance), short posterior facial dimensions (especially short mandibular ramus length), and a steep mandibular plane angle. It is possible to recognize this pattern in some children at an early age. Although not all individuals who will become long-face adults have this facial pattern prior to puberty. (Proffit & Fields, 1983)

Force of occlusion was compared between normal and long face adults. The data make it understandable that there is a significant difference in the force of occlusal contact between normal and long-face adults. On maximum effort, the normal adults bite two to three times harder. (Proffit et al., 1983)

Schudy was among the first to emphasize the importance of vertical growth for understanding the anteroposterior chin position. More recently, moderate relationships have been reported between the anteroposterior and vertical mandibular changes that occur during growth, suggesting that most individuals who become more hyperdivergent over time also become more retrognathic. (Buschang & Martins, 1998); (Schudy, 1964)

4.1.4.2. Prevalence

While the prevalence of the high angle vertical pattern has not been precisely quantified, many of the subjects with open-bite malocclusions, who have been estimated to comprise approximately 3.5% of the population, might be expected to be hyperdivergent and retrognathic. More importantly, at least half of Class IIs, who comprise approximately 15% of the population, are retrognathic and hyperdivergent. Children with Class II molar relationships show a slightly but not statistically significant greater tendency toward hyperdivergence than Class Is. Based on the

prevalence of open-bite and Class II malocclusions, it can be conservatively estimated that approximately 10%of the population is both retrognathic and hyperdivergent. (Proffit et al., 1998)

Studying a sample which was composed of 5,020 individuals of Brazilian nationality, of both genders, aged 10 years to 16 years and 11 months. The prevalence of long face was 14.06% (Cardoso et al., 2011). Two of the most extensive studies that investigated the prevalence of skeletal facial types were undertaken in the United States and involved the evaluation of a large orthodontic based patient sample. In both studies, the prevalence of the long face pattern was approximately 22%. This extreme form of vertical craniofacial growth was also reported to be the second most common cause for seeking and receiving orthodontic/surgical treatment. (Bailey et al., 2001); (Proffit et al., 1990)

The prevalence of these vertical growth patterns differed significantly according to Angle's classification of malocclusion, with the highest proportion occurring in the Class III sample (35%), followed by Class I (32%), Class II Division 1 (30%) and Division 2 (18%) groups. (Willems et al., 2001)

In another study investigating the distribution of dentofacial deformities in an ethnically diverse Asian population receiving orthognathic surgery and found that the overall prevalence of vertical maxillary excess (VME) was nearly 22%. However, significant differences existed in the distribution of VME among the three Angle classes. The highest prevalence of VME occurred in the Angle Class I (50%) and Class II malocclusions (48%), followed by the Class III group (10%). (Ming, 2006)

4.1.4.3. Etiology

Variations in the long face morphology have so far been discussed in terms of skeletal growth imbalances and mandibular rotations, although there remains a great deal of uncertainty as to what causes or "triggers" these growth patterns. The

multiplicity of growth theories suggests a complex multifactorial etiology that involves genetic, environmental and epigenetic regulation. Several local environmental factors have been implicated in the etiology of the long face morphology, including nasal obstruction, parafunctional habits and weak muscle activity. (Bansal, 2015); (Opdebeeck et al., 1978)

Enlarged adenoids and a narrow nasopharynx are common causes of nasal obstruction that can prompt an individual to become a mouth breather. Theoretically, the downward and forward tongue position needed for oral respiration may also displace the mandible inferiorly and lead to an increase in the vertical dimension. The long face morphology of mouth breathing children may also result from the effects of soft tissue stretching that commonly occur when these individuals overextend their heads to compensate for impaired nasal respiration. (Linder-Aronson, 1970)

Reduced masticatory muscle forces have provided the best explanation for the prevalence of hyperdivergence retrognathic phenotypes. Anthropological studies have consistently shown that the prevalence of malocclusion is much lower for subjects living under primitive conditions than for their counterpart eating processed foods. (Corruccini, 1999)

Weak jaw muscle in humans is directly connected with hyperdivergent growth tendencies. Skeletal hyper divergence has been directly related to reduced muscle size, low EMG activity, and reduced muscle efficiency. (Ueda et al., 1998)

Different heritability estimates have been reported for various vertical dimensions of the face. For instance, the heritability of total face height is reported to range from 0.8 to 1.3, while that of the lower anterior face is between 0.9 and 1.6. In contrast, the heritability of the posterior and upper anterior face height ranges from 0.2 to 0.9 and 0.2 to 0.7, respectively. (Amini & Borzabadi-Farahani, 2009)

4.1.5. Low angle vertical pattern

4.1.5.1. Definition

The short face pattern is determined by facial and skeletal changes that characterize the individual who presents vertical deficiency of the lower third of the face and compressive lip sealing. They present nose breathing and swallow with the mouth closed with no tongue interposition, resulting in intra- and perioral functions. (Beckmann et al., 1998b)

Terms have been introduced to describe extreme vertical facial types, according to what is considered to be the most important clinical manifestation or possible etiologic factor. Faces with reduced lower facial height have been designated either “low-angle type” to express the positive correlation between the SN : MP angle and the reduced lower facial height or “skeletal deep-bite type” to indicate that the lower face height is reduced and associated with deep overbites. Other names take possible etiologic factors into account. “Vertical maxillary deficiency” and ‘idiopathic short face” attribute the reduced lower face height to a reduced maxillary dentoalveolar height, which allows the mandible to rotate in a counter clockwise manner. Hence, the use of another term, “extreme counter clockwise rotation type,” has evolved. (Opdebeeck & Bell, 1978)

The facial form of the deep bite patient is typically characterized by more convergent horizontal facial planes and presents with a reduced lower anterior facial height to total facial height ratio (N-Me). The patient frequently presents with a concave profile, a deep labiomental sulcus, and an over closed appearance. (Nielsen, 1991)

In a study of the facial morphology of three groups of subjects, low angle, average, and high angle, Isaacson et al. found that high angle and low angle subjects had similar upper facial height development. Vertical height as measured from the

palatal plane to the maxillary molars was significantly higher in the high angle subject group when compared to the other two groups, indicating that dentoalveolar eruption is greater in high angle cases. (J. R. Isaacson et al., 1971)

The maxillary molars usually contribute more than the mandibular molars toward an overall increase in lower face height. Schudy contends that the downward movement of the maxillary molar teeth within the facial complex is the most crucial growth factor in reducing the amount of overbite, and the most critical factor in establishing facial height. (Schudy, 1968)

Differences in posterior dental eruption have been attributed to weaker musculature in high angle cases compared to stronger musculature in the low angle cases. (Moller, 1966) A common finding is that the masseter and medial pterygoid muscles have larger cross-sections in those with short lower anterior facial heights and small gonial angles compared to longer-faced individuals. (Kiliaridis & Kälebo, 1991) Möller, Ingervall, and Ingervall and Thilander described the facial shape of individuals with great bite force or with high masticatory muscle activity as rectangular. (B Ingervall, 1976); (Bengt Ingervall & Thilander, 1974) The facial morphology in these individuals was characterized by short anterior face height, anterior inclination of the mandible, convergence of the facial planes and a curved mandible. Ingervall found that strong muscles seemed to bring about forward rotation of the mandible, reducing the height of the lower anterior part of the face. (Ingervall 1978) (B. Ingervall & Helkimo, 1978) In contrast, Van Spronsen et al. reported no significant correlations between either anterior facial height or posterior facial height and jaw elevator cross sectional areas. (van Spronsen et al., 1991)

4.1.5.2. Prevalence

In a study aimed at assessing the prevalence and severity of short face pattern. It was found that short face pattern accounted for 3.15% of individuals of Bauru,

Brazil. White individuals had the highest prevalence than other background ethnics. (Bastos et al., 2017)

A study to evaluate the anterior face height (AFH) of a native African ethnic group detected significant differences among age groups and between the sexes. AFH was higher in men than in women. The post hoc Bonferroni test showed that NPP, NANS, and OrbPP were significantly different at ages less than 22 and more than 25. The older age group had shorter heights than the younger group. The AFH of the Shona was significantly lower than that of African Americans. All AFHs for the men and only TAFH for the women were significantly shorter for the Shona than for the whites. (Dandajena et al., 2006) Another investigation examined facial vertical dimension in an African ethnic group and reported a reduced LAFH compared to White Americans and African-Americans. (Dandajena et al., 2006)

The prevalence of anterior deep bite among the sample of Egyptian orthodontic patients was 65.6%. The majority (80.8%) had mild deepbite, 15% had moderate deep bite and 4.2% had severe deep bite. Skeletal contributing factors were associated with 15% of patients with deep bite. There was no difference in the prevalence of specific skeletal patterns between males and females with deep over bite. (Abuelazayem et al., 2014)

4.1.5.3. Etiology

Vertical malocclusions such as the short face may be due to a combination of etiological factors, such as variability in growth intensity, mandibular soft tissue and muscle function, and dentoalveolar development. Although it is clear that mandibular growth is the most important of these factors, all must be taken into account, including soft-tissue aspects and cephalometric parameters. (Nielsen, 1991)

Some cephalometric studies investigated the association between facial morphology and malocclusion and revealed that hypodivergent individuals with a

closed gonial angle develop deep overbite and are predominantly affected by Angle Class II, Division 2 malocclusions (Siriwat & Jarabak, 1985).

Patients with upward and forward growth of the mandibular condyle often have reduced anterior face height, if they develop malocclusion, its nearly always characterized by deep bite. Patients with an anterior condylar growth pattern usually have a more considerable amount of vertical growth than patients with posteriorly directed growth. (Nielsen, 1991)

4.2. Muscles

4.2.1. Muscles and malocclusion

The masticatory muscular dystrophy can generate malocclusions and craniofacial deformities. Patients with Duchenne muscular dystrophy (DMD) have an increased occurrence of open-bites and cross-bites with their severity aggravate over time. Eckardt L et al. believed that the deterioration of masseter muscles and the enlargement of hypotonic tongue might be the reason for sagittal underdevelopment and transverse overdevelopment of dentomaxillary complex respectively. Another craniofacial deformity could be seen from patients with facioscapulohumeral muscular dystrophy (FSHD), which involves progressive facial muscle atrophy and weakness. Patients with FSHD have narrow arches, high palates and different degrees of malocclusion. (Eckardt & Harzer, 1996); (Kriwalsky et al., 2008); (van den Engel-Hoek et al., 2016)

Severe modifications of cranium, mandible and dental arches were detected when one side of the masseter muscle in growing rats was removed. Veccione L et al. also found morphological changes in skulls and mandibles in myostatin-deficient mouse models, which suggested hypermuscularity significantly correlated with skeletal growth. (Nanda et al., 1967); (Vecchione et al., 2010)

In another study, patients with unilateral posterior crossbite had decreased activity of both temporalis and masseter muscle as compared to those without malocclusion, and the imbalance torque of the two pairs of muscle was responsible for the lateral functional shift of the mandible. Raoul G et al. analyzed the phenotype of masseter muscle in patients with asymmetric mandible and found that the percentage of type II fibers on the jaw deviation side significantly increased than the contralateral side. (Raoul et al., 2011a); (Woźniak et al., 2015)

It was detected that Class III patients have a higher resting temporal muscle activity and their masseter muscle activity increased less when clenching compared with other skeletal types. As for vertical malocclusion types, its association with masticatory muscle activity levels is also unclear. Some researchers found that the activity duration of masseter muscle was remarkably longer in individuals with hypodivergent discrepancy, while some detected no significant difference between individuals with short and long faces. (Bong et al., 2007); (Farella et al., 2005); (Ueda et al., 2000)

4.2.2. Muscles and Bone development

The process of endochondral ossification is an efficient way to build a new bone over a pre-existing cartilaginous template and to allow it to grow in length. However, the main feature of this process also represents its limitation, as it cannot form a shape that is missing from the template or operate in places along the shaft from which the growth plate has already retreated. Hence, the rapid growth and morphological changes that occur during bone development must be brought about by a process other than endochondral ossification. (Sharir et al., 2011)

When considering the mechanisms that underlie the developmental process that shapes bones, of special interest is the question of tissue-autonomous versus non-autonomous regulation, in other words, the possible involvement of signals from other tissues. Muscle-induced mechanical signals provide an example of such an external

contribution. Post-developmentally, there is a large body of evidence that supports the involvement of muscles and the mechanical load that they apply on the bone in determining its 3D morphology. Bones remodel throughout life in order to adapt to their mechanical environment. (Frost, 2001)

Accordingly, increased physical activity in children affects the geometry and composition of their bones, whereas decreased loads due to enforced rest or muscle dysfunctions result in thinner bones. Also, it has been shown that the relationship between bone morphology and muscle force is reciprocal, as the shape of a bone determines the load that it can tolerate (Frost, 2001); (Schoenau, 2005); (Weiner & Wagner, 1998). Furthermore, Astronauts lose both muscle and bone mass due to immobilization and lack of gravity. The recovery of muscle mass is much faster than the bone in astronauts. Several lines of evidence have shown that low-magnitude mechanical signals are anabolic to bone and muscle. (Evans et al., 2013); (Keyak et al., 2009)

During development and growth, a close relationship between bone and muscle is observed. Studies suggest that the Indian Hedgehog pathway and fibroblast growth factor (FGF)-2 may play essential roles in the interactions between muscle and bone during development. The peak velocity for lean body mass (LBM) precedes that of bone mineral density (BMD), indicating that an increase in muscle mass during growth stimulates the increase in bone mass. Circulating insulin-like growth factor (IGF)-I promotes bone mass accrual during puberty, although muscle secretes IGF-I as one of the potential sources. (Bonewald et al., 2013); (Sharir et al., 2011)

Numerous studies indicate that higher LBM is related to increased BMD and reduced fracture risk, especially in postmenopausal women. Age-related sarcopenia is affected by two components for diagnosis: low muscle mass and function. Decreased muscle mass does not often parallel functional disability. Muscle mass and muscle strength were also independently associated with postmenopausal osteoporosis, and they should be considered separately in clinical practice. besides, middle-aged and elderly community-dwelling European men with reduced muscle mass had significantly lower BMD and higher prevalence of osteoporosis. (Ambrose et al., 2015); (Kaji, 2013); (Rikkonen et al., 2012)

According to Kaji, local bone growth is stimulated by stretching of collagen fibers due to an increase in muscle mass. Alternatively, higher blood flow to bone might lead to an increase in bone strength, since blood flows to limbs at a level proportional to muscle mass. (Kaji, 2014)

Clinical studies also suggested that low-intensity vibration signals stimulate bone and muscle formation as well as increase muscle force activity. They stimulate mesenchymal stem cell proliferation and bias their differentiation toward osteoblastogenesis and away from adipogenesis, suggesting that mechanical signals can determine the outcome of the hematopoietic progenitors. (Henrotin, 2011)

Fractures that are covered with relatively intact muscle were found to improve more rapidly than fractures associated with more severe damage. Muscle flaps applied to autogenous bone grafts also improved healing. Proinflammatory cytokines, in particular tumor necrosis factor (TNF)-α, at the site of fracture induced the differentiation of stromal cells present in muscle into osteoprogenitor cells and promoted bone fracture healing. (Glass et al., 2011)

Another study also demonstrated that muscle-derived stem cells take on a primary role in the reparative response in the setting of severe injury to the periosteum. These findings suggest that muscle tissues play essential physiological and pathological roles through specific interactions between muscle tissues and bone metabolis. (Henrotin, 2011)

Bone formation and resorption are sensitive to both external loads arising from gravitational loading as well to internal loads generated by muscular activity. High-frequency mechanical signals may provide benefits at low- (<1g) and high- (>1g) acceleration magnitudes. While the mechanisms by which cells perceive high-frequency signals are mostly unknown, higher magnitude vibrations can cause large muscle loads and may, therefore, be sensed by pathways similar to those associated with exercise. (Judex & Rubin, 2010)

Bones are exposed to thousands of repetitive forces each day. By bone formation or resorption, the skeleton optimizes its architecture by adaptations to these

mechanical loads. Structural changes can be predicted, to some extent, by mathematical formulas derived from three fundamental rules:

(1) bone adaptation is driven by dynamic, rather than static loading.

(2) extending the loading duration has a diminishing effect on further bone adaptation.

(3) bone cells accommodate to a mechanical loading environment, making them less responsive to routine or customary loading signals. (Turner & Pavalko, 1998)

The dose-response relationship between loading frequency and the bone formation response closely resembles the relationship between loading frequency and stress-generated potentials (SGP) within a bone. The qualitative similarity between these two relationships suggests that increased bone formation is associated with increased SGP, which are caused by interstitial fluid flow. Bone cells are known to be sensitive to electric fields and may respond directly to SGP. Also, fluid shear forces have been shown to stimulate bone cells in culture, so it is possible that increased interstitial fluid flow directly affects bone formation. (Turner et al., 1994)

4.3. Cephalometry

The word cephalometrics means the measurement of the head. Radiography eased the measurement of the face and head by the way of lateral and frontal cephalometric analyses. The measurement of the head from the shadows of the bony and soft-tissue landmark on the radiographic image is known as radiographic cephalometry. Moyers defines cephalometrics as a radiographic technique for abstracting the human head into a geometric shape. (Premkumar, 2015)

A lateral cephalogram is a standardised lateral skull view with known magnification that is taken to help orthodontic diagnosis and treatment planning. The first lateral head film was taken by Pacini in 1922, but it was the independent invention of the cephalostat by Broadbent (USA) and Hofrath (Germany) in 1931 that allowed

accurate radiographic measurements to be taken from lateral skull views. The cephalostat is a machine that holds the head in a set position in relation to the X –ray tube and the film. For taking lateral the patient's midsagittal plane is placed vertical and parallel to the film cephalogram and ear rods are placed in the external auditory meatus. Broadbent originally used the Frankfort plane (keeping it parallel to the floor) to orientate the head in the cephalostat and this is still widely used in radiographic departments. Natural head position was suggested by (NHP) Solow and Tallgren (1971) as being a more physiological method, and this is most easily simulated by asking the patient to look into their own eyes in a mirror on the opposite side of the room when taking the cephalogram. The teeth are kept in centric occlusion with lips at rest. (Gill & Naini, 2013); (Solow & Tallgren, 1971)

The cephalostat obviously is one of the most important contributions made so far to the study of growth and development and to the science of orthodontics in general. Since the exact distance between the patient, tube and film is known, it helps to determine the image magnification usually 7-8%. The reproducibility of the head position enables sequential images to be superimposed on each other. This makes the cephalogram of great importance to the orthodontist because it allows growth and treatment effects to be accurately assessed.

Since then, numerous points, planes and analyses were described in order to assess the lateral cephalometric radiographs. However, all analyses rely on the relatively stable elements in the cranial base to serve as reference points and planes by which to measure changing or growing structures. Downs and colleagues presented various cephalometric and soft tissue analyses of facial harmony. Many of these analyses present normative values for different parameters of facial or dental measurement and numerically compare a person's findings with these normative values. (El Kholy et al., 2018)

4.3.1. Uses of cephalometry

The original purpose of cephalometry was to study growth patterns in the craniofacial complex.

- Morphological analysis – sagittal and vertical relationship of dentition, facial skeleton and soft tissue profile. This helps diagnosis especially where there is a skeletal discrepancy or where anteroposterior movement of the incisors is planned. However, it may not be required in some Class I malocclusions and the benefits need to be weighed against the potential damaging effects of ionising radiation. (K. Isaacson & Thom, 2015)
- Growth analysis – growth analysis helps in deciding the treatment timing especially in patient with skeletal III malocclusion. It is done by superimposition of cephalograms taken over a period of time.
- Treatment analysis – a lateral cephalogram can be taken during treatment to assess progress by comparing it to the pre-treatment view
- Assessment of unerupted teeth – used for detecting the position of the impacted teeth.
- Research - post retention records to assess the stability of orthognathic procedures.
- Other – assessment of growth stage (cervical spine maturation); assessment of patients with OSA (airway analysis); identification of pathology. (Gill & Naini, 2013)

When planning orthodontic therapy, it may be desirable to assess the ability of the patient to breathe through the nose. It is therefore, the use of lateral cephalometry is of value to supplement clinical techniques for establishing the mode of breathing. Hence the greatest use of lateral cephalogram as a screening tool for diagnosing obstructed upper airways before more rigorous ear-nose-throat follow up is suggested. (Godhane & Grewal, 2010)

4.4. Genetics

The branch of biology which deals with hereditary and variation, making us understand how life is existing at all levels ranging from the molecular to the population level. Variation in genetics is the root of natural diversity among members of the same and different species. Genes are the center of genetics, which are defined as the unit of heredity. At the molecular level, gene is part of DNA which produce a functional product, which is polypeptide in most genes. A polypeptide is a linear sequence of amino acids that fold into a unit which form proteins. (Brooker, 2017)

Genetic factor is a gene or a specific gene variation affecting characteristic of an individual or the offspring, gene variation maybe simple single change of nucleotide in DNA code at a specific location (i.e., polymorphism), or they can be of amplifications/duplications, insertions, deletions, inversions, and/or transposition of more substantial portions of the DNA code.(Hartsfield & Morford, 2016)

The human genome project provided a better understanding of the genetic aspects of diseases and approaches to prevent, diagnose, and manage diseases. Giving us opportunities to understand the role of genetics for treating, diagnosing, and preventing diseases (Johnson et al., 2008).

Understanding the effect of genetics is essential in diagnosing and treatment planning. When the genetic factor is determined, the dentist can easily distinguish the environmental factor and carrying out the treatment plan according to the etiology. Therefore, it is important to outline the association between orthodontics and genetics. (Cakan et al., 2012)

4.4.1. Gene acquiring methods

Recent research advances in genomic disorders have necessitated the collection of large amounts of suitable quality DNA that needs to be obtained from different sample sources. In a wide variety of genetic studies, the commonly used method is to obtain genomic DNA from nucleated cells of peripheral blood; as a result of the invasiveness of this approach, it could be harder to obtain samples from the subjects. (Hansen et al., 2007)

Another way for collecting DNA samples is the Buccal swab, which is a simple, non-invasive, and reliable source for obtaining genomic DNA. Swabs stored for 12 months at -20°C provide a similar amount of genomic DNA as the freshly collected swabs.(Xu et al., 2012)

Buccal cell collection can be performed easily by a buccal swab with a cotton swab or using a mouth-wash procedure. DNA isolation using buccal swabs provides many advantages, such as cost-effective processing, lower sample volume requirement, long-term archiving, and suitability of self-collection. It is more comfortable for the patient, and the buccal swabs provide sufficient DNA for the PCRs, as they demand only a few nanograms of DNA. (Van Wieren-De Wijer et al., 2009)

Cytology brushes, mouthwash and treated cards for DNA collection were compared and concluded that cytobrush appears to be the most appropriate method with good quality and high security in multicenter studies. On the other hand, another study comparing oral rinse, cytobrush and swab using OrageneTM DNA collection kit to extract DNA showed that oral rinse provided sufficient DNA quantity and quality better than the buccal swab and brush techniques.(Mulot et al., 2005); (Rogers et al., 2007)

Another study by Hansen et al. shows that group of Danish nurses had higher response rate for self-collection saliva samples and buccal cell samples than the response rate of blood samples. However, only the quality of genomic DNA from

saliva samples was comparable with blood samples as accessed by purity, genotyping, and PCR amplification. It was concluded that to increase response rate and obtaining a high quality DNA, the use of saliva samples was an excellent alternative to blood samples in epidemiologic studies. (Hansen et al., 2007)

4.4.2. α-actinin

Humans are defined by inheritance of the more than 20 000 genes. However, variation exists between individual human genomes, including, amongst others, replication of gene sequences (copy number variation, tandem repeats), or changes in individual base pairs (defined as mutations if frequency <1% and Single Nucleotide Polymorphisms (SNPs) if frequency >1%). One of these is the ACTN3 R577X (rs1815739) SNP. The ACTN3 gene is located on the long arm of chromosome 11(11q13-q14) and encodes for the α-actinin-3 protein (K. N. North et al., 1999a) The α-actinins are an ancient family of actin-binding proteins that are believed to have arisen from a single gene prior to the eukaryote-prokaryote divergence (MacArthur & North, 2004). The α-actinin isoforms have been characterised from a large variety of taxa, including protists, invertebrates, birds and mammals (Lek and North, 2010).

Interestingly, this diversity is most marked in mammalian cells where four α-actinin encoding genes (α-actinin-1 to -4) produce different protein products, each with a specific expression profile. The sarcomeric α- actinins, α-actinin-2 (encoded by the ACTN2 gene) and α-actinin-3 (encoded by the ACTN3) evolutionarily are the youngest α-actinins, sharing 90% sequence similarity and are primarily expressed in skeletal muscle, (Beggs et al., 1992). Of these, α-actinin-3 appears to have a more specialised role and pattern of expression, being present only in type 2 fast-twitch skeletal muscle fibers.

4.4.2.1. The functional role of α-actinins

According to evidence, α-actinins have an essential role in the maintenance of muscle structure, contraction, and connection of the sarcomere to the plasma membrane. α- actinins are found at the Z-line, where it anchors actin-containing thin filaments from adjacent sarcomeres. (Salmikangas et al., 1999).

α-actinins also associate with a number of cytoskeletal and signalling molecules, cytoplasmic domains of ion channels and transmembrane receptors, rendering its important structural and regulatory roles in cytoskeleton organization and muscle contraction (MacArthur & North, 2004).

Other roles of α-actinins are interaction with integrin, zyxin, actin, vinculin and filament capping protein Cap Z (Papa et al., 1999). Binding of metabolic enzymes to cytoskeletal proteins is a recognised mechanism of enzyme regulation, and tethering the metabolic enzymes at the sarcomeric Z-disk contributes to the local availability of the metabolites required for energy generation. Also, α-actinins have been shown to be indirectly regulated by calcium levels by forming a signalling complex with calcineurin (Faulkner et al., 2000).

4.4.2.2. ACTN3 Gene

ACTN3 is the gene that encodes for alpha-actinin-3, which is a protein expressed only in type-II muscle fibers. The human sarcomeric α-actinins (ACTN2 and ACTN3) are major structural components of the Z line in skeletal muscle; they play a role in the maintenance of sarcomeric integrity and also interact with a wide variety of structural, signalling and metabolic proteins. ACTN2 is expressed in all muscle fibers, and the expression of ACTN3 is restricted to the type 2 (fast glycolytic) fibers that are responsible for forceful contraction at high velocity. There is a common stop codon polymorphism R577X in the ACTN3 gene. The frequency of homozygosity for the

R577X null-allele, which results in the absence of α-actinin-3 in fast muscle fibers, varies from < 1% in Africans to ~18% in Caucasians.(K. N. North et al., 1999a); (Yang et al., 2009)

Skeletal muscles have two types of muscle fibers, type I (slow twitch and type II (fast twitch). The slow twitch muscle fibers are more efficient at using oxygen to generate more adenosine triphosphate (ATP) fuel for continuous, extended muscle contractions over a long time. They can go for a long time before they fatigue and fire more slowly than fast twitch fibers. Thus, slow twitch fibers are great for athletes who run marathons and bicycle for hours. Fast twitch fibers (type II) create fuel by using anaerobic metabolism, and they are better at generating short bursts of strength or speed. Though, they fatigue more quickly. Both fibers generally produce the same amount of force per contraction, the fast twitch fibers get their name because they are able to fire more rapidly. Having faster twitch fibers will quickly generate a lot of force, therefore, it can be an asset to a sprinter. (Zierath & Hawley, 2004)

The fast twitch muscle fibers can be subdivided into fast twitch muscle Fibers (Type IIa). These fast twitch muscle fibers are also known as intermediate fast-twitch fibers they are a combination of type I and type II muscle fibers since they can use both aerobic and anaerobic metabolism almost equally to create energy. The fast twitch muscle fibers (Type IIb), they are the classic fast twitch fibers since they use anaerobic metabolism to create energy that excel at producing quick, powerful bursts of speed. This muscle fiber has the highest rate of contraction of all the muscle fiber types, but it also has a faster rate of fatigue and can't last as long before it needs rest. (Zierath & Hawley, 2004)

A common stop codon polymorphism in the ACTN3 gene, R577X, was discovered serendipitously while searching for possible causative genes for muscular dystrophy. Individuals who are homozygous for the 577X allele are completely deficient in α-actinin-3. Interestingly, homozygosity for the null polymorphism is very common in humans. The frequency of this null polymorphism varies between human populations, with the 577X allelic frequency being around 0.093 in African populations to 0.764 in the Americas and an average worldwide frequency of 45%. Overall, it is estimated that 20% of the world population, or approximately 1.5 billion

people, are ACTN3 577XX genotype and thus deficient in α-actinin-3. (Amorim et al., 2015); (K. North, 2008); (K. N. North et al., 1999b)

α-Actinin-3 deficient humans, with a shift towards slower muscle properties in the absence of a shift in fiber-type, may have been at a selective advantage due to resistance to cold or famine. The shift towards slower fiber properties and altered adaptation to physical demands also explains the association with athletic performance and muscle function. Powerful muscle contractions required for sprint and strength activities rely on fast muscle fibers, which do not function optimally in the absence of α-actinin-3. (Lee et al., 2016)

4.5. Genetics and Orthodontics

Many orthodontics think of genetic as defining the limits of what can be changed with treatment by controlling and therefore predicting facial growth. They may be limited in what they can do to improve the outcome if the phenotype is genetically programmed (Manfredi et al., 1997). But, this concept has misunderstood when authors misinterpret these estimates for determining whether a phenotype is of “genetic origin”; particularly when a malocclusion or other anatomic morphology exhibits a complex inheritance pattern, or etiology, as most phenotypes do. (Shroff, 2016)

Malocclusions have a complex or multifactorial pattern of inheritance, where more than one gene is involved in the development of the phenotype. There is also the possibility that the environment influences malocclusions. (Vieira, 2019)

Skeletal structure development is partly under genetic control and partly under environmental factors. Therefore, the importance of the genetic impact on malocclusion can't be denied. The polygenic nature of craniofacial traits makes the understanding of the genetic component of most malocclusions and dental anomalies very challenging. Data provided by the human genome project have made it feasible

to map inherited conditions related to dentofacial development. However, further genetic studies are required. (Cakan et al., 2012)

4.5.1. Genetics of malocclusion

In theory, there are two main ways that genetics can cause malocclusion. The first main way is by inheriting disproportion between teeth and jaws sizes and the second is the inheritance of a disproportion in the position, size, or shape of the mandible and maxilla. (Hartsfield, 2016)

The inheritance of a disproportion between the size of the teeth and the jaws results in crowding or spacing issues. Posterior and anterior Bolton discrepancies are defined as a disproportion among the sizes of individual teeth between the two arches. To achieve good occlusion with the ideal overbite and overjet, the maxillary and mandibular teeth must be proportional in their mesial-distal dimensions. If some teeth are not proportional, then there could be crowding, spacing issues, and/or an effect on overbite and or overjet, even if the arches are in a favourable relationship. Certainly hypodontia, as well as the often-associated smaller size of teeth that are often agenic, or other teeth, can contribute to malocclusion. This is particularly evident in patients with hypodontia or even oligodontia, which may occur spontaneously or be part of a syndrome such as hypohidrotic ectodermal dysplasia, which can be caused by mutations in the EDA gene. (Hartsfield, 2016)

The other way is the inheritance of a disproportion in the position, size, or shape of the mandible and maxilla. Bony morphology may be influenced secondary by heritable effects, including those on cartilage, collagen, muscle fibers, other connective tissue components, and growth factors. (Hartsfield, 2016)

Variability in expression of malocclusion is caused by interaction of genetic and environmental factors. The etiological complexity lies not only in unpredictable expression but also in the wide spectrum of dentofacial variation present in affected

individuals. This complexity justifies why most treatments of malocclusion are focused on the symptoms rather than to etiology. However, to understand the biology underlying craniofacial growth and dental relations, it is fundamental to study etiology of malocclusion. Understanding the biology will aid progress toward effective treatment, prevention, thereby decreasing the burden of this condition. (Moreno Uribe & Miller, 2015)

Genetic factors contribution to malocclusion susceptibility is suggested by multiple data sources. many dental and facial features reported to have moderate to high heritability proportions (>60%) such as mid and lower facial dimensions, dental spacing, arch dimensions and Bolton type tooth size discrepancies. Contrariwise, overbite (53%) and overjet (28%) have lower heritability, which suggests they are highly susceptibility to environmental factors. (Naini & Moss, 2004); (Townsend et al., 2009)

In a study conducted in normal Japanese population consisting of 50 men and 50 women which aimed to study the association of the Pro561Thr (P56IT) variant in the growth hormone receptor (GHR) gene with craniofacial measurements on lateral cephalometric radiographs, it was found that individuals without the GHR P56IT allele had a significantly greater mandibular ramus length (condylion-gonion) than did those with the GHR P56IT allele. (Yamaguchi et al., 2001)

In a study of twins with class II div. 2 showed that monozygotic twins displayed high concordance within malocclusion features, while dizygotic twins showed 90% discordance in these features. Also, in a study by Mosey showed strong genetic influence in patients with Class II division 2 malocclusion (Mossey, 1999); (S. Peck et al., 1998).

Familial aggregation studies suggested that Class III pedigrees have an autosomal dominant model with incomplete penetrance, including the royal Habsburg family. But polygenic inheritance and autosomal dominance models, with incomplete penetrance and variable expressivity, have been suggested for Class II subdivision 1 and 2, respectively. (J. E. Harris, 1975); (Morrison et al., 2008); (Wolff et al., 1993)

In another study, a high correlation was found between parents and their offspring in Class II and Class III population. Thus, suggesting a strong familial tendency in the development of the malocclusions (Nakasima et al., 1982). Also, when the families of fifty-one probands having Class III malocclusion were examined, around 13% of the siblings exhibited the trait showing Class III malocclusion has a strong genetic influence (Litton et al., 1970).

Patients with craniofacial birth defects are frequently presented with malocclusion suggesting a strong genetic etiology. About 150 genes/loci are associated with craniofacial conditions presenting malocclusion. (Moreno Uribe & Miller, 2015)

Association studies suggest positive associations for mandibular prognathism and genes EPB41, MATN1, SSX2IP, and PLXNA, located within the 1p22-p36 locus. Also, positive associations have been found for genes COL2A1, MYO1H, TGFB3, and LTBP2 within the 12q13-q24 locus. Also, variants in Matn1 (1p35) were associated with mandibular prognathism and anterior cross bites in donkey. (Moreno Uribe & Miller, 2015); (Rodrigues et al., 2013)

Also, in Class I Chinese subjects a significant associations were reported between crowding of more than 5 mm and genes EDA (rs3764746 and rs3795170), XEDAR (rs372024), and BMP2 (rs1005464). (Ting et al., 2011)

Generally, there are fewer genetic studies for Class II and Class I malocclusion than Class III. In a study of four Colombian families, individuals with mandibular hypoplasia were homozygous for the rare allele on SNP rs1348322, within the Noggin gene. This gene is essential for mandibular formation in mice. For Class I malocclusion, delayed tooth eruption and occlusion irregularities that required orthodontic therapy were associated with a SNP rs6504340 within the HOXB cluster. (Gutiérrez et al., 2010); (Pillas et al., 2010)

4.5.2. Actn3 polymorphism and malocclusion

A common polymorphism in the ACTN3 gene is R577X (rs1815739), where a C-to-T base substitution results in the transformation of an arginine base (R) to a premature stop codon (X). X allele homozygotes are deficient in the alpha-actinin-3 protein, which is associated with a lower fast-twitch fiber percentage but does not result in disease. (MacArthur & North, 2004); (Vincent et al., 2007)

According to the functional matrix theory of craniofacial growth, the skeletal development is secondary to muscle function, airway requirement and other causes extrinsic to the bone. So, according to that theory, genes that have an effect on the muscle will contribute to skeletal development. (Melvin L. Moss, 1987)

Variations in masseter muscle fiber type, gene expression in the masseter muscle, and epigenetic changes that alter gene expression are associated with anterior open versus deep bites, mandibular retrognathism versus prognathism, and mandibular asymmetry. (Huh et al., 2013); (Sciote et al., 2013)

Genetic factors determine about 45% of the muscle fiber type proportions. The ACTN3 variant, through its interaction with calcineurin, could be one of the contributing genes in the heritability of fiber type distribution. (Vincent et al., 2007)

Elite sprint athletes had significantly higher frequencies of the R allele than controls, showing ACTN3 genotype is associated with speed and power phenotypes, a finding that has been replicated multiple times in speed, power and strength athletes. (Yang et al., 2003)

Investigating fiber composition in master muscle tissue from patients of mandibular asymmetry showed difference in muscle fiber composition. Significant increase in fast muscle fibers type II area and frequency on the same side as the deviation were discovered as compared to muscle fiber in the opposite side. When the right and left sides of symmetrical patients were compared, no significant differences were noted. (Raoul et al., 2011b)

ACTN3 577XX is underrepresented in subjects with deep bite malocclusion, suggesting that muscle differences contribute to variations in vertical facial dimensions (Zebrick et al., 2014a). in another study, it was concluded that genetic variant rs1815739 (R577X) is involved in vertical malocclusions, in which the TT genotype (that results in the impairment of the actinin-3 protein) is overrepresented in dolichofacial phenotype while the CC genotype is overexpressed in brachyfacial group of patients that require orthognathic surgery. (Cunha et al., 2019)

Rowlerson et al. showed that vertical bite characteristics vary according to the fiber type composition of masseter muscle. Type I fiber occupancy increased in open bites, and conversely, type II fiber occupancy increased in deep bites. (Rowlerson et al., 2005)

Among Class III cases, differences were observed in the average fiber area when comparing normal, open bite and deep bite cases. Class III deep bite cases showed an increased amount of type I and hybrid type I/II muscle fiber areas in the masseter muscle, compared to normal and open bite cases. (Hartsfield et al., 2017)

In contrast to the muscle fiber variations observed in vertical dimension malocclusions, muscle fiber compositions varied to a lesser degree in malocclusions affecting the sagittal dimension. Interestingly, when the α-actinin-3 gene (ACTN3) alleles were homozygous for the polypeptide to stop being made at the 577th amino acid (577Stop/Stop), the patient was more likely to have a Class II malocclusion, while this genetic variation was less likely to be found in patients with a deep bite. (Rowlerson et al., 2005); (Zebrick et al., 2014a)

A study conducted by Zebrick et al. to examine ACTN3 expression and genetic variation in the masseter muscle, which was composed of sixty patients (42 females and 18 males) undergoing orthognathic surgery from the University of Lille also thirty-one nontreated Class I subjects were included. They organized the subjects into one of six craniofacial morphological groups that include one variation of vertical skeletal jaw malocclusion, open, deep, normal bite relation, and one variant of sagittal relationship Class II, Class III. DNA extracted from saliva samples, and they also collected muscle samples from the deep area of the masseter muscle.

Two SNP in ACTN3 were selected for genotyping on all subjects rs1815739 (the R577X SNP) and rs678397 then tested to determine if specific allelic variants are over presented in subjects with malocclusion. They used chi-square or fisher's exact test to asses Hardy-Weinberg equilibrium and compare alleles and genotype distribution between individuals with malocclusions and non treated Class I control subjects.

R577X genotype frequencies for the entire malocclusion group was 14% CC, 63% TC, 23% TT. The group was composed of 18 Class II open bite, 7 Class III open bite, 13 Class II deep bite, 6 Class III deep bite, 12 Class II normal bite, 4 Class III normal bite malocclusion.

They compared SNP frequency for Cl II and Class III to Class I and secondly between open, normal and deep bite. No significant allele frequency differences were found between cases and controls in the 31 anonymous SNPs genotyped, and they proceeded to test differences in ACTN3 genotypes between cases and controls. There were no divergences from the Hardy-Weinberg equilibrium in the samples and no statistically significant differences between cases and controls considering age and sex. Chi-square tests revealed very significant differences between Class II malocclusion vs. controls for genotypes and alleles at rs1815739 and rs678397. There were no significant differences in Class III malocclusion. In vertical dimension comparisons, there were highly significant differences for genotypes and alleles at rs678397. For rs1815739, the TT and CC genotypes were underrepresented in subjects with deep bite malocclusion.

They also assayed mRNA levels for ACTN 2 and ACTN3 to determine if gene expression levels differed from the SNP. Furthermore, they compared the difference in fiber type properties from masseter muscle to determine how normal or absent of actininn -3 protein expression affected muscle phenotype

In conclusion, they found that Two SNPs in ACTN3 at rs1815739 and rs678397 had statistically significant differences between Class II malocclusion and controls. These SNPs had no significant association with Class III malocclusion, which may be the result of limited numbers of Class III subjects in the study. These findings suggest

that bone growth may be altered with the ACTN3 genotype for Class II subjects in our population. (Zebrick et al., 2014b)

In another cross-sectional study by Cunha et al. to evaluate the association of genetic variants in ACTN3 and MYO1H with craniofacial skeletal pattern. The sample was composed of 646 patients in Brazil. Phenotype determined by pre-orthodontic or pre-orthognathic digitalize lateral cephalography.

Steiner's ANB and Ricketts' NBa-PtGn angles in centric jaw relationship were analyzed to determine the presence of skeletal malocclusion (sagittal skeletal pattern) and the facial type (vertical pattern), respectively. Patients were classified according to ANBo values in: Class I (0.0^o – 4.0^o), Class II (> 4.0^o), or Class III (< 0.0^o); and, according to NBa-PtGno values in: mesofacial (87.0^o – 93.0^o), dolichofacial (< 87.0^o), or brachyfacial (> 93.0^o).

DNA sample obtained from buccal epithelial cells from saliva samples. Two genetic Variants in the ACTN3(rs678397 and rs1815739) and one in MYO1H(rs10850110) were genotyped by real time polymerase chain reaction (PCR). Chi square or fisher's exact test were used to asses Hardy-Weinberg equilibrium and to evaluate the association between genotype and allele frequencies for each genetic variant.

They observed in rs678397 genetic variant in the sample of orthognathic patients, that the TT genotype was over presented in mesofacial phenotype. In the genetic variant rs1815739 it was observed that TT genotype was more common in skeletal Class II then Class I subjects. In addition, they concluded that genetic variant rs1815739 might be involved in vertical malocclusion. Since TT genotype (that cause impairment of the actinin-3 protein) is over presented in dolichofacial phenotype, but in brachyfacial group of patients CC genotype was over presented.

They also found an association between skeletal Class II malocclusion and the genetic variant rs10850110 in MYO1H.(Cunha et al., 2019)

5. MATERIALS & METHODS

5.1. MATERIALS

Our study and study protocol were prepared in accordance with the Helsinki Declaration-2 (2015) guidelines and approved by Üsküdar University Non-Interventional Ethics Committee. The volunteers participating in the study were given detailed information about the analyzes and outputs before the study and their consent forms were obtained from them. Records of a total number of 49 patients were selected from Department of Orthodontics, School of Dentistry at Marmara University. These patients were undergoing orthodontic treatment in the department.

Our study sample consisted of 49 patients which 18 of them were male and 31 were females. All the patients had malocclusion in sagittal and/or vertical planes. Sagittal 17, 15 and 17 patients had Class I, Class II and Class III malocclusion respectively. Vertically 19 were high angle and 21 were Low angle vertical pattern.

Table 5.1.1: patient Distribution

Malocclusions	Gender		Total
	Male	Female	
Class I	5	12	17
Class II	6	19	15
Class III	7	10	17
High angle pattern	6	13	19
Low angle pattern	9	12	21
Normal pattern	3	6	9

5.2. Patient Selection

The following inclusion and exclusion criteria were followed to include or discard patients who were not in line with the purpose of this study.

Inclusion criteria

- Age ranging above 18 years old.
- Being in permanent dentition.
- No previous history of orthodontic correction.
- Availability of pre-orthodontics records.

Exclusion criteria

- History of orthodontic or orthognathic treatment.
- History of an aesthetic / plastic operation or trauma in the facial area,
- Any genetic disease that has been diagnosed in him and his first-degree relatives.
- Uncontrolled medical systemic disease
- Cleft lip and palate patient
- Lack of more than one permanent tooth

5.3. DNA Collection Procedure

Buccal swap was used to collect DNA from the volunteers. DNA isolation using buccal swabs provides many advantages, such as cost-effective processing, lower sample volume requirement, long-term archiving, and It is more comfortable for the patient, and the buccal swabs provide sufficient DNA for the PCRs (Van Wieren-De Wijer et al., 2009)

After obtaining informed consent forms from individuals participating in the study, DNA were isolated from buccal cells of the volunteers. These subjects were asked not to consume food or drink for at least 30 minutes before providing a sample. Buccal swabs were collected by a member of the research team by firmly rubbing a brush against inside of cheek at least 15 seconds, then in the maxillary and mandibular buccal sulcus (the upper and lower furrows between the gingiva and the inner cheek).

5.3.1. Buccal swab sample collection protocol

1) Writing the name of patient and date of collection on the tube.

2) Rinsing the patient's mouth with water

3) Removing the swabs from its package. Hold it by its plastic shaft

4) Asking patient to open their mouth and vigorously rub the inside of each cheek and over the gums of the test subject15 seconds. Holding the swab as close to its head as is comfortable for the test subject. While rubbing the cotton swab, turn the plastic shaft. This will ensure the entire tip is covered with cells from the cheek

5) carefully placing the swab back into its original tube.

5.4. DNA Isolation

Oral epithelium cells were collected by DNA collection sticks from the volunteers who participated in the study, and DNA isolation was completed by using PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA). Briefly, 20 µL proteinase K was vortexed by adding 10µL of RNAase to 200 µL of DNA

isolation. After 2 min at room temperature, 200µL of binding buffer was added and homogenized with stirring. After incubation for 10 minutes in a 55 ° C water bath, 200 µL of ethanol was added and vortexed for 5 seconds. It was taken to the filtered tube and centrifuged at 10000g for 1 minute. The supernatant was discarded and 500 µL of washing buffer was added to the pellet and centrifuged at 10000 g for 1.15 seconds. 80µL of elution buffer was added and incubated and centrifuged at maximum speed for 1 minute. The DNA samples obtained were stored at -20 ° C until the analysis of the respective gene regions was completed.

5.5. Genotyping of ACTN3 rs1815739

Genotyping of ACTN3 rs1815739 was performed from the isolated DNA by using 7500 Fast Real-Time PCR System (Applied Biosystems). Taqman Genotyping Assays (Applied Biosystems Foster City, CA, USA) genotyping kit was used for allelic determination. Genotyping was completed using 5 µL master mix, 3.75 µL H2O, 0.25µL assay and 1µL (10 ng) DNA

5.6. Cephalometric Analysis

Pre-orthodontics cephalometric radiographs was used in our study to evaluate the sagittal and vertical malocclusion of the patients. All cephalometric radiographs were traced using NemoTech Cephalometric tracing software (Version 10.4.2) and all were traced by single examiner to avoid bias.

5.6.1. Skeletal Points

1. S: Sella. The geometrical midpoint of Sella Turcica
2. N: Nasion. The most anterior intersected point of the nasofrontal suturen in the sagittal plane
3. Po: Porion. The most superior point of external acoustic meatus.
4. Or: Ortbitale. The most inferior point of the orbital floor.
5. Ba: Basion. The most posterior and inferior part of foramen magnum.
6. PNS: The posterior nasal spine. The most posterior extent of the hard palate.
7. ANS: The anterior nasal spine. The most anterior extension of the maxilla
8. Go: Gonion. The most inferior and posterior point of the mandibular symphysis.
9. Pg: Pogonion. The most anterior curvature of the mandible in the sagittal plane.
10. Me. Mention. The most inferior point of the lower surface of mandibular symphysis.
11. A: Subspinale. The deepest concavity of the mid contour of the alveolar process located between prosthion and anterior nasal spine.
12. B: Supramentale. The deepest concavity seen between the mandibular infra-dental point and Pogonion.
13. Gn: Gnathion. The midpoint of Pogonion and Menton.

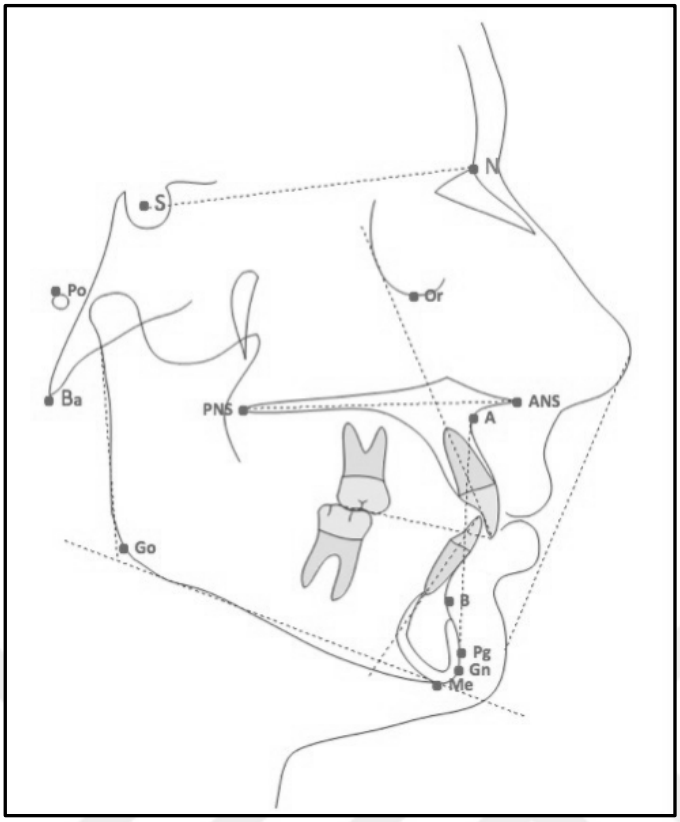

Figure 5.6.1.1: Cephalometric Points

5.6.2. Dental Points

1. U1i: Incisal edge of maxillary central incisors
2. U1a: Apex of maxillary central incisor root
3. L1i: Incisal edge of mandibular central incisor
4. L1a: Apex of mandibular central incisor
5. U6t: Mesial cusp tip of maxillary first molar
6. U6a: Mesial root apex of maxillary first molar

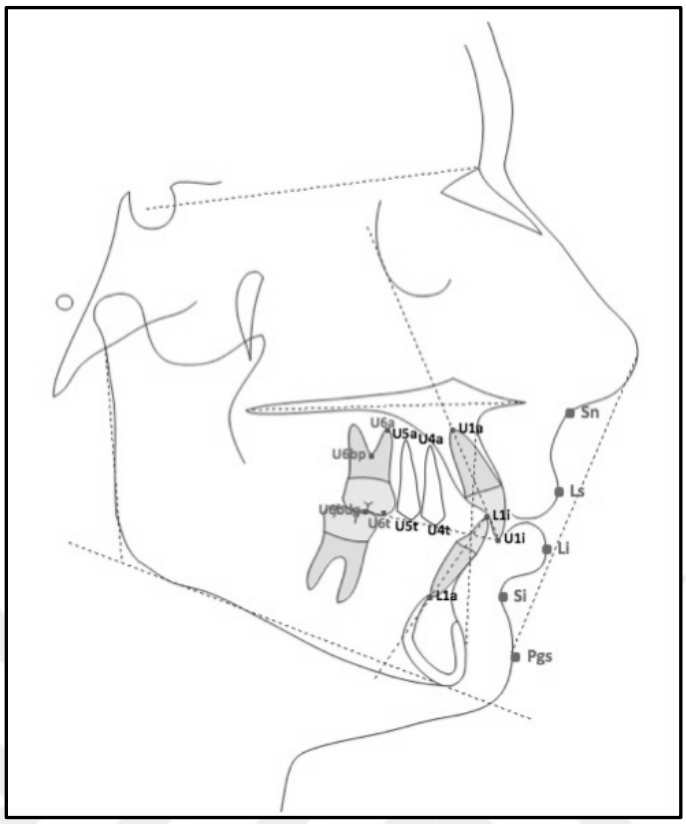

Figure 5.6.2.1 : Cephalometric Dental and Soft Tissue points

5.6.3. Cephalometric and Reference Planes

As for reference planes for tracing and using measurements, the following 5 planes were used:

1. Sella Nasion (SN) Plane: The plane crossing between the sella turcica and nasion point
2. Palatal Plane (PP): The plane passing from the anterior nasal spine to posterior nasal spine.
3. Occlusal Plane (OP): The plane passing from the posterior occlusal contact point of the maxillary and mandibular molars, and anterior between the occlusal contact point of the first premolars.
4. Mandibular Plane (MP): The plane intersecting the Gonion point posteriorly and the Menton point anteriorly.

5. Frankfurt Hortizontal Plane (FHP): The plane crossing from the superior point of Porion to the most inferior point of the Orbitale.

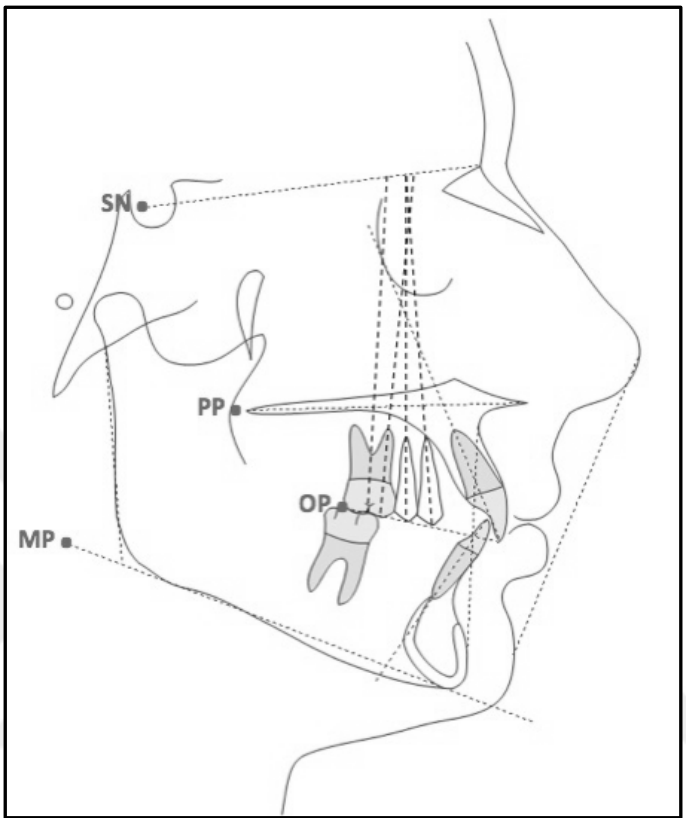

Figure 5.6.3.1: Cephalometric reference planes

5.6.4. Cephalometric Measurements Used in this Study

For this study for all cephalometric measurements and comparison of results, the Marmara Analysis was used. This analysis is followed for all patients at Department of Orthodontics, School of Dentistry at Marmara University. The Marmara analysis comprises of the following points

5.6.4.1. Vertical

1. Sum of Inner Angles: The collective sum of the saddle angle, articulare angle and the gonial angle.

2. Gonion Menton-SN: The angle between the anterior cranial base and the plane passing through the gonion and menton points.
3. ANS Me/ Nme: The ratio of anterior facial height to lower anterior facial height.
4. Jarabak Ratio: The ratio of posterior facial height to anterior facial height.
5. FMA: The angle between the Frankfurt horizontal plane and the mandibular plane.
6. Maxillary Height: The angle between the Nasion, Centre of Face and A point.

5.6.4.2. Sagittal

1. SNA: the angle between the S-N and the N-A plane.
2. SNB: the angle between the S-N and the N-B plane.
3. ANB: the angle between the N-A and the N-B plane that determines the sagittal relationship between maxilla and mandible.
4. Witts: a measure of jaw relationships in an anteroposterior plane, by drawing perpendiculars from A and B points on the maxilla and mandible on the occlusal plane.
5. N per A: Distance between A point from a line drawn perpendicular from Nasion with respect to the Frankfurt Horizontal Plane.

5.6.4.3. Dental

1. UI-NS: The angle between the axis of maxillary central incisor and the SN plane.
2. IMPA: The angle between the axis of the lower central incisors and the mandibular plane.
3. LI- UI angle: The inter-incisal angle, the angle between the axis of maxillary and mandibular incisors.
4. UI-OP angle: The angle between the axis of upper incisor and occlusal plane.
5. LI-OP angle: The angle between the axis of lower incisors and occlusal plane.

Table 5.6.1: Normal values of cephalometric analysis

Analyisis	Normal	Standard deviation
Total inner angles	396°	+/- 3
GoMe-SN	32°	+/- 7
Jarabak	60%	+/- 1
ANSMe/NMe	55%	+/- 1
Frankfort mandibular plane angle	25°	+/- 1
Maxillary height	53°	+/- 1
SNA	82°	+/- 2
SNB	80°	+/- 2
ANB	2°	+/- 2
Witts	-1 mm	+/- 3
Maxillary depth	90°	+/- 1
N perpendicular A	1 mm	+/- 2
I-SN	103°	+/- 2
IMPA	90°	+/- 1
MxI-OP	56	+/- 3
MndI-OP	64	+/- 3

6. Statistical Analysis

Statistical analyses was performed using SPSS software version 25. Descriptive analyses was presented using means, standard deviations, median, minimum and maximum values for contionus data and frequecies and percentages for categorical data. The variables investigated using Kolmogorov Smirnov test to determine whether or not they are normally distributed. The Fisher's exact test was used to compare the proportions of the groups. Since the variables are not normally distributed Kruskal-Wallis test was used to compare medians of the groups. A 5% type-I error level was used to infer a statistical significance

7. Results

7.1. Vertical Analysis

For vertical discrepancy, cephalometric radiographs were used to categorize the patient into normal, high and low angle vertical pattern. The analysis that were used are (total inner angle, GoMe-SN, Jarabak, ANSMe/Nme, Frankfort mandibular plane angle, Maxillary height)

Since there are many variations in these analyses, all of the measurment were used to categorize the patient to overal vertical pattern. This way will allow more accurate diagnosis for the patients.

Table 7.1.1: Overall vertical pattern analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
Overall Vertical pattern	Low angle	Count	1_a	2_a	18_a	21	0,560
		% within patient vertical	4,8%	9,5%	85,7%	100,0%	
		% within genetic results ACTN3	16,7%	50,0%	46,2%	42,9%	
	Normal angle	Count	2_a	0_a	7_a	9	
		% within patient vertical	22,2%	0,0%	77,8%	100,0%	
		% within genetic results ACTN3	33,3%	0,0%	17,9%	18,4%	
	High angle	Count	3_a	2_a	14_a	19	
		% within patient vertical	15,8%	10,5%	73,7%	100,0%	
		% within genetic results ACTN3	50,0%	50,0%	35,9%	38,8%	
Total		Count	6	4	39	49	
		% within patient vertical	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and patient vertical pattern $p>0,05$.

Table 7.1.2: Total inner angle analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
total inner angles	Low angle	Count	2_a	2_a	25_a	29	0,494
		% within total inner angles	6,9%	6,9%	86,2%	100,0%	
		% within genetic results ACTN3	33,3%	50,0%	64,1%	59,2%	
	Normal angle	Count	3_a	1_a	7_a	11	
		% within total inner angles	27,3%	9,1%	63,6%	100,0%	
		% within genetic results ACTN3	50,0%	25,0%	17,9%	22,4%	
	High angle	Count	1_a	1_a	7_a	9	
		% within total inner angles	11,1%	11,1%	77,8%	100,0%	
		% within genetic results ACTN3	16,7%	25,0%	17,9%	18,4%	
Total		Count	6	4	39	49	
		% within total inner angles	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using total inner angles $p>0,05$.

Table 7.1.3: GoMe-Sn analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
GoMe-SN	Low angle	Count	0_a	0_a	3_a	3	0,880
		% within GoMe-SN	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	7,7%	6,1%	
	Normal angle	Count	4_a	3_a	28_a	35	
		% within GoMe-SN	11,4%	8,6%	80,0%	100,0%	
		% within genetic results ACTN3	66,7%	75,0%	71,8%	71,4%	
	High angle	Count	2_a	1_a	8_a	11	
		% within GoMe-SN	18,2%	9,1%	72,7%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	20,5%	22,4%	
Total		Count	6	4	39	49	
		% within GoMe-SN	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using GoMe-SN p>0,05.

Table 7.1.4: Jarabak analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
Jarabak	low	Count	3_a	3_a	30_a	36	0,493
		% within Jarabak	8,3%	8,3%	83,3%	100,0%	
		% within genetic results ACTN3	50,0%	75,0%	76,9%	73,5%	
	normal	Count	2_a	1_a	4_a	7	
		% within Jarabak	28,6%	14,3%	57,1%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	10,3%	14,3%	
	high	Count	1_a	0_a	5_a	6	
		% within Jarabak	16,7%	0,0%	83,3%	100,0%	
		% within genetic results ACTN3	16,7%	0,0%	12,8%	12,2%	
Total		Count	6	4	39	49	
		% within Jarabak	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using Jarabak $p>0,05$.

Table 7.1.5: ANSMe/NMe analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
ANS Me/NMe	low	Count	3_a	2_a	13_a	18	0,604
		% within ANS Me/NMe	16,7%	11,1%	72,2%	100,0%	
		% within genetic results ACTN3	50,0%	50,0%	33,3%	36,7%	
	normal	Count	2_a	0_a	10_a	12	
		% within ANS Me/NMe	16,7%	0,0%	83,3%	100,0%	
		% within genetic results ACTN3	33,3%	0,0%	25,6%	24,5%	
	high	Count	1_a	2_a	16_a	19	
		% within ANS Me/NMe	5,3%	10,5%	84,2%	100,0%	
		% within genetic results ACTN3	16,7%	50,0%	41,0%	38,8%	
Total		Count	6	4	39	49	
		% within ANS Me/NMe	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using ANS Me/NMe p>0,05.

Table 7.1.6: Frankfort mandibular angle analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
FMA	low	Count	0_a	2_a	15_a	17	0,337
		% within FMA	0,0%	11,8%	88,2%	100,0%	
		% within genetic results ACTN3	0,0%	50,0%	38,5%	34,7%	
	normal	Count	1_a	0_a	6_a	7	
		% within FMA	14,3%	0,0%	85,7%	100,0%	
		% within genetic results ACTN3	16,7%	0,0%	15,4%	14,3%	
	high	Count	5_a	2_a	18_a	25	
		% within FMA	20,0%	8,0%	72,0%	100,0%	
		% within genetic results ACTN3	83,3%	50,0%	46,2%	51,0%	
Total		Count	6	4	39	49	
		% within FMA	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using FMA p>0,05.

Table 7.1.7: maxillary height analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
Max Height	normal	Count	0_a	0_a	2_a	2	0,765
		% within Max Height	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	5,1%	4,1%	
	high	Count	6_a	4_a	37_a	47	
		% within Max Height	12,8%	8,5%	78,7%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	94,9%	95,9%	
Total		Count	6	4	39	49	
		% within Max Height	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and vertical pattern using Max Height $p>0,05$.

7.2. Sagittal Analysis

For the Sagital relationship ANB and Wits analysis were used to classify the patients into Class I, Class II and Class III relationship.

Table 7.2.1: ANB analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
ANB	Class I	Count	3_a	2_a	12_a	17	0,828
		% within ANB	17,6%	11,8%	70,6%	100,0%	
		% within genetic results ACTN3	50,0%	50,0%	30,8%	34,7%	
	Class II	Count	1_a	1_a	13_a	15	
		% within ANB	6,7%	6,7%	86,7%	100,0%	
		% within genetic results ACTN3	16,7%	25,0%	33,3%	30,6%	
	Class III	Count	2_a	1_a	14_a	17	
		% within ANB	11,8%	5,9%	82,4%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	35,9%	34,7%	
Total		Count	6	4	39	49	
		% within ANB	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and sagital relation using ANB $p>0,05$.

Table 7.2.2: Wits analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
wits	Class I	Count	1_a	2_a	12_a	15	0,788
		% within wits	6,7%	13,3%	80,0%	100,0%	
		% within genetic results ACTN3	16,7%	50,0%	30,8%	30,6%	
	Class II	Count	2_a	1_a	8_a	11	
		% within wits	18,2%	9,1%	72,7%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	20,5%	22,4%	
	Class III	Count	3_a	1_a	19_a	23	
		% within wits	13,0%	4,3%	82,6%	100,0%	
		% within genetic results ACTN3	50,0%	25,0%	48,7%	46,9%	
Total		Count	6	4	39	49	
		% within wits	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and sagital relation using witts p>0,05

Maxillary Position Analysis

For maxillary position SNA, Maxillary depth and Nasion perpendicular A were used to categorize the patients into normal, prognathic and retrognathic maxillary position and an overal maxillary position of the patients were decided by considering all the three analysis.

Table 7.2.3: Overall maxillary position analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
max. position	normal	Count	1_a	0_a	16_a	17	0,044*
		% within max. position	5,9%	0,0%	94,1%	100,0%	
		% within genetic results ACTN3	16,7%	0,0%	41,0%	34,7%	
	prognathic	Count	0_a	0_a	9_a	9	
		% within max. position	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	23,1%	18,4%	
	retrognathic	Count	$5_{a, b}$	4_b	14_a	23	
		% within max. position	21,7%	17,4%	60,9%	100,0%	
		% within genetic results ACTN3	83,3%	100,0%	35,9%	46,9%	
Total		Count	6	4	39	49	
		% within max. position	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is statatistically significant frequency distribution difference between ACTN3 results and max position $p<0,05$. the people who have retrognathic max have significant proportion difference between XX and RX and between XX and RR. In prograthic group 100% of people genotypes are RR. In normal group 94.1% of people genotype RR.

Table 7.2.4: SNA analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
SNA	normal	Count	2_a	1_a	20_a	23	0,215
		% within SNA	8,7%	4,3%	87,0%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	51,3%	46,9%	
	prognathic max	Count	0_a	0_a	7_a	7	
		% within SNA	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	17,9%	14,3%	
	retrognathic max	Count	4_a	3_a	12_a	19	
		% within SNA	21,1%	15,8%	63,2%	100,0%	
		% within genetic results ACTN3	66,7%	75,0%	30,8%	38,8%	
Total		Count	6	4	39	49	
		% within SNA	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and maxillary position using SNA p>0,05. In prognathic max group 100% of people have RR genotype

Table 7.2.5: Maxillary depth analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
Max depth	normal max	Count	1_a	0_a	11_a	12	0,188
		% within Max depth	8,3%	0,0%	91,7%	100,0%	
		% within genetic results ACTN3	16,7%	0,0%	28,2%	24,5%	
	prognathic max	Count	0_a	0_a	9_a	9	
		% within Max depth	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	23,1%	18,4%	
	retrognathic max	Count	5_a	4_a	19_a	28	
		% within Max depth	17,9%	14,3%	67,9%	100,0%	
		% within genetic results ACTN3	83,3%	100,0%	48,7%	57,1%	
Total		Count	6	4	39	49	
		% within Max depth	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and maxillary position using Max depth p>0,05. In prognathic max group 100% of people have RR genotype. In normal group 91,7% of people genotype RR.

Table 7.2.6: N perpendicular A analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
N per A	normal max	Count	1_a	1_a	15_a	17	0,070
		% within N per A	5,9%	5,9%	88,2%	100,0%	
		% within genetic results ACTN3	16,7%	25,0%	38,5%	34,7%	
	prognathic max	Count	0_a	0_a	12_a	12	
		% within N per A	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	30,8%	24,5%	
	retrognathic max	Count	5_a	$3_{a, b}$	12_b	20	
		% within N per A	25,0%	15,0%	60,0%	100,0%	
		% within genetic results ACTN3	83,3%	75,0%	30,8%	40,8%	
Total		Count	6	4	39	49	
		% within N per A	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and maxillary position using N per A p>0,05. Only the people who have retrognathic max have significant proportion difference between RX and RR and between RX and XX. In prognathic max group 100% of people have RR genotype. In normal group 88,2% of people genotype RR.

Mandibular Position Analysis

Mandibular position is determined by using SNB and categorazing the patients into normal, prognathic and retrognathic mandibuar position.

Table 7.2.7: SNB analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
SNB	normal mand.	Count	0_a	0_a	12_a	12	0,359
		% within SNB	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	30,8%	24,5%	
	prognathic mand.	Count	1_a	1_a	7_a	9	
		% within SNB	11,1%	11,1%	77,8%	100,0%	
		% within genetic results ACTN3	16,7%	25,0%	17,9%	18,4%	
	retrognathic mand.	Count	5_a	3_a	20_a	28	
		% within SNB	17,9%	10,7%	71,4%	100,0%	
		% within genetic results ACTN3	83,3%	75,0%	51,3%	57,1%	
	Total	Count	6	4	39	49	
		% within SNB	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and mandibular position using SNB $p>0,05$.

7.3. Incisor Inclination

Maxillary Incisor Inclination

Incisor SN angle and Maxillary Incisor – occlusal plane angle are used to determine Maxillary Incisor Inclination and categorizing them into normal, proclined and retroclined incisor inclination.

Table 7.3.1: Incisor SN analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
I-SN	normal incisor angle	Count	4_a	$0_{a, b}$	5_b	9	0,024*
		% within I-SN	44,4%	0,0%	55,6%	100,0%	
		% within genetic results ACTN3	66,7%	0,0%	12,8%	18,4%	
	Proclined Upper Incisor	Count	1_a	3_a	24_a	28	
		% within I-SN	3,6%	10,7%	85,7%	100,0%	
		% within genetic results ACTN3	16,7%	75,0%	61,5%	57,1%	
	Retroclined Upper Incisor	Count	1_a	1_a	10_a	12	
		% within I-SN	8,3%	8,3%	83,3%	100,0%	
		% within genetic results ACTN3	16,7%	25,0%	25,6%	24,5%	
Total		Count	6	4	39	49	
		% within I-SN	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is statatistically significant frequency distribution difference between ACTN3 results and incisor inclination using I-SN $p<0,05$. the people who have normal incisior angle have significant proportion difference between RX and XX and between RX and

RR. In proclined group 85,7% of people genotypes are RR. In retroclined group 83,3% of people genotype RR.

Table 7.3.2: Maxillary incisor occlusal plane analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
UI-OP	normal incisor angle	Count	4_a	1_a	10_a	15	0,233
		% within UI-OP	26,7%	6,7%	66,7%	100,0%	
		% within genetic results ACTN3	66,7%	25,0%	25,6%	30,6%	
	Proclined Upper Incisor	Count	0_a	2_a	16_a	18	
		% within UI-OP	0,0%	11,1%	88,9%	100,0%	
		% within genetic results ACTN3	0,0%	50,0%	41,0%	36,7%	
	Retroclined Upper Incisor	Count	2_a	1_a	13_a	16	
		% within UI-OP	12,5%	6,3%	81,3%	100,0%	
		% within genetic results ACTN3	33,3%	25,0%	33,3%	32,7%	
Total		Count	6	4	39	49	
		% within UI-OP	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and incisor inclination using UI-OP $p>0,05$.

Mandibular Incisor Inclination

Incisor mandibular plane angle and Mandibular Incisor – occlusal plane angle are used to determine Mandibular Incisor Inclination and categorizing them into normal, proclined and retroclined incisor inclination

Table 7.3.3: Incisor Mandibular plane angle analysis

			genetic results ACTN3				P
			XX	RX	RR	Total	value
IMPA	normal incisor angle	Count	0_a	0_a	5a	5	0,571
		% within IMPA	0,0%	0,0%	100,0%	100,0%	
		% within genetic results ACTN3	0,0%	0,0%	12,8%	10,2%	
	Proclined Upper Incisor	Count	2_a	2_a	20_a	24	
		% within IMPA	8,3%	8,3%	83,3%	100,0%	
		% within genetic results ACTN3	33,3%	50,0%	51,3%	49,0%	
	Retroclined Upper Incisor	Count	4_a	2_a	14_a	20	
		% within IMPA	20,0%	10,0%	70,0%	100,0%	
		% within genetic results ACTN3	66,7%	50,0%	35,9%	40,8%	
Total		Count	6	4	39	49	
		% within IMPA	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and incisor inclination using IMPA p>0,05. In normal group 100% of people genotype RR.

Table 7.3.4: Mandibular incisor occlusal plane analysis

			genetic results ACTN3				P value
			XX	RX	RR	Total	
LI-OP	normal lower incisor angle	Count	0_a	1_a	7_a	8	0,570
		% within LI-OP	0,0%	12,5%	87,5%	100,0%	
		% within genetic results ACTN3	0,0%	25,0%	17,9%	16,3%	
	Proclined lower Incisor	Count	2_a	2_a	18_a	22	
		% within LI-OP	9,1%	9,1%	81,8%	100,0%	
		% within genetic results ACTN3	33,3%	50,0%	46,2%	44,9%	
	Retroclined lower Incisor	Count	4_a	1_a	14_a	19	
		% within LI-OP	21,1%	5,3%	73,7%	100,0%	
		% within genetic results ACTN3	66,7%	25,0%	35,9%	38,8%	
Total		Count	6	4	39	49	
		% within LI-OP	12,2%	8,2%	79,6%	100,0%	
		% within genetic results ACTN3	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results ACTN3 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statatistically significant frequency distribution difference between ACTN3 results and incisor inclination using LI-OP $p>0,05$.

Kruskal Wallis test

Since the variables are not normally distributed Kruskal-Wallis test was used to compare medians of the groups.

Table 7.4.: Kruskal Wallis test

	genetic results ACTN3	N	Mean	Std. Deviation	Median	Minimum	Maximum	P value
total inner angles	XX	6	393,9167	4,45664	394,1500	388,20	400,90	0,510
	RX	4	390,8750	8,13690	390,6500	382,40	399,80	
	RR	39	389,1077	11,64373	390,3000	339,20	403,20	
	Total	49	389,8408	10,77686	390,5000	339,20	403,20	
GoMe-SN	XX	6	37,1667	4,57894	37,0000	30,00	43,00	0,554
	RX	4	33,5000	6,45497	33,5000	27,00	40,00	
	RR	39	34,2564	6,36073	34,0000	24,00	46,00	
	Total	49	34,5510	6,15109	35,0000	24,00	46,00	
ANS Me/NMe	XX	6	54,4167	1,36002	54,0500	53,00	56,20	0,312
	RX	4	54,2000	2,79523	54,1000	51,60	57,00	
	RR	39	55,4974	2,25907	55,8000	51,00	59,70	
	Total	49	55,2592	2,22476	55,6000	51,00	59,70	
Jarabak	XX	6	65,6667	3,52628	65,4000	61,60	70,90	0,484
	RX	4	68,1250	3,92545	67,4000	64,70	73,00	
	RR	39	67,8641	4,72699	67,7000	59,60	77,20	
	Total	49	67,6163	4,52706	67,1000	59,60	77,20	
FMA	XX	6	28,3333	1,96638	29,0000	25,00	30,00	0,685
	RX	4	27,2500	9,21502	28,0000	17,00	36,00	
	RR	39	26,5897	5,74785	26,0000	16,00	40,00	
	Total	49	26,8571	5,67524	27,0000	16,00	40,00	
Max Height	XX	6	61,3333	4,17931	60,5000	57,00	68,00	0,747
	RX	4	60,5000	1,91485	61,0000	58,00	62,00	
	RR	39	60,1282	3,70760	60,0000	53,00	71,00	
	Total	49	60,3061	3,61826	60,0000	53,00	71,00	

Kruskal Wallis test

There is no statistically significant median difference between the genetic results $p>0.05$.

8. DISCUSSION

8.1. Discussion of Aims

The purpose of this study is to draw a relation between skeletal malocclusion and ACTN3 577XX polymorphism in order to determine if it plays a role in skeletal malocclusion. And to be able to statistically quantify this relation, hence provide an opportunity to use the effects advantageously to reproduce them in general orthodontic setting as a reliable treatment option.

The ACTN3 577XX genotype which is one of common nonsense mutation which has been mostly studied for comparing general population with elite athletes. (Alfred et al., 2011) Presence of alpha-actinin-3 protein in elite athletic is advantageous for sprinting and power lifting and limited evidence show lack of no α-actinin-3 protein enhances human endurance activities. (Ahmetov et al., 2011)

In mouse models however, there is clear evidence that α-actinin-3 co-localizeswith glycogen phosphorylase and enhances oxidation of carbohydrates during exercise. In knockout mice, type II muscle fibers have reduced free glucose and shift from glycolytic metabolism towards a more oxidative metabolism similar to type I fibers. (Berman & North, 2010) According to the studies conducted on human muscles we can not rule out the probability of ACTN3 genotypes may effect the cranial muscles in similar way.

In orthodontics the concept of the effect of muscle forces on the facial morphology and on the type of malocclusion is not new (Graber, 1984) Many researches pointed out that muscles have effect on facial bones. (Johnson et al., 2008). Muscle effects are translated into mechanical forces on areas of bone insertion, and this leads to modification of skeletal structures. Therefore, genetic variations that affect muscle composition or properties related to muscle activity also would affect the skeletal configuration. (Klingenberg et al., 2004)

Although the role of muscles on the type of malocclusion have been widely reviewed, only recently studies begun to explore the association between variants in genes involved in muscular activity with skeletal malocclusion or particular facial characteristics; and these studies pointed to ACTN3 as gene impacting the likelihood of developing malocclusion. (Zebrick et al., 2014a)

Etiology of malocclusion can be the cause of deviation in the skeleton, dental, and soft tissue development in children. Identifying etiology of malocclusion and dominant orthodontic problems as well as early detection could help in future effective treatment, management, and public health planning. (Rapeepattana et al., 2019)

Genotype-Phenotype correlation studies contribute important knowledge that could translate into preventive approaches, better clinical outcomes, and potentially more rewarding clinical practice. In orthodontics, it is favorable to have balance, harmony, and symmetry in skeletal growth to provide a good base to align teeth into the proper occlusion. Mandibular plane angles, maxillary jaw width, lower anterior facial height, and the anterior posterior position of the maxilla as it relates to the mandible are all integral parts in the determination of a proper treatment plan for a given patient. Therefore, any knowledge of the genetic determinants of these dimensions will contribute greatly to the understanding of craniofacial growth and development. (Moreno Uribe & Miller, 2015)

Our study aimed to replicate these initial findings in ACTN3 using sagittal and vertical and sagittal craniofacial skeletal patterns also the inclination of the incisors. Replications are a key part of the genetic epidemiologic research. However, we attempted, not only to replicate, but also to test additional facial phenotypes not explored in the previous studies.

8.2. Discussion of Materials and Methods

The final sample, following exclusion criteria, was 49 patients. The patients had a variety of skeletal malocclusions with mean age above 18 years old. The sample consisted of 17 male and 32 female patients.

For measurement of parameters in our study we used cephalometric radiographs which obtained from the archives of Department of Orthodontics, Faculty of Dentistry at Marmara University. The reason for using cephalometric in our study was that it's an inexpensive yet powerful tool which helps orthodontists in treatment planning and diagnosis of orthodontic cases. Since it's based on degrees and linear measurements, with respect to the Sella-Nasion position, it gives us information about sagittal relationship, thus explaining the skeletal relationship of patients. It also explains the vertical height of the face and the inclination of the maxillary and mandibular relationship.

The only drawback of cephalometric is that it's a 2-dimensional image of a 3-dimensional structure, however, still its reproducible and accurately depicts the changes. In our study we used digital tracing, which is considered more reliable and decreasing the human error and parallax errors which may have an increased tendency using hand tracing. Digital tracing is less time consuming and increased accuracy of superimposition and results are comparable. This is supported by studies from Celik and colleagues, who found that digital tracing was preferred due to its friendly and time saving characteristics(Celik & Cebi, 2009). Albarakati et al. concluded that conventional and digital methods are equally reliable in comparing the cephalometric measurements.(Albarakati et al., 2012)

Genetic analysis conducted in this study was done using DNA obtained from patient's saliva sample. Saliva is an excellent source of DNA for many types of genetic studies. Research has revealed that salivary DNA is equivalent in quantity and purity to DNA obtained from blood(Rylander-Rudqvist et al., 2006); (Turner et al., 1994)

and that the stability of salivary DNA is good when proper methods of handling are employed. (Quinque et al., 2006)

Whole saliva is a mixture of the secretions from all of the various salivary glands located in the mouth, and it may also contain nasal and bronchial secretions, tears, blood from micro injuries in the mouth, serum exudates from the gums, and food and cellular debris. The DNA in saliva originates from cells that are shed from the inner linings of the mouth and from white blood cells. These DNA containing cells are collected, and the DNA is then extracted by various methods.

Saliva collection is a simple process. There are three methods of collecting oral DNA samples which are dry, wet and non-invasive procedure. Dry procedures require the donor to insert a cytobrush, buccal swabs or other collection device into the mouth where tissue is scraped from the gum and cheek surfaces. These methods collect primarily buccal cells which are of lower quality and are potentially contaminated with bacteria from the teeth and other surfaces.

Wet procedures include swishing liquids in the mouth and spitting them into a collecting vessel. Mouthwash, which can contain a high percentage of alcohol content, is typically used for this procedure. The protocol, which can request the donor to swish for up to one minute, can burn and be uncomfortable for the donor. Mouthwash is also designed to remove bacteria from teeth and other mouth surfaces which results in a high amount of bacterial content being released into the sample.

Both the dry and wet methods do not prevent bacteria from growing in the sample and do not actively stabilize DNA. These methods also involve the insertion of an object or substance into the mouth. While it is less invasive than venepuncture, it does not quite meet the definition of ‘non-invasive'. Taken all these into consideration, saliva collection was done for the purpose of DNA extraction. The procedure is not invasive, simple and easy, and the DNA is stable for a very long period of time.

There are several methods used to find which genes are involved in causing disease; linkage analysis; association studies; sib-pair analyses, however these all rely on large a patient sample to be collected and will usually only identify the gene

involved and not the specific pathological mutation. In small scale studies like this one, when the probable causative gene is known genomic sequence is the best method to get any information about specific mutations. Due to the lack of genomic data for causative mutations it has been impossible to look for distinct phenotypic results from the mutations.

8.3. Discussion of the Results

The concept of facial morphology is affected by muscle force is not new in orthodontics (Graber,1963). There are several studies showing the effect of muscle on the facial bone. Different pathways have been suggested in order to understand the mechanism of how muscles affect the bone morphology. One mechanism is that the muscles exert mechanical force on areas of bone insertion, leading to skeletal structure modification. So, it can be assumed that genetic variations that affect muscle activity can affect the skeletal structure.

Different studies have been conducted to find the effect of ACTN3 polymorphism on the facial skeleton and developing malocclusion(Cunha et al., 2019); (Zebrick et al., 2014). There are also studies showing the effect of ACTN3 polymorphisms on muscle composition and its function.

In our study, we aimed to replicate findings of ACTN3 polymorphism on facial form and tested several additional phenotypes. Keeping in mind that replication is considered a vital part of genetic epidemiologic researches

Different phenotypes were used in this study. For vertical discrepancy, cephalometric radiographs were used to categorize the patient into normal, high and low angle vertical pattern. The analysis that were used are (total inner angle, GoMe-SN, Jarabak, ANSMe/Nme, Frankfort mandibular plane angle, Maxillary height)

Since there are many variations in these analyses, all of the measurment were used to categorize the patient to overal vertical pattern. This way will allow more accurate diagnosis for the patients.(Luiz Paranhos et al., 2012)

When the total Inner angle analysis was compared with the genotypes, the RR genotype is associated with higher frequency of patients with low angle vertical pattern. The same result was found when the genotypes were compared to the Jarabak results, both comparisons were statistically insignificant.

When maxillary height, Frankfort mandibular angle and ANSMe/NMe were used for comparing the vertical pattern with the genotypes, it was found out that the patients with RR genotype are associated with higher frequency of patient with high vertical pattern phenotype, which contradict the finding of the previous results where Jarabak and total inner angle where used to determine the skeletal vertical pattern of the patient. All the results were statistically not significant.

The RR genotype were associated with higher frequency of patient with normal vertical pattern when GoMe-Sn were used to determine the vertical pattern of the patients and Low angle vertical pattern were only associated with RR genotype. When Frankfort mandibular angle and maxillary height were used it showed patients having XX genotype had high frequency of high angle vertical pattern, which showed the same result as Cunha et al.(Cunha et al., 2019)

When comparing the genotype and the overall vertical pattern, it showed that patient with RR genotype were associated with higher frequency of low angle vertical pattern and patient with XX genotypes were associated with higher frequency of High vertical pattern patients in which it was coinciding with the result of the Cunha et al. (Cunha et al., 2019). Low angle vertical pattern had the highest frequency of RR and lowest frequency of XX genotypes in most of the analysis, which is the same finding as Zebric et al., showing the effect of mucle on the vertical pattern of the patient. Theses results were statistically not significant.

ANB and Wits were used for determining skeletal sagittal relationship, it was found that patients with RR genotypes were associated with higher frequency of Class

III patient. Furthermore, Class II patients were associated with higher frequency of RR genotype this relation was statistically not significant. In study of cunha et al., it suggested a relationship between patient with XX to higher frequencies of Class II than Class I phenotypes.(Cunha et al., 2019)

For maxillary position SNA, Maxillary depth and Nasion perpendicular A were used and an overal maxillary position of the patients were decided by the three analysis.

There were association between RX and XX and higher frequencies of retrognathic maxilla phenotypes when SNA, maxillary depth and N perpendicular A where used for determining maxillary position. These relationships were statistically insignificant.

When overall maxillary position was used for comparison, prognathic maxilla group were only associated with RR genotype. Furthermore, XX genotype were associated with higher number of maxillary retrognathic group. This relationship was statistically significant with P value < .05.

For the mandibular position SNA analysis were used. It showed that Normal mandibular position is associated only with RR genotype. Additionally, XX genotype is associated with higher frequency of retrognathic mandible, which is the same finding as in maxillary retrognathic group. The relationship was statistically not significant.

XX genotype were associated with normal incisor inclination phenotype and RR genotype was associated with proclined upper incisor inclination when both incisor SN and incisor-occlusal plane were used to determine incisor inclination. The relationship was statistically significant with P value < .05 when Incisor SN analysis were used.

For the lower incisor inclination when incisor mandibular plane angle and incisor occlusal plane were used, the RR genotype was associated with higher frequency of proclined mandibular incisor inclination phenotype and the XX genotype

was associated with higher frequency of retroclined lower incisor inclination. The relationship was statistically not significant.

RR genotype was associated to higher frequency of proclined both maxillary and mandibular incsor inclination, which could suggest the effect of tongue muscle on them.

9. CONCLUSION

- This study shows that there is Statistically significant association between prognathic maxilla patients with RR genotype .
- There is Statistically significant association of RR genotype with proclined maxillary incisor inclination phenotype.
- Low angle vertical pattern patients has tendency to higher frequency of RR genotypes.

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