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**INVESTIGATION OF THE DISTRIBUTIONS OF COL1a1
rs1800012 POLYMORPHISM IN ADULT INDIVIDUALS WITH
MALOCCLUSION**

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BEYAN

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20.03.2020

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YAZID ALI DAMA

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III. ABBREVIATIONS

- BMD : Bone mineral density
- bps : Base pairs
- CFM: Craniofacial Microsomia
- CMT: Congenital Muscular Torticollis
- COL1A1: The gene encoding the $\alpha 1$ chain of type I collagen
- DAM: Disease-associated mutation
- DI: Dentinogenesis Imperfecta
- DNA: Deoxyribonucleic acid
- FAS: Fetal Alcohol syndrome
- GHR: Growth hormone receptor
- NCC: Neural crest cell
- PDL: Periodontal ligament
- PRS: Pierre Robin sequence
- SNP: Single Nucleotide Polymorphisms
- TCS: Treacher Collins syndrome
- TMJ: Temporomandibular joints

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COL1A1 rs1800012 polimorfizminin maloklüzyonu olan erişkin bireylerdeki dağılımlarının incelenmesi

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1. ÖZET

Amaç: Kollajenler, ekstrasellüler matris ve bağ dokuda yer alan ve üçlü fiberler protein yapısında olan mekanik destek sağlayan yapılardır. Günümüzde 27 farklı tipi olduğu bilinmektedir. İnsandaki kollajenlerin %80-90'ını tip I, II ve III kollajen oluşturmaktadır. Bu proteinleri kodlayan genlerde görülebilen varyasyonlar ve polimorfizmler, aşırı elastikiyet gibi fizyolojik durumlara neden olarak işlev bozukluklara neden olabilmektedirler. Bu çalışmamızda *COL1A1* geni rs1800012 polimorfizminin maloklüzyonu olan erişkin bireylerdeki dağılımlarını belirlemeyi amaçladık.

Gereç-yöntemler: Çalışmamıza maloklüzyonu olan 49 birey dahil edilmiştir. Çalışmaya gönüllü olarak katılan bireylerden çalışma öncesi yapılan analizler ve çıktıları hakkında detaylı bilgi verilmiş, kendilerinden imzalı bilgilendirme formları alınmıştır. DNA izolasyonu için swab (Invitrogen, Van Allen Way Carlsbad, CA, USA) vasıtası ile bukkal mukoza hücreleri temin edilmiştir. Tüm polimorfizmlerin genotiplemesi gerçek zamanlı PCR (RT-PCR) yöntemi ile belirlenmiştir.

Bulgular ve Sonuç: *COL1A1* rs1800012 polimorfizmi için CC, AC ve AA genotiplerinin sayı ve yüzdeleri sırasıyla 26 (%53,1), 18 (%36,7), 5 (%10,2) olarak tespit edilmiştir. Allel dağılımlara bakıldığında ise A ve C allelleri 28 (%28) ve 70 (%72) olarak bulunmuştur. *COL1A1* rs1800012 polimorfizmi incelendiğinde CC genotipi ve C allelinin çalışma grubunda baskın olarak gözlenmiştir. Bu çalışma, üst ve alt kesici diş inklınasyonları ile iskeletsel, sagittal ve vertikal ilişkilerin fenotipinin CC, AC ve AA genotipleri ile muhtemel ilişkilerine işaret etmektedir.

Anahtar kelimeler: COL1A1, polimorfizm, Maloklüzyon, ortodonti, genetik.

Investigation of the distributions of COL1A1 rs1800012 polymorphism in adult individuals with malocclusion

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2. SUMMARY

Aim: Collagen is a structure in the extracellular matrix and connective tissue that provides mechanical support in the form of triple fibrillar protein. Today it is known that there are 27 different types. Type I, II and III collagen form 80-90% of human collagen. Variables and polymorphisms that can be seen in genes encoding these proteins can cause functional disorders such as excessive elasticity, causing physiological conditions. In this study, we aimed to analyze *COL1A1* rs1800012 polymorphism in patients with malocclusion.

Materials and methods: In our study, 49 patients with malocclusion were included. The volunteers participated in the study were given detailed information about pre-study analyzes and outputs, and signed informative forms were obtained from them. DNA isolation from buccal swabs by using PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA). Genotyping of all polymorphisms was determined by real-time PCR (RT-PCR) method.

Results and conclusion: The number and percentages of CC, AC and AA genotypes for *COL1A1* rs1800012 polymorphism were determined as 26 (%53.1), 18 (%36.7), 5 (%10,2) respectively. When allelic distributions are examined, A and C alleles were found as 28 (28%) and 70 (72%). When the *COL1A1* rs1800012 polymorphism was examined in our study group, it was observed that CC genotype and C allele were more dominant. This study shows the possible relationship between upper and lower incisors inclination and skeletal sagittal and vertical relationship phenotypes with the CC, AC and AA genotypes.

Keywords: *COL1A1*, Polymorphism, Malocclusion, Genetics, Orthodontics.

3. INTRODUCTION AND AIMS

Collagen is a structure in the extracellular matrix and connective tissue that provides mechanical support in the form of triple fibrillar protein. Today it is known that there are 27 different types. Type I, II, and III collagen form 80-90% of human collagen. Variables and polymorphisms that can be seen in genes encoding these proteins can cause functional disorders such as excessive elasticity, causing physiological conditions.

Dental and skeletal malocclusions generally occur in a wide spectrum with the combined effect of transcription and growth factors on bone, teeth, skeletal muscles, and other soft tissues and the addition of environmental factors. Our knowledge of the etiology of many of the resulting malocclusions is very limited as of today. Our study aims to investigate the potential relationship between three-dimensional malocclusion phenotypes and genes that may play a role in craniofacial development. For this purpose, it is the examination and determination of the clinical effects of COL1A1 rs1800012 polymorphism in adults with malocclusions, which are expressed in muscle cells, related to muscle structure and have important functions in providing appropriate muscle movement.

Previous studies stated that the development of malocclusion was affected by human evolution and some genetic factors, including the muscle fibers plasticity and heterogeneity of both jaw skeletal morphology and masticatory muscle, which can be inherited (Isola et al., 2018).

Our knowledge of the etiology of malocclusions as of today, although it is quite limited, the effect of muscle tissues on the bone structure of the face and effect on malocclusions is a subject that has been included in textbooks for years (Graber 1963). In studies conducted in the last decade, facial muscles affect facial morphology (Brotto and Johnson 2014; Saccomanno et al. 2012). As an example, the study conducted by Sciote where they describe the forces expressed by the masseter muscle are transmitted to the skeletal structure of the face through the muscle attachments, although they

concluded that skeletal structures undergo structural changes under repetitive muscle forces (Sciote et al., 2013).

It has been long recognized that if there would be a battle between muscle and bone in the field of orthodontics then the winner would be always the muscle, and as a consequence, the muscles are a key element in the development of the face in all three dimensions.

Since muscles affect the way repetitive activities take place, muscle composition and gene variants related to each specific activity have been studied (Klingenberg et al., 2004). One of these genes is COL1A1. There is a close relation between proteins expressed by these genes and collagen tissues. Da Fontoura suggested that the presence of COL1A1 is associated with a type of skeletal malocclusion (Da Fontoura et al., 2015). Doraczynska-Kowalik suspected that COL1A1 expression may be involved in the epigenetic regulations behind the mandibular prognathism phenotype (Doraczynska-Kowalik et al., 2017).

These genes are related to temporomandibular joint growth and development, as it is important in determining mandibular length and height. Condylar growth, as a part of TMJ, is a result of endochondral ossification, which occurs at the deep surface of human condylar cartilage. Human condylar cartilage arises from the edge of bone membrane after (secondary) bone formation. COL1A1 are related to temporomandibular joint growth and development, also The main components of extracellular discs are collagen type I (Ardani et al., 2020).

In this study, we aimed to analyze *COL1A1* rs1800012 polymorphism in patients with malocclusion, to achieve a better understanding of the rule of genetic influence in malocclusion and to predict if there is a direct relation between *COL1A1* rs1800012 and specific type of malocclusion.

4. LITERATURE REVIEW

4.1 General Information

4.1.1 What is Orthodontics?

Is a specialty of dentistry that deals with the diagnosis, prevention and correction of malpositioned teeth and jaws. It can also focus on modifying facial growth, known as dentofacial orthopedics. It includes three main tissue systems, which are the craniofacial system, the dentition and the jaw mastication.

4.2 Occlusion and Malocclusion

Orthodontics addresses the diagnosis of malocclusion and its management. A malocclusion, in most cases, does not represent a state of illness, but rather a variation from what people generally consider ideal. It is therefore important for an orthodontist to be able to define ideal occlusion, as this is the basis for proper diagnosis and precise planning of the treatment.

4.2.1 Malocclusion

Malocclusion is the manifestation of complex genetic and environmental interactions on the development of the oral-facial region (Hartsfield Jr. et al., 2012). Abnormalities of the craniofacial complex affect the standard of life and have negative social and psychological effects on an individual. orthodontic treatment offers correction of those abnormalities within the face caused by dental or skeletal discrepancies to attain functional, aesthetic and psychosocial improvements (Zhou et

al.,2014). There were conflicting views on the criteria for determining severity and the types of malocclusions.

Angle's classification is the most commonly accepted for the quantification and evaluation of malocclusions. Angle suggested that the first upper molars were the key to occlusion, and that the upper and lower molars must be correlated in such a way that the upper molar's mesio-segment occludes in the lower molar's buccal groove(Proffit et al., 2013).

4.2.2 Classification of malocclusion

Numerous methods of defining malocclusion have been used, ranging from measures of result and care requirements to specific classifications. This is not the same as a cycle of disease, because only the presence of a specific feature classifies the presence of the disease. A wide range of occlusal characteristics are required here to create a malocclusion. Nevertheless, for classification purposes, we can recognize certain features which allows better communication and provides us with a basis for diagnosis. The method to be used for classification needs to be simple, accurate and objective.

4.2.3 Prevalence of malocclusion

For many years, studies have been conducted to determine the prevalence of malocclusion in different populations. A comparison of these results is almost impossible, and the results of studies, even those studies conducted in a population of the same origin, may show great variability. Variables such as the differences in classification of occlusal relationships, the developmental period of the study sample, examiner differences in determining the bounds of normal, and differences in sample sizes can affect the results. Instead of differentiating normal and abnormal in a

population, determining frequencies of different types of malocclusions in a referred population may also give valuable information (M. Özgür and Türkkahraman, 2004).

Although, many studies of the prevalence of malocclusion in different populations have been reported, a review of the literature indicates that only a few studies evaluated malocclusion in a referred population (Jones, 1987)(W. S. Yang, 1990). Jones investigated malocclusion and facial types in a group of Saudi Arabian patients referred for orthodontic treatment and reported that there were indications amongst Saudi Arabian patients of a tendency for bimaxillary proclination and a greater proportion of Class III malocclusion than in Western communities(Jones, 1987). Yang evaluated patients who visited the Department of Orthodontics at Seoul National University Hospital from 1985 to 1989 and reported that the percentage of Class III malocclusion has been increasing but that of Class I has been decreasing(W. S. Yang, 1990).

4.2.4 Etiology of malocclusion

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).

Malocclusion can be seen as a disorder that does not represent a particular individual and is indeed developmental. It is the number of occlusal characteristics that are summarized and relatively complex, displaying multifactorial inheritance. Given the fact that some causes and pathologies may be identified as causing a malocclusion in particular cases; the etiology of malocclusion is less evident in the majority. During the development and growth of both dentition and jaws there is a relatively close relationship between environment and genetics in each adult.

In most instances, malocclusion and dentofacial deformity are caused, not by some pathologic process, but by moderate (occasionally severe) distortions of normal

development. Occasionally, a single specific cause is apparent. More often, these problems result from a complex interaction among multiple factors that influence growth and development, and it is impossible to describe a specific etiologic factor (Proffit et al., 2013)(Figure 4.1).

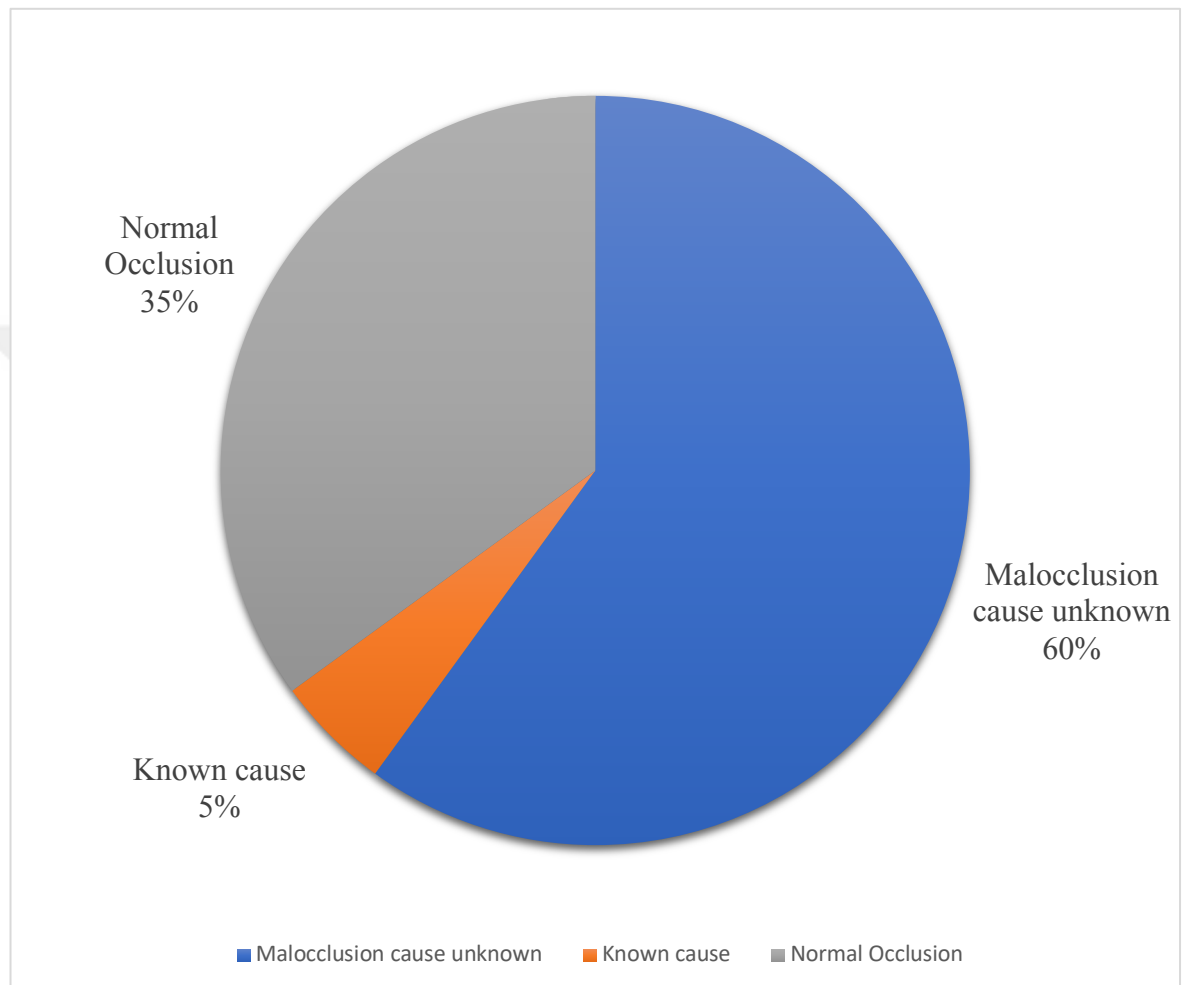


Figure 4.1 : Etiology of malocclusion

4.2.5 Cause of malocclusions

While the exact cause of most malocclusions is difficult to determine, we do recognize in general what the possibilities are, and these must be considered when care is considered. The etiological factors for malocclusion can be investigated under three

major headings: specific causes, hereditary influences and environmental influences(Proffit et al., 2013).

4.2.5.1 Specific causes of malocclusion

4.2.5.1.1 Disturbances in embryologic development

Deficiencies in embryological development typically lead to embryo death. Because of lethal embryological defects as many as 20 percent of early pregnancies terminate, often so early that the mother is not even aware of conception. Although most embryo defects are of genetic origin, environmental effects are also important. Chemical and other agents able to produce embryological defects are called teratogens if given at the critical time. Most drugs do not interfere with normal development or kill the embryo without causing defects and are not teratogenic as a result(Proffit et al., 2013)(Table 4.1).

The likelihood of birth defects in orofacial tissues is high due to the structural and developmental complexity of the face and the susceptibility to intrinsic and extrinsic perturbations. Skeletal malocclusion is caused by the distortion of the proper mandibular and/or maxillary growth during fetal development (Joshi et al., 2014).

Table 4.1 : Teratogens Affecting Dentofacial Development

Teratogens	Effect
Aminopterin	Anencephaly
Aspirin	Cleft lip and palate
Cigarette smoke (hypoxia)	Cleft lip and palate
Cytomegalovirus	Microcephaly, hydrocephaly, microphthalmia
Dilantin	Cleft lip and palate
Ethyl alcohol	Central midface deficiency
6-Mercaptopurine	Cleft palate
13-cis Retinoic acid (Accutane)	Similar to craniofacial microsomia and Treacher Collins syndrome
Rubella virus	Microphthalmia, cataracts, deafness
Thalidomide	Malformations similar to craniofacial microsomia, Treacher Collins syndrome
Toxoplasma	Microcephaly, hydrocephaly, microphthalmia
X-radiation	Microcephaly
Valium	Similar to craniofacial microsomia and Treacher Collins syndrome
Vitamin D excess	Premature suture closure

There are five principal stages in craniofacial development (Table 4.2), and effects on the developing face and jaws can arise during each stage:

- Germ layer formation and initial organization of craniofacial structures.
- Neural tube formation and initial formation of the oropharynx.
- Origins, migrations, and interactions of cell populations, especially neural crest cells.
- Formation of organ systems, especially the pharyngeal arches and the primary and secondary palates.
- Final differentiation of tissues (skeletal, muscular, and nervous elements)(Proffit et al., 2013).

The best example of a problem that can be traced to the very early first and second stages is the characteristic facies of fetal alcohol syndrome (FAS).

FAS consists of multisystem abnormalities and is caused by the excessive intake of alcohol during pregnancy. The teratogenic effect of alcohol on the human fetus has now been established beyond reasonable doubt and FAS is one of the most important human teratogenic conditions known today (Naidoo et al., 2006).

Although such blood levels can be reached only in extreme intoxication in chronic alcoholics, the resulting facial deformity and developmental delay occur frequently enough to be implicated in many cases of midface deficiency (Moore et al., 2002), In these unfortunate children, the delay in dental development matches the skeletal delay (Naidoo et al., 2006).

Table 4.2 : Stages of Embryonic Craniofacial Development

Stage	Time in humans (post fertilization)	Related syndromes
Germ layer formation and initial organization of structures	Day 17	Fetal alcohol syndrome (FAS)
Neural tube formation	Days 18-23	Anencephaly
Origin, migration, and interaction of cell populations	Days 19-28	Craniofacial microsomia Mandibulofacial dysostosis (Treacher Collins syndrome) Limb abnormalities
Formation of organ systems	Days 28-38	Cleft lip and/or palate, other facial clefts
Primary palate Secondary palate	Days 42-55	Cleft palate
Final differentiation of tissues	Day 50-birth	Achondroplasia Synostosis syndromes (e.g., Crouzon's, Apert's)

Many of the problems that result in craniofacial anomalies arise in the third stage of development and are related to neural crest cell origin and migration. Since most structures of the face are ultimately derived from migrating neural crest cells, it is not surprising that interferences with this migration produce facial deformities.

The importance of neural crest migration and the possibility of drug-induced impairment of the migration have been demonstrated clearly by unfortunate experience. In the 1980s, severe facial malformations related to the anti-acne drug isotretinoin were reported. Retinoic acid plays a crucial role in the ontogenesis of the midface, and recent work suggests that loss of retinoic acid receptor genes affects post migratory activity of crest cells, clarifying the timing of Accutane effects (Dupé and Pellerin, 2009). The danger with isotretinoin is that it affects a developing embryo before the mother knows she is pregnant.

Altered development of cells derived from the neural crest also has been implicated in Treacher Collins syndrome (TCS), which is a rare congenital disorder of craniofacial development that arises as the result of mutations in the TCOF1 gene, which encodes a nucleolar phosphoprotein known as Treacle (Trainor et al., 2009).

TCS is a severe congenital disorder of craniofacial development characterized by numerous developmental anomalies that are restricted to the head and neck. Hypoplasia of the facial bones, particularly the mandible and zygomatic complex, is an extremely common feature of TCS. Hypoplasia of the facial bones may result in dental malocclusion, with anterior open bite a common finding. The teeth may be widely spaced, malpositioned or reduced in number. In a large proportion of cases, the palate is high, arched and occasionally cleft and in severe cases, the zygomatic arches may be completely absent (Trainor et al., 2009).

Craniofacial microsomia (CFM) is characterized by a lack of development in lateral facial areas. Typically, encapsulates congenital anomalies of the external and middle ear, maxilla, mandible, facial and trigeminal nerves, and surrounding soft tissues on the affected side (Birgfeld and Heike, 2012). Although facial asymmetry is always seen, cranial as well as facial structures are affected.

The popular assumptions for the pathogenesis of CFM include neural crest cell (NCC) disturbance and vascular disruption (Luquetti et al., 2012). The NCCs originate from the neural ectoderm, migrate over long distances to participate in the formation of the first and second pharyngeal arches, and give rise to craniofacial structures (Trainor et al., 2009).

NCCs migrating to lower regions are important in the formation of the great vessels (aorta, pulmonary artery, aortic arch), and they also are likely to be affected by problems in crest cell migration. For this reason, defects in the great vessels (as in the tetralogy of Fallot) are common in children with craniofacial malformations (Proffit et al., 2013).

The most common congenital defect involving the face and jaws is clefting of the lip, palate, or (less commonly) other facial structures. Clefts arise during the fourth developmental stage. Exactly where they appear is determined by the locations at which fusion of the various facial processes failed to occur and this in turn is influenced by the time in embryologic life when some interference with development occurred.

Clefting of the lip occurs because of a failure of fusion between the median and lateral nasal processes and the maxillary prominence, which normally occurs in humans during the sixth week of development. Since the fusion of these processes during primary palate formation creates not only the lip but the area of the alveolar ridge containing the central and lateral incisors, it is likely that a notch in the alveolar process will accompany a cleft lip (Proffit et al., 2013).

Closure of the secondary palate by elevation of the palatal follows that of the primary palate by nearly 2 weeks, which means that an interference with lip closure that still is present can also affect the palate. About 60% of individuals with a cleft lip also have a palatal cleft. An isolated cleft of the secondary palate is the result of a problem that arose after lip closure was completed. Incomplete fusion of the secondary palate produces a notch in its posterior extent. This indicates a very late-appearing interference with fusion.

The width of the mouth is determined by fusion of the maxillary and mandibular processes at their lateral extent, so a failure of fusion in this area could produce an exceptionally wide mouth, or macrostomia. Failure of fusion between the maxillary and lateral processes could produce an obliquely directed cleft of the face. Other patterns of facial clefts are possible, based on the details of fusion (Tessier, 1976).

Another significant group of craniofacial malformations occurs much later than the ones described so far, during the final stage of facial development and in the early fetal instead of the embryological period of prenatal life. These are the craniosynostosis syndromes, which results from premature fusion of one or several sutures of the skull and it called synostosis (Hehr and Muenke, 1999).

In fetal life, normal cranial and facial development depends on growth adjustments at the sutures in response to growth of the brain and facial soft tissues (Proffit et al., 2013). Synostosis leads not only to secondary distortion of skull shape but to various complications including neurologic, ophthalmic and respiratory dysfunction (Ko, 2016).

Crouzon's syndrome is the most frequently seen member of this group (Proffit et al., 2013). Crouzon syndrome characterized by brachycephaly, ocular proptosis, and maxillary retrusion. The hypothesis has been forwarded that an alteration in anterior cranial base synchondrosis activity is responsible for the skeleton abnormalities which are associated with this disorder (Carinci et al., 1994).

Although the characteristic deformity is recognized at birth, the situation worsens as growth disturbances caused by the fused sutures continue postnatally. Surgery to release the sutures is necessary at an early age.

4.2.5.1.2 Growth disturbances in the fetal and perinatal period

Birth injury is defined as the structural destruction or functional deterioration of the neonate's body due to a traumatic event at birth. Amniocentesis and intrauterine transfusions can cause injuries before birth, and these and any injuries that occur following neonatal resuscitation procedures are not considered birth injuries (Akangire and Carter, 2016).

Head molding and trauma to the mandible during the birth process consider as the most common birth-related injuries.

Intrauterine head molding does not consider as birth injury. Molding refers to the normal change in shape of the baby's head that results from pressure on the head during delivery. In most births, the head is the first part to enter the birth canal. Because a fetus's skull bones are not rigidly fixed in position, the head elongates as it is pushed through the birth canal, which allows the fetus to pass through more easily. Molding does not affect the brain and does not cause problems or require treatment. The head shape gradually becomes more rounded over several days (Stavis, 2019).

Occasionally, a fetus' head is flexed tightly against the chest in utero, preventing the mandible from growing forward normally. This is related to a decreased volume of amniotic fluid, which can occur for any of several reasons. The result is an extremely small mandible at birth, usually accompanied by a cleft palate because the restriction on displacement of the mandible forces the tongue upward and prevents normal closure of the palatal shelves, this condition called Pierre Robin anomaly or sequence (PRS)(Suri et al., 2010).

PRS is classically described as a triad of micrognathia, glossoptosis, and airway obstruction. Infants frequently present at birth with a hypoplastic mandible and difficulty breathing. The smaller mandible displaces the tongue posteriorly, resulting in obstruction of the airway. Typically, a wide U-shaped cleft palate is also associated with this phenomenon. PRS is not a syndrome in itself, but rather a sequence of disorders, with one abnormality resulting in the next. However, it is related to several

other craniofacial anomalies and may appear in conjunction with a syndromic diagnosis, such as velocardiofacial and Stickler syndromes (Gangopadhyay et al., 2012).

Birth Trauma to the Mandible, in certain difficult births the use of forceps to the head to assist in delivery might damage one or both of the temporomandibular joints (TMJ). High pressure in the area of the TMJ could cause internal hemorrhage, loss of tissue, and eventual underdevelopment of the mandible. This had been common explanation for mandibular deficiency at one time. Of course, if the mandibular condyle cartilage were an important growth center, the risk from damage to a presumably critical area would appear far greater. Bearing in mind the contemporary understanding that the condylar cartilage is not critical to proper mandibular growth. It is not as easy to blame underdevelopment of the mandible on birth injuries. Children with deformities involving the mandible are much more likely to have a congenital syndrome (Proffit et al., 2013).

4.2.5.1.3 Progressive deformities in childhood

Progressive deformity is the ones that are progressively getting worse, suggesting of course early intervention. Fortunately, these conditions occur much less often than the serious yet permanent deformities that compose much of the jaw conditions that children face. Most common deformities in childhood are fractures of the jaw and muscle dysfunction.

Childhood fractures of the Jaw, exuberant activity and lack of fine physical control are the main factors predisposing to injuries in childhood, and the facial skeleton is a particularly susceptible part of the body in the early years of life as the young child explores his environment. The presence of developing bones and teeth in the facial skeleton of the child, together with the occurrence of etiologic factors may result in patterns of damage peculiar to children (Hall, 1972).

Mandibular fractures are reported to be the most frequent type of maxillofacial fractures in children (Iida and Matsuya, 2002), Mandibular fractures in children occur less frequently or else are less often recognized than comparable fractures in adults (Frederick et al., 2015). The relative elasticity of the young child's skeleton, and the lesser exposure of the child to the common causes of fracture in adults, where high velocity impact is frequent, are the two main factors responsible for the low incidence of severe skeletal fractures in children (Hall, 1972).

Studies have shown that fractures of the mandibular condylar region may account for as much as 72% to 80% of all mandibular fractures in children and adolescents (Thorén et al., 1992)(Zerfowski and Bremerich, 1998).

Fractures of the mandibular condyle in children may lead to later mandibular asymmetry, altered facial appearance, and occlusal changes due to trauma-induced disturbances in the condylar growth region (Lekven et al., 2011). Luckily, the condylar process tends to regenerate well after early fractures. The best data indicate that approximately 75% of children with early mandibular condylar process fractures have normal mandibular growth afterward and therefore do not develop malocclusions that they would not have had in the absence of such trauma (Proffit et al., 2013).

Mandibular growth is the main factor in facial growth, and as the main mandibular growth center is situated in the condyle, it follows that severe trauma to the condyles may result in damage to these centers, with ankylosis of the temporomandibular joint, and maldevelopment of the mandible on the affected side resulting in facial asymmetry and micrognathia (Hall, 1972).

Muscle dysfunction, the facial muscles can have two ways of affecting jaw growth. First, the formation of the bone is dependent on muscle activity at the point of the muscular attachment. Second, the musculature is a major component of the entire matrix of soft tissue whose growth normally takes the jaws downward and forward. Loss of some part of the musculature is most likely due to damage to the motor nerve. The result would be the underdevelopment of the face, with a soft and hard tissue deficiency (Proffit et al., 2013). There is a correlation between the functional condition of the orofacial muscles and craniofacial growth(Kiliaridis and Katsaros, 1998).

In one hand, excessive muscle contraction can restrict growth as in Congenital Muscular Torticollis (CMT), CMT caused by idiopathic fibrosis of the sternocleidomastoid muscle that restricts movement and pulls the head toward the involved side. Deformation of the craniofacial skeleton will develop if the restriction is not released and result in aesthetic and functional problems (Yu et al., 2004).

Torticollis is one of the most common congenital anomalies. The facial deformity in torticollis has been reported to include deviation and flattening of the face on the affected side with recessed eyebrow and zygoma, inferior displacement of the orbit and ear on the affected side, and deviation of the chin point and nasal tip (Keller et al., 1986)(Hollier et al., 2000), Ferguson described it as “subcarinal torsional rotation of face toward the affected site.”(Ferguson, 1993)

The severity of the torticollis increased with age, this indicates that the early release of the muscle restriction is advised to prevent craniofacial deformation (Yu et al., 2004).

On the other hand, the significant reduction in muscle tonic activity as with myotonic and dystrophic muscles allows the mandible to drop away from the remaining facial skeleton. Their impacts are also significant. The result is increased anterior face height, distortion of facial proportions and mandibular form, excessive eruption of the posterior teeth, narrowing of the maxillary arch, and anterior open bite (Kiliaridis and Katsaros, 1998).

4.2.5.1.4 Disturbances arising in adolescence or early adult life

The postnatally-induced disturbance of development and growth can be the consequence of an adverse event during growth affecting one hemimandible only, or both unequally. In other cases, the abnormal growth seems to be the consequence of mis regulation of growth without any recognized cause. It characterized by surplus growth in length , mass or both of the affected hemimandible, resulting from postnatal condylar mis regulation of growth (Obwegeser, 2000).

Hemi mandibular hypertrophy previously known as condylar hyperplasia is a developmental asymmetry characterized by three-dimensional enlargement of one half of the mandible. The hyperplastic side usually involves the condyle, condylar neck, ramus, and body, with the anomaly terminating abruptly at the symphysis(Xu et al., 2014). The etiology is uncertain, but hormonal influences, heredity, hypervascularity, infection, or trauma are factors that can contribute to irregular growth activities in one of the condyles. The excessive growth may stop spontaneously, but in severe cases removal of the affected condyle and reconstruction of the area are necessary (Proffit et al., 2013).

In acromegaly, caused by an anterior pituitary tumor that secretes excessive amounts of growth hormone, excessive mandible growth may occur, creating a Class III malocclusion of the skeleton in adult life. Mandibular growth often again accelerates to the levels seen in the spurt of adolescent growth, years after adolescent growth has been completed (Fleseriu et al., 2010). Although the excessive growth stops when the tumor is removed or irradiated, the skeletal deformity persists and orthognathic surgery to reposition the mandible is likely to be necessary.

4.2.5.1.5 Disturbances of dental development

The majority of dental development disorders contribute to isolated class I malocclusion and these conditions, like drifting of the permanent teeth following early loss of primary teeth. Dental problems associated with major congenital or health problems include:

Malformed and Supernumerary Teeth, Anomalies in tooth size and shape result from disturbances during the developmental stage of morpho differentiation, with possibly some carryover from the stage of histodifferentiation. The most common abnormality is a change in the size, especially of maxillary and second lateral incisors. Due to the disproportionate sizes of the upper and lower teeth, about 5 per cent of the

total population has a significant "tooth size discrepancy." Normal occlusion is not possible if the teeth are not matched for size(Proffit et al., 2013).

Supernumerary or extra teeth are also caused by disturbances during the initiation and proliferation stages of dental development. In the maxillary midline, the most common supernumerary tooth is called mesiodens. Occasional additional premolars appear. Some patients may have fourth and third molars. Overcounting lateral incisors are also present. The occlusal development is obviously interfered with by an additional tooth, and early intervention to remove the occlusal tooth normally requires an appropriate alignment and occlusal relations. In congenital cleidocranial dysplasia, multiple supernumerary teeth are often observed, which is characterized by missing clavicles (collar bones), many supernumerary and unerupted teeth, and failure of the succedaneous teeth to erupt (Park et al., 2013).

Congenitally missing teeth, the congenital absence of teeth is the result of disturbances during the initial stages of tooth formation. The extreme shape is anodontia, the complete absence of teeth. The term oligodontia refers to the congenital absence of many teeth, while hypodontia, which is occasionally used, means the absence of only a few teeth (Horowitz, 1966).

Anodontia and oligodontia are usually related to ectodermal dysplastic systemic abnormality. Individuals with ectodermal dysplasia have thin, sparse hair and lack of sweat glands in addition to their characteristically missing teeth. In patients without any apparent systematic problem or congenital syndrome, oligodontia occasionally takes place. In these children, it seems that the missing teeth have a random pattern. Anodontia and oligodontia are rare, but hypodontia is quite frequent. The best explanation for etiology seems to be a polygenic multifactorial etiology model (Proffit et al., 2013).

Malformed and supernumerary teeth, almost everyone in their early years falls and hits their teeth. There are two possible results if a trauma to a primary tooth moves the permanent bud of the tooth. First, if the damage happens when the permanent tooth's crown is developing, the development of enamel will be interrupted and a defect in the permanent tooth's crown will appear.

Second, if the trauma occurs on completion of the crown, the crown will be displaced relative to the root. Root formation can cease, leaving the root shortened permanently. Root formation continues more frequently, but the remaining part of the root then develops at an angle to the traumatically displaced crown. This root-form distortion is called dilaceration, which is characterized as a distorted root-form. If the root position distortion is severe enough, the crown cannot take its correct position this may mean that the root extends through the alveolar bone (Proffit et al., 2013).

4.2.5.2 Environmental influences

Environmental factors during face, jaw and teeth formation and development consist primarily of physiological activity-related stresses and forces.

On the other hand, there is every reason to suspect that relationships between form and function may be important to a person's development during his or her lifetime. Although the changes of body form are minimal, a person with heavy physical activity has both heavier and stronger muscles and a stronger skeletal system than a sedentary person. If the function can influence the jaw's growth, a modified function would become an important cause of malocclusion and an important part of orthodontic treatment would make it logical for chewing and other forms of physical therapy.

However, if function makes little or no difference to the pattern of development, it would have little or no etiological or therapeutic impact if it altered its jaw function. Because of its importance in contemporary orthodontics, the evaluation of the potential functional contribution to malocclusion etiology and possible recurrence following treatment places particular emphasis here (Proffit et al., 2013).

4.2.5.2.1 Masticatory function

The pressures caused by mastic operation might potentially affect dentofacial development in two ways: (1) decreased use of the jaws might lead to underdeveloped dental arches and crowded and irregular teeth and the resulting decreased biting force could affect how much the teeth erupt, thereby affecting lower face height and overbite/ open bite relationships, or (2) increased use of the jaws, with a higher and/or longer bite force, could increase jaw dimensions and arcs of the denture.

Biting force and eruption, in patients with excessive overbite or anterior open bite, posterior teeth usually have infra or supra-erupted . It seems reasonable to note that the amount of strength that the teeth erupt depends on how much strength it is placed against. Is it possible that differences in muscle strength and consequently in biting strength are involved in short- and long-term etiologies.

It was noted that short-face persons have higher and long-face persons have lower biting forces than those with normal vertical dimensions. For occlusal tooth contacts during swallowing, simulation chewing, and maximum biting, the difference between long face and normal face patient is statistically significant (Ciochon et al., 1997). Such an association between facial morphology and occlusal force does not prove a cause-and-effect relationship. In the rare muscle weakness syndromes there is a downward and backward rotation of the mandible associated with excessive eruption of the posterior teeth, but this is almost a caricature of the more usual long-face condition, not just an extension of it (Nakamura et al., 2013).

A long-face growth pattern in prepubescent children can be identified. Occlusal forces measurement in this group yields a surprising result: there are no differences between children with long faces and normal faces, nor between either group of children and long-face adults. The three groups are well below normal adults in terms of strength. Hence, the differences in occlusal force appear to arise at puberty when the normal group gains masticatory muscle strength and the long-face group does not. Because before differences in occlusal strength appear, the long face growth pattern

can be identified. it seems more likely that the different biting force is an effect rather than a cause of the malocclusion (W. R. Proffit and Fields, 1983).

Function and dental arch size Muscle activity should be reflected in the size and shape of the jaw muscles. Enlargement of the mandibular gonial angle in the humans can be seen with hypertrophy of the mandibular elevator muscles, and changes in the form of the coronoid processes can occur in kids when the temporal muscle function is affected by injury. The heavy intermittent forces generated during chewing should have little direct effect on tooth positions in order to affect the dimension of the dental arches (Proffit et al., 2013).

The differences between human-racial groups appear to reflect somewhat of the dietary differences and the accompanying chewing effort. The typical craniofacial morphology of Eskimos that includes broad arches is best explained because of the adaptation to the extent to which they traditionally put stress on their jaws and teeth. Changes in craniofacial dimensions from ancient civilizations to modern mankind have been linked to the accompanying dietary changes (Larsen, 2015).

Some studies by physical anthropologists have shown changes in dental occlusion and malocclusion as well as changes in the primitive diet and modern lifestyle (Kelley and Larsen, 1991). As a result of adjustments to dietary changes over even a couple of generations, it seems that dietary changes probably play a role in modern malocclusion development. Vertical jaw relations are clearly influenced by muscular activities during the development of a single individual. It is not so clear whether mastication influence the size of the dental arches and the amount of space for the teeth (Kiliaridis, 1995).

In humans, where dietary consistency affects the dental arch size and the amount of space for the teeth as an individual develops, this must be done early in life because the dental arch dimensions are established early, but the precise relationship remains unknown (Ciochon et al., 1997).

4.2.5.2.2 Equilibrium considerations

Physics laws state that an object subject to unequal forces is accelerated and thus shifts to another position in space. Therefore, if any object undergoes a set of forces but remains in the same position, any force must be balanced or equally balanced. From this point of view, the dental dentition obviously has a balance since teeth are subject to various forces, but in the usual circumstances do not move to another location (Weinstein et al., 1963).

The efficacy of orthodontic treatment itself shows that teeth forces are normally balanced. Teeth usually have forces of chewing, swallowing, and talking, but don't move. When an orthodontic appliance subjects a tooth to a continuous force, the tooth moves, so that the orthodontist 's force has altered its previous balance (W. Proffit, 1978).

Four major primary factors in the dental equilibrium can be identified at present. The factors are intrinsic forces by tongue and lips, extrinsic forces like habits and orthodontic appliances, forces from dental occlusion and forces from the periodontal membrane (W. Proffit, 1978).

One key consideration is that the supporting structures of the teeth, periodontal ligament (PDL) and alveolar bone, are designed to withstand heavy forces such as chewing forces of short duration. During mastication, the fluids in the PDL area act as the shock absorber to prevent compression of the soft tissues on the PDL, even if the alveolar bone bends. There is an impact on the soft tissues only if pressure is maintained sufficiently long to squeeze out fluid. Then the pressure is released since it hurts and the fluid returns before the next stroke. The result is that only light force of long duration (6 hours or so per day) is important in determining whether there is enough of an imbalance of forces to lead to tooth movement, which means if the balance between tongue versus lip/cheek pressure changes, tooth movement would be expected.

Since the teeth are positioned between the lips and cheeks on one side and the tongue on the other, the opposing force or pressures from these organs should be major determinants of the dental equilibrium (W. Proffit, 1978). For example, if a damage to the lip's soft tissue leads to scarring and contracture, the incisors in this area will be lingually moved as the lip tightens against them. On the other hand, if restraining pressure is removed from the lip or cheek, the teeth move outwards in response to tongue-free pressure. Pressure from the tongue, whether from an enlargement of the tongue from a tumor or another source, or because its posture has changed, will result in the labial displacement of the teeth, even though the lips and cheeks are intact due to altered equilibrium (Straub, 1961).

These observations clearly indicate that the light lingering pressure from the lips, cheeks and tongue during rest, contrary to the forces of mastication, is an important determinant of tooth position. However, it seems unlikely that the intermittent short-duration pressures created during swallowing or speaking when the tongue and lips contact the teeth would have any significant impact on the position of the dentation. As with chewing forces, the pressure magnitudes would be large enough to move a tooth, but the duration is insufficient (Table 4.3).

Table 4.3 : Possible equilibrium influences magnitude and duration of force against the teeth during function

Possible equilibrium influence	Force magnitude	Force duration
Tooth Contacts		
Mastication	Very heavy	Very short
Swallowing	Light	Very short
Soft Tissue Pressures of Lip, Cheek, and Tongue		
Swallowing	Moderate	Short
Speaking	Light	Very short
Resting	Very light	Long
External Pressures		
Habits	Moderate	Variable
Orthodontics	Moderate	Variable
Intrinsic Pressures		
Periodontal ligament fibers	Light	Long
Gingival fibers	Variable	Long

The skeleton, including the facial skeleton, also has considerations of equilibrium. Skeletal modifications often arise in response to functional requirements and in rare experimental conditions are magnified. The osseous processes to which muscles attach are influenced in particular by the muscles and the place of the attachment. The shape of the mandible is particularly susceptible to change because it is largely dictated by the shape of its functional processes. Like the skeleton as a whole, the density of the facial bones increases when hard work is performed and decreases in its absence (Solow and Tallgren, 1976).

4.2.5.2.3 Sucking and Other Habits

Almost all young children indulge in non-nutritious sucking of a thumb or pacifier, and as a general rule, sucking habits have little, if any, long-term effect during the primary years of dentition. However, as these behaviors last longer than the eruption of permanent teeth, malocclusions with flared and spaced maxillary incisors, lower incisors lingually positioned, anterior open bite and a narrow maxillary arch are the probable outcome (Kelly et al., 1973). A combination of direct pressures on the teeth and alteration in resting cheeks and lip pressures contributes to characteristic malocclusion associated with sucking habit.

If a child puts a thumb or finger between the teeth, it is generally placed at an angle such that it rests against the lower incisors lingually and the upper incisors labially. The effects on teeth and how much may differ considerably. The principle of equilibrium implies that how much of the teeth are displaced will best correspond with the number of hours per day of sucking rather than the magnitude of the pressure implying while sucking. The kids who are intensely but intermittently sucking cannot displace incisors too much while others may cause a serious malocclusion, especially those who are sleeping with a thumb or finger between teeth overnight.

4.2.5.2.4 Respiratory Pattern

The primary determinant of the posture of the jaws and tongue (and, to a lesser degree, of the head itself) are respiratory needs. It seems therefore entirely reasonable for the posture of the head, jaw and tongue to be altered by a modified respiratory pattern, such as breathing through the mouth instead of nose. This could shift the balance of pressure on the jaws and teeth and affect both jaw development and tooth position.

To breathe through the mouth the mandible and tongue must be lowered and the head extended (tip back). If these postural changes were sustained, face height

would increase and posterior teeth would super-erupt if there were no irregular vertical growth of the ramus, the mandible would rotate down and back, opening the bite anteriorly and increasing overjet and increased pressure from the stretched cheeks may create a narrower maxillary dental arch. This very form of malocclusion is also related to breathing throughout the mouth is exemplified by the long-face syndrome “adenoidal facies” (Vig and Columbus, 1998).

In evaluating these factors, it is necessary first of all to remember that while people are primary nasal respirators, everybody partially respire through the mouth under certain physiological conditions.

Under rested conditions, it requires more energy to breathe through the nose than through the mouth — due to the tortuous nasal passages they add an aspect of airflow resistance that warms and moisturizes inspired air. The increased nasal breathing function is physiologically appropriate to an extent and indeed, with the modest resistance in the system, breathing is most effective. When the nose is partly obstructed, the nasal breathing function increases and the person switches to partial mouth respiration at a certain degree of resistance to nasal airflow (Warren et al., 1996).

4.3 Genetics

The word genetics comes from the word “gene,” and genes are the focus of the subject. Whether geneticists’ study at the molecular, cellular, organismal, family, population, or evolutionary level, genes are always central in their studies. Simply stated, genetics is the study of genes. A gene is a section of a threadlike double-helical molecule called deoxyribonucleic acid (DNA) (Griffiths et al., 2004).

4.3.1 Genome, genotype, phenotype, modes of inheritance and epigenetics

The **genome** of an individual is defined as genetic information that has been inherited from both parents. Information encoded in a patient's genome can influence growth and development by translating the coded information into protein form (and/or regulatory molecules such as microRNAs (miRNAs)). Such information is encoded by residues of ~3.2 billion nucleotide base pairs (bps), consisting of adenine (A), thymine (T), cytosine (C) and guanine (G), arranged into sequences on 23 chromosome pairs.

Each individual has 22 pairs of autosomal chromosomes (chromosomes in both males and females showing the same copy number) and one pair of sex chromosomes (XX or XY). One chromosome in each pair is inherited from the mother of the individual and the other pair from the father of the individual. This genetic information is often collectively referred to as a DNA or genetic code for a person. Surprisingly, all human's genetic sequences seem to be ~99.9 percent identical, so it's just 0.1 percent of the sequence information that codes for our individual differences.

The human genome is estimated to consist of 25,000 genes (representing only about ~2 percent of the entire genome), with an average gene length of ~3,000 bps of information. A **gene** is a specific information sequence that provides the instructions for creating a unique protein or set of related proteins. The location or "address" for any gene within a genome is called its **locus** (plural loci: i.e., reference to more than one gene's physical location).

For a specific location within a person's genome, a determination of the actual DNA code (A, T, C or G) describes their **genotype** for that location. Since there is natural variation in the DNA sequence, a specific gene at a locus in the same individual can still vary between individuals and homologous chromosomes. Those various forms of the "same" gene are called alleles. When the alleles are the same on homologous pairs of chromosomes, they are said to be **homozygous**. When the alleles are different on homologous chromosome pairs they are said to be **heterozygous**. The

inheritance mode describes how genetic information is transmitted from one generation to the next (Mossey, 1999a).

The majority of cells in the body within a single individual will contain a complete copy of the genome the individual has inherited from their parents. Only a few specific types of cells (e.g. mature erythrocytes, mature immune system T and B cells, sperms and egg cells) remove portions of inherited DNA to enable the cell to carry. Apart from these specialized cell types, the majority of cells within a person's body either become a particular type of cell or form part of a greater tissue or organ based on the pattern of genes "on" or "off" in each cell. The "on" process is known as the "gene expression" and more forms of gene expression lead to the production of a protein or a set associated protein (Bourzgui, 2012).

An individual's visible or measurable characteristics are his / her **phenotype**. A phenotype is determined on the basis of: (1) the inherited genetic information expressed through cells in an individual (e.g. the genotype of an individual), (2) the environment in which proteins are expressed, and (3) the interactions between the genotype and environment that could influence the expression or function of protein (Edward F. Harris, 2008). By contrast, a **trait** is a particular aspect or feature of the phenotype as a whole. An **inherited trait** is one that has the ability to pass from one generation to the next. A **syndrome** is a combination of traits that occur in non-random patterns together that is different from the usual pattern (Hartsfield, Jr. et al., 1984)(Hartsfield, 2011).

A trait or syndrome is considered to be monogenic because the information in a single gene locus is ultimately responsible for the creation of a trait or syndrome. If the gene locus is located on one of the 22 autosomal pairs, and one copy on the autosomal pair of a particular gene allele is adequate for creating a trait or syndrome, the individual will typically be heterozygous to this allele and the effects on the inheritance pattern of the trait or syndrome will be **autosomal dominant**. If the trait or syndrome cannot be developed when there is only one copy of a certain allele present at the locus in a pair of autosomes, but it happens when a pair of autosomes includes two copies of that allele, then the inheritance pattern of the trait or syndrome is **autosomal recessive**. In this situation they suggest that the "recessive" alleles are

homozygous. The peculiar case of X chromosome genes produces even different pedigrees. Given the impossibility of transmission of male to male, and since females do not express the disease while bearing only one copy of the disease gene, the standard inheritance consists of an affected male with clinically normal parents and children, but with affected brothers, maternal uncles, and other male relatives. This mode of inheritance is referred as X-linked recessive (Mossey, 1999a).

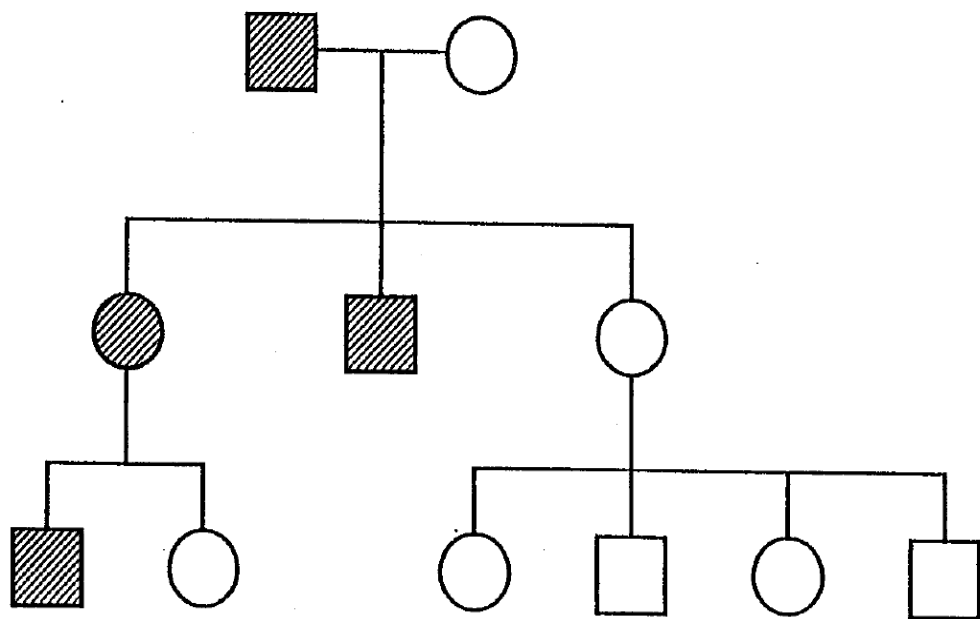


Figure 4.2 : *Autosomal dominant Inheritance.*

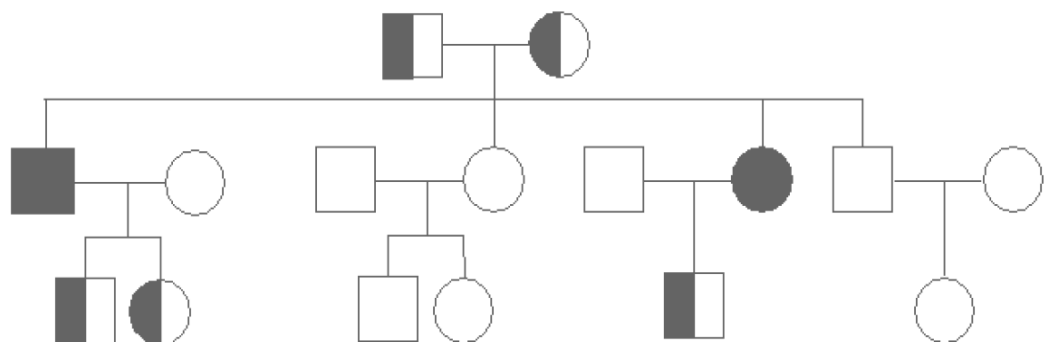


Figure 4.3 : Autosomal recessive inheritance.

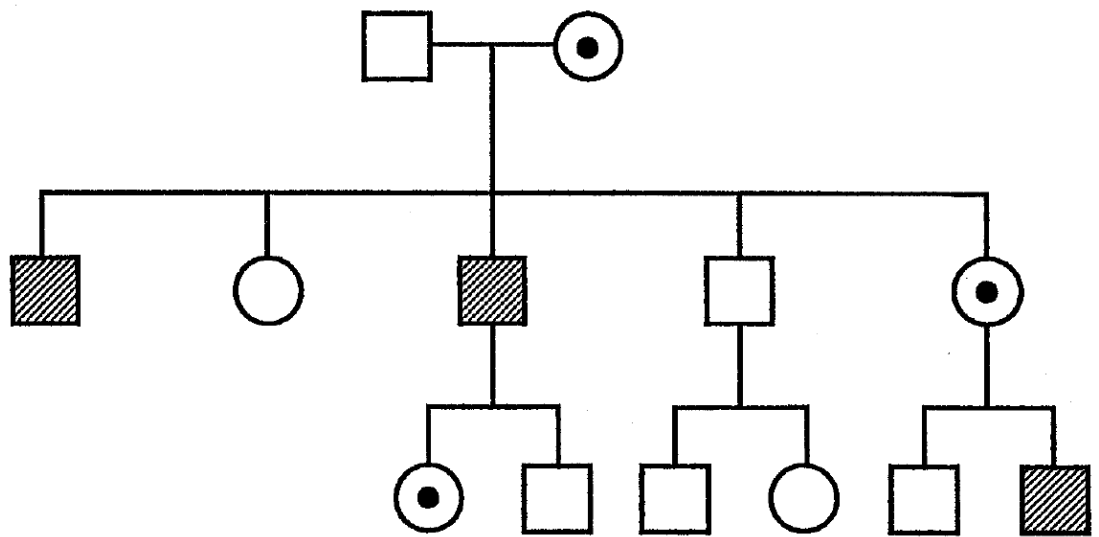


Figure 4.4 : X-linked recessive inheritance.

The following are characteristic of **autosomal dominant inheritance**: (1) when an adult has the gene allele resulting in the trait or syndrome, each child has a 50 percent chance of inheriting the gene allele; (2) the trait or syndrome develops in successive generations; (3) parents who do not have the trait or syndrome have offspring who do to have the trait or syndrome; and (4) males and females are equally likely to have the trait or syndrome. Those features, however, have significant caveats. Just because an person has the "dominant" gene allele that would usually lead to the development of a specific trait or syndrome, such as Class III malocclusion, Treacher Collins syndrome or Crouzon syndrome, the presence of the trait or syndrome may "skip a generation" in what is referred to as person non-penetrance, or incomplete penetrance in a group of individuals with the trait or syndrome (Everett et al., 1999)(Cruz et al., 2008).

Usually in **autosomal recessive inheritance** the pedigree transmission is horizontal (present only in siblings). Parents of a child with a trait or syndrome with an autosomal recessive mode of inheritance are usually heterozygous "carriers". Heterozygous parents will also have a 25 percent autosomal recessive trait or syndrome with each of their children.

For X-related traits, recessive genes on the one male X chromosome manifest themselves phenotypically as being dominant genes as a male normally only has one X chromosome (hemizygous). In this case, the genotype of males in the pedigree is affected, while in some instances even the females may be affected. Females who are heterozygous for the gene associated with the recessive X-linked phenotype may show some phenotype expression. This is because a mechanism called **lyonization** (or X chromosome inactivation) would usually inactivate most of the genes on one of the X chromosomes in each female cell.

Most of the traits don't follow Mendelian inheritance patterns. These features are known as complex or common diseases and traits and reflect the complex interacting of genes from more than one locus and environmental factors. Polygenic traits infer the effect on the phenotype of multiple genes but can also be affected by environmental factors. The distinction between polygenic traits and multifactorial traits was made for some discrete multifactorial traits that occur in an individual once the developmental threshold of genetic and environmental factors for producing the phenotype is reached.

Epigenetics is the study of inherited and heritable gene functional modifications that arise without altering the DNA sequence. Epigenetic mechanisms, such as DNA modification, histone modification and post-transcriptional silencing may be affected by environmental factors. Each of these processes can lead to activation and inactivation of the genes (Lambert and Herceg, 2011). Epigenetic mechanisms can mediate the environmental effect on the human genome through the control of the transcriptional activity of certain genes at specific times of certain organs (Gabory et al., 2009)(I. V. Yang and Schwartz, 2011). Malocclusion is a trait of which environmental factors can greatly influence. Corruccini indicated that rapid growth of malocclusion in the indigenous people of Australia is triggered by dietary factors which are in parallel with industrialization and stressed the importance of environment influences on variation in occlusal conditions and variability in the environmental or population of apparent genetic determinants in the measured population (Robert S. Corruccini, 1984), (1990) (R. S. Corruccini et al., 1990).

When considering the environmental impact on malocclusion growth, it should not be overlooked that the genome will influence the response to environmental factors. The variation in the shape of the mandibular condyles being "slightly greater" between four different inbred strains of the mice is confirmed by the discrepancies between hard diets and a soft food for six weeks. When the environment changed significantly, animals with different genotypes had different responses before the change in the environment (Lavelle, 1984). Siblings often experience similar anomalies not only due to common genetic or environmental factors but also because of their specific genetic factors that affect their response to shared environmental factors (King et al., 1993).

4.3.2 Heritability and malocclusion

Most orthodontic disorders are not exclusively the product of just genetic or just environmental causes, unless they are caused through trauma. The optimal condition for occlusion indicates a proportionate growth from the cranial base to the maxilla and the mandible and the harmonious connections of soft and skeletal bases (Mossey, 1999b). Genetic determination of the general morphology of craniofacial bones and teeth is largely dictated, although variation is partly due to environmental factors (R. S. Corruccini et al., 1990)(Townsend et al., 2003)(Klingenberg et al., 2004)(Edward F. Harris, 2008)(Kraus and Lufkin, 2006).

During embryonic craniofacial morphogenesis and the etiology of many craniofacial abnormalities genetic mechanisms prevail, therefore in malocclusion etiology genetic factor should be considered. However, the environment, especially during facial growing, is supposed to influence postnatal dentofacial morphology. The Functional Matrix Hypothesis from Moss theorized the principal role of function in craniofacial growth and development as the presumption of the genome being the pre-determining force for facial development and by inference skeletal malocclusion (Moss, 1997b), (1997a).

The calculation of the heritability of a feature is a method used to estimate this relative contribution of genetic and environmental factors. Heritability in the broader context, since (H^2) covers all genetic and environmental additive, interactive and other influences. This cannot be derived because all factors and their interactions are unknown. Hence in literature heritability estimates are narrow in the meaning (h^2) and represent the proportion in a sample that is supported by a genetic additive variance of the total phenotypic variance. However, gene-gene or gene-environment interaction does not include the estimated ratio of genetic variation (Hartsfield, 2011).

The cause of the majority of skeletal and dentoalveolar malocclusions is basically multifactorial, as many various causes converge in order to achieve the observed results (King et al., 1993). The majority of studies (especially those which attempt to take into account bias due to the environmental effects, unequal means and unequal fluctuations in twin samples)(E. F. Harris and Potter, 1997), usually involve a moderate or high degree of genetic variation in cephalometric skeletal dimensions, whereas in general the fluctuation in the occlusal relationship has little or no association with genetic variation (Edward F. Harris, 2008).

While veritableness estimates are low, most studies that look at occlusal characteristics have found a positive correlation between genetic variations and phenotypical variations for arch width and arch length rather than overjet, overbite and molar interaction. However, the size and form of the arch are related to environmental differences more than to genetic differences (Cassidy et al., 1998). As many occlusal variables reflect the combined changes in tooth position, and in basal and alveolar bone development, those variables cannot be less variable than the supportive structures. They vary in position and in the basil structures due to their own variations (Edward F. Harris and Johnson, 1991). Heritability trials shall be supplemented and replaced by studies which link or associate certain features to genetic marker variations such as single nucleotide polymorphisms (SNPs), variable number of tandem repeats, or other types of specific variation in DNA.

4.3.3 Use of family data to predict growth

Each child receives half of his genes from each parent. However, unless they are monozygotic twins, they may not get a combination of the same genes as siblings. When looking at parents with a different skeletal morphology, it is difficult to see which genes are present in what combination from each parent in the infant before the child's phenotype evolves with continuous environmental influences. The highest phenotypic correlation that can be predicted based on genes that are specific from parent to child, or between siblings, when considering polygenic traits is 0.5. As the phenotype of your child is likely to be influenced by the interaction of two parents' genes, the "mid-parent" value may rise to 0.7 by the return to parental dimension mean in your kids. Squaring the correlation between the two variables results in the amount of variation for one variable that is projected in correlation with another. Therefore, at the very best, by considering average parental parent values, only 49 percent of a variability of any facial dimension can be predicted in a child. In the best case, the same dimensions in a sibling or a parent can be predicted by only 25% of the variability of any facial dimensions in a child. Due to the different effects of environmental factors that interact with many genetic factors, approximately 30 percent of the normal correlation between parents and children in facial dimensions produces even less predictive power (Šešelj et al., 2015).

Siblings with similar malocclusion were reported as frequently. A parent and older sibling examination was proposed to provide information on a child's therapy requirement, including early malocclusion treatment (J. E. Harris and Kowalski, 1976); (Litton et al., 1970); (Niswander, 1975); (Saunders et al., 1980). Niswander observed a decrease in the prevalence of malocclusion among siblings with normal occlusion, whereas siblings with malocclusive index cases appear to have the same malocclusive form more frequently (Niswander, 1975).

Of Class II and Class III malocclusions there are high coefficient values of association between parents and their descendants (Nakasima et al., 1982). It has been shown that children with Class II Division 1 malocclusions are familial with craniofacial skeletal patterns and that a strong similarity to skeletal patterns occurs

with normal occlusion in their siblings (J. E. Harris et al., 1975). Even though Class II (division 1) was credited to being "heritable," it failed to take into account specific environmental factors. This led to the hypothesis that the genetic basis for this similarity could be polygenic and family skeletal patterns were used as a predictor of the child's Class II malocclusion care prognosis, although it was acknowledged that current morphology in patients is the primary source of knowledge about future growth (J. E. Harris and Kowalski, 1976).

The inheritance mode of the craniofacial skeleton is polygenic (complex) in the majority of patients. However, in certain families (e.g. the inheritance mode is not polygenic, with a relatively prognathic jaw compared to the maxilla). Unfortunately, orthodontists don't have enough information to accurately predict occlusion by simply studying the frequency of occlusion among parents or even siblings. Certainly, family similarity patterns are frequently apparent, and familial tendencies should not be ignored. Nevertheless, predictions must be made cautiously, since it is unknown and difficult to precisely evaluate and predict genetic and environmental factors and their interaction (Hartsfield, 2011).

4.3.4 Genetic markers associated with variations in growth of complex etiology

Craniofacial and skeletal growth is influenced by growth hormone. When there is a proline amino acid instead of threonine in the 561st residue of the protein, a variation in the growth hormone receptor and its gene (GHR) is referred to as the GHR P56IT allele. In a typical Japanese group in 50 men and 50 women, those without the GHR P56IT allele had a substantially greater length of the mandibular ramus (condylion-gonion) than those with the GHR P56IT allele did. A further 80 women reported this significant association between the GHR P56IT allele and shorter mandibular ramus height (Yamaguchi et al., 2001).

Surprisingly, the relation was with the height of the mandibular ramus but not the length of the mandibular bone, maxillary length or the length of the anterior cranial base. That indicates an impact unique to the site, area, or region. The study showed that the allele GHR P56IT can be correlated and can be a genetic marker with the growth of a mandibular height. Nevertheless, it is not clear if the effect is directly on the mandible or any other surrounding tissue or on a separate matrix. GHR variants P561 T and C422F were proposed to be correlated with mandibular ramus height in the Japanese population and that the GHR gene SNPs correlated with variations in the height of mandibular ramus in the Japanese were likely to differ in other ethnic groups (Tomoyasu et al., 2009).

This is confirmed by the finding that while there is a potential correlation between the GHR polymorphisms P561 T, C422F and "haplotype 4" in a Korean population, in African-Americans, European-Americans and Hispanics there was no substantial correlation between these markers and mandibular height. (Kang et al., 2009). This group proposed the different craniofacial morphologies of various ethnicities could be partly explained by this result. There was no difference between mandibular protrusion and normal occlusion in the analysis of the potential associating P561 T variant in the GHR gene with early childhood mandibular development (Sasaki et al., 2009). In order to explore genetic and environmental associations, it would be important to see what effect different diets have on individuals with and without the GHR P56IT allele. Unquestionably several other genes could be found that can affect craniofacial structure, including ramus height, and their variability could be studied along with various environmental factors and the resulting phenotype.

Improved precision in predicting pubertal facial growth would be of great advantage before using various treatment approaches, including orthodontics, modifying orthopedic growth and surgery. Research and discussion in the literature on facial growth and treatment has centered either on the timing of the greatest amount of facial growth, particularly for the mandible (Gu and McNamara, 2007); (Hunter et al., 2007); (Verma et al., 2009), or the approximate degree of facial growth to be achieved (Chvatal et al., 2005); (Turchetta et al., 2007). A more accurate prediction must include and represent the variability linked to individual genetic factors, particularly those

highly pertinent to pubertal growth spurts, as useful as average facial growth forecasts based on predicted growth curves are. The response of the pubertal growth spurt is regulated by the combination of sex hormones, growth hormone, insulin-like growth factor (IGF-I), and other endocrine, paracrine, and autocrine. In mice, testosterone and estradiol have a strong, sex-specific stimulating effect on the proliferation of male and female related chondroprogenitor cells. Testosterone has stimulated growth and local production of IGF-I and IGF-I-R in the chondrocyte cell layers of an isolated mice mandibular condyle organ culture (Maor et al., 1999).

Research on the effect on craniofacial growth of rats of neonatal surgical castration and prepubertal chemical castration has shown that craniofacial growth is linked to testosterone concentrations. Administering low testosterone doses not only speeds up the statural growth rate of children with delayed puberty, but their craniofacial growth rate as well (Verdonck et al., 1998), (1999).

CYP19A1 is the gene that encodes aromatase. This enzyme catalyzes the rate limiting step in estrogen biosynthesis by converting androgens. In order to best diagnose and treat the child or adolescent patient, the orthodontist needs to know as much as possible about the patient's growth potential. As useful as predictions based upon expected growth models starting from early in the patient's life may be, prediction must incorporate and account for the variation associated with individual genetic factors, especially those that are highly pertinent to the pubertal growth spurt.

4.4 Collagen : The Fibrous Proteins of the Matrix

In the extracellular matrix and in the connective tissue, collagen is the major insoluble fibrous protein. It is in fact the most abundant single protein in the animal kingdom. There are at least 16 types of collagen, but 80-90% of the body's collagen is made up of I, II, and III types. These collagen molecules pack together to form long, thin, similarly structured fibrils. Type IV, by contrast, forms a two-dimensional reticulum; several other types associate with collagens of the fibril type, linking them

to each other or to other components of the matrix. At one time it was thought that all collagens were secreted by fibroblasts in connective tissue, but we now know that numerous epithelial cells make certain types of collagens. The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching (Lodish et al., 2000).

4.4.1 The Basic Structural Unit

Type I collagen it was the first to be characterized because its abundance in tendon-rich tissue such as rat tail makes fibrous easy to isolate. Its basic structural unit is a long (300-nm), thin (1,5-nm-diameter) protein consisting of three coiled subunits: two $\alpha 1(I)$ chains and one $\alpha 2(I)$ chains. Each chain contains precisely 1050 amino acids wound in a characteristic right-handed triple helix around each other. Eventually all collagens were shown to contain three-stranded helical segments of similar structure; the unique properties of each type of collagen are mainly due to segments that interrupt the triple helix and fold into other types of tridimensional structures.

Collagen's triple helical structure derives from an extraordinary abundance of three amino acids: glycine, proline, and hydroxyproline. Both amino acids make up the popular Gly-Pro-X repeat sequence, where X can be any amino acid. Each amino acid is accurate in function. Glycine's side chain, a H atom, is the only one that can fit into a three-stranded helix's crowded center. In an adjacent polypeptide, hydrogen bonds linking the peptide bond NH of a glycine residue with a peptide carbonyl (CO) group help hold the three chains together. The fixed angle of the bond between C – N peptidyl-proline or peptidyl-hydroxyproline allows each polypeptide chain to fold into a helix with a geometry such that three polypeptide chains can twist together to form a three-stranded helix. Interestingly, although the rigid peptidyl- proline connections interfere with the packing of amino acids in an α helix, they stabilize the rigid three-stranded helix of collagen (Shoulders and Raines, 2009).

4.4.2 Type I Collagen

Type I collagen is the collagen most abundant and is expressed in virtually all connective tissue. It is a component of the interstitial matrix and the major structural protein of bone, skin, tendon, ligament, sclera, cornea, blood vessels as well as a significant component of other tissues. Of these tissues, the organs with the most prominent functional role for type I collagen are the bone and skin. Type I collagen comprises approximately 95 percent of the total bone collagen content and approximately 80 percent of the total bone protein content, thus representing the tissue with, by far, the largest type I collagen content (Niyibizi and Eyre, 1994).

The genes encoding Type I collagen are COL1A1 and COL1A2 (Table 4.4), and their importance is emphasized by genetic studies that show that mutations in these genes can lead to imperfecta osteogenesis, Ehlers–Danlos syndrome, or Caffey disease (Morello, 2018).

Biosynthesis of type I collagen follows a rather general route, involving propeptides removal and the formation of lysyl-crosslinks, as described in the introduction.

In general, type I collagen molecules consist of heterotrimers made up from two $\alpha 1$ chains and one $\alpha 2$ chain, but a low level of $\alpha 1$ homotrimers has also been reported. The full-length type I collagen is around 300 nm long and has a width of 1–5 nm, while each individual collagen chain is built from more than 1000 amino acids.

Table 4.4 : Overview of type I collagen.

Type I collagen	Description	References
Gene name and number	COL1A1, location 17q21.3-q22 COL1A2, location 7q21.3-22.1	Gene ID 1277 Gene ID 1278
Mutations with diseases in man	Osteogenesis imperfecta I–IV Ehlers-Danlos Caffey disease	(Malfait et al., 2007)
Tissue distribution in healthy states	Ubiquitous	(Niyibizi and Eyre, 1994)
Tissue distribution in pathological affected states	Ubiquitous	(Niyibizi and Eyre, 1994)
Special domains	Like other fibrillar collagens it consists of three NC domains (1–3) + two Col domains (1–2)	(Yamauchi and Sricholpech, 2012)
Special neoepitopes	N- and C-terminal propeptides, and N- and C-terminal degradation peptides	(Viguet-Carrin et al., 2006)
Protein structure and function	Type I collagen is a heterotrimer molecule in most cases composed of two $\alpha 1$ chains and one $\alpha 2$ chain, albeit an $\alpha 1$ homotrimer exists as a minor form. Each chain consists of more than 1000 amino acids, glycine at every third position of the helical domain are crucial for the helix Essential component for the mechanical competence of the bone extracellular matrix, but also a key structural component of many other tissues. Full function not yet clear	(Nistala et al., 2014)
Binding proteins	Integrins, proteoglycans, and many more	(Shoulders and Raines, 2009)
Known central function	The main organic component of bone, indispensable for bone integrity	(Yamauchi and Shiiba, 2002)
Animals models	COL1A2-deficient mice (oim mice), collagenase-resistant collagen I mouse	(Yamauchi and Sricholpech, 2012)(Viguet-Carrin et al., 2006)

4.4.2.1 Type I collagen and the COL1a1 subunit

Type I collagen, which is encoded by two subunits of *COL1a1* and one subunit of *COL1a2* wound together to form a triple-helix, is the most abundant protein in vertebrates and is the main structural protein of bone, teeth, and tendon (Viguet-Carrin et al., 2006). As such, several skeletal and connective-tissue disorders have been associated with mutations in these genes (Dalglish, 1997). Within COL1a1 alone, > 600 disease-associated mutations (DAMs) have been identified (Marini et al., 2007), most of which are associated with I-IV types of osteoporosis, osteogenesis imperfecta, and I and VIIA types of Ehlers-Danlos syndrome, affecting ~200 million, ~500,000, and 200,000 people worldwide, respectively (Stoll et al., 1989)(Burrows, 1999)(Reginster and Burlet, 2006). These DAMs primarily affect protein-coding regions, especially within the triple-helix domain, which consists of a repeating amino acid sequence with glycine, the smallest of the amino acids, in each third position, whose repetition is crucial to allowing the domain to wind into its compact type I collagen form (Yamada et al., 1980)(Wada et al., 2006). Thus, the most phenotypically severe DAMs are often the substitutions of these glycine's (Rauch et al., 2010).

However, the phenotypic severity associated with COL1a1 mutations can be very variable depending on the location of the affected amino acid and how the mutation affects the Type I collagen thermostability, both of which were previously used to predict the phenotypic outcome of novel mutations affecting glycine residues (Persikov et al., 2005)(Bodian et al., 2008)(Bodian et al., 2009). However, those earlier approaches do not integrate an evolutionary approach. For instance, genome-wide studies using evolutionary site models have shown that DAMs are typically located more frequently at locations of amino acids that are highly 4 preserved across organisms (Subramanian and Kumar, 2006). Applying similar means to examine the long-term evolutionary history of COL1a1 in vertebrates that allow for greater accuracy in predicting the phenotypic severity of novel mutations, especially those affecting non-glycine positions that previous predictive models have largely ignored (Miller, 2001).

4.4.2.2 Potential importance of noncoding *COL1a1* polymorphism

In the natural population (i.e., individuals who lack clinical symptoms of skeletal disease) the variability of COL1a1 amino acids is unusual based on a study of 48 individuals (96 chromosomes) from each of four U.S populations (European-, African-, Mexican, and Chinese-Americans) in which only 3 amino acid mutations were found, each with an allele frequency of < 2% in the total sample (Chan et al., 2008). As such, the variability in COL1a1 protein is unlikely to explain this gene's association with the bone phenotypic variation among populations. Instead, genetic variation in non-coding regions affecting COL1a1 expression that significantly contribute to these population differences in bone-related phenotypes, as has been hypothesized for phenotypic variation among populations in general (Kasowski et al., 2010)(Ge et al., 2009).

For example, a mutation in an Sp1 transcription factor binding site in the first COL1a1 intron has been shown to increase gene expression and probably accounts for the associated change in the COL1a1 and COL1a2 subunit ratio which reduces the structural integrity of type I collagen (Grant et al., 1996)(Mann et al., 2001a)(Jin, van't Hof, et al., 2009). This mutation reaches > 20 percent levels among ancestral Western European populations but is rarely found among Africans and is absent among Asians (Beavan et al., 1998)(Lau et al., 2004). Therefore, this single non-coding mutation was found to significantly contribute to the difference in bone strength and fracture frequency in the population.

In addition, this mutation is present in the promoter's major bonding disequilibrium with two other mutations that are also associated with decreased bone mineral density (BMD). Recently, it has been shown that these three polymorphisms have haplotype-specific effects on COL1a1 expression resulting not only in low BMD but also in a decrease in overall bone strength. Nevertheless, aside from these polymorphisms, little is known about non-coding variation at COL1a1, particularly in the natural population, since these regions were largely overlooked in earlier studies in favor of screening individuals for amino acid mutations. As such, it would be interesting to investigate the extent of potentially functional genetic differentiation in

noncoding regions of *COL1a1* in ethnically and geographically diverse populations (Jiang et al., 2007)(Jin, Stewart, et al., 2009).

4.4.2.3 Bone phenotypic variation among primates

While studying *COL1a1* variability in vertebrate species, in general, will shed light on this gene's ancient evolutionary history, in order to better understand the origin of skeletal disease in humans, we must also establish how evolutionary conditions have changed in our lineage more recently. In particular, while there are phenotypic bone variations among human populations, this doesn't mean that this variability is unique to our species. Rather, evolutionary processes that led to the prevalence of the skeletal disease in humans could be shared with other closely-related primate species. Though there is limited bone phenotypic data for non-human primates, two general trends have already emerged. First, BMD varies among other species, partly due to the underlying genetic variation (Havill et al., 2003)(Havill et al., 2005). Second, slight differences in bone morphology among species have been documented, including differences between humans and our closest living relative, the chimpanzee, in osteoporotic-like symptoms, such as bone loss patterns and age-accumulation microfractures (X. Wang et al., 1998)(Mulhern and Ubelaker, 2003)(Mulhern and Ubelaker, 2009).

These data suggest that our lineage may not be isolated from the phenotypic bone variation common among humans. However, with the limited availability of phenotypic data, the magnitude of this variation within species is difficult to assess. Instead, population genetic comparisons of candidate genes can enable us to make inferences about possible functional differences that may exist within and between species, as has been done for other phenotypes such as color vision and viral infection resistance. This locus is a perfect candidate for such an approach given the link between *COL1a1* and phenotypic bone variation in humans. For example, comparing patterns of genetic variation at *COL1a1* in chimpanzees to those observed in humans would reveal whether the evolutionary constraints at this locus recently shifted in our

8 lines since their divergence from the last common human ancestor and chimpanzees ~4-6 million years ago (Wooding et al., 2005)(Verrelli et al., 2008).

4.4.2.4 Polymorphism rs1800012 in COL1A1

COL1A1 gene, located on chromosome 17 at position 17q21.33.7, encodes the α_1 chain of type I collagen (Myllyharju and Kivirikko, 2004). COL1A1 gene has arisen as a vital candidate gene of interest in many diseases recently. Single nucleotide polymorphisms (SNPs) in the collagen type I (COL1A1) gene have been shown to be associated with several complex connective tissue disorders. The relationship between gene polymorphism of COL1A1 gene and osteoporosis is mostly reported (Wu et al., 2017)(Xie et al., 2015). Some articles also found the connection between COL1A1 gene and other diseases in musculoskeletal system, such as sports-related tendon and ligament injuries (C. Wang et al., 2017), osteogenesis imperfecta (Bardai et al., 2016)(Stephen et al., 2014), osteosarcoma (He et al., 2014), idiopathic carpal tunnel syndrome (Dada et al., 2016). And among various polymorphisms within the COL1A1 gene, the most frequently studied polymorphism is the +1245G/T polymorphism (rs1800012, Sp1), which is a guanine (G) to thymine (T) polymorphism sitting within the first intron of COL1A1 gene influencing a binding site for the transcription factor Sp1 (Mann et al., 2001b).

In a study conducted by Ardani in Airlangga University's Dental Hospital in Surabaya, they analyzes the relationship between single nucleotide polymorphisms (SNPs) of COL1A1 and COL11A1 with class II skeletal malocclusion in Javaneseethnic group patients with mandibular micrognathism.

Class II skeletal malocclusion was determined using the digital cephalometric tracing (OrthoVision - 2017, South Korea). Anatomical landmarks (such as: N, S, A, B, Ba, Gn, Co) were determined manually using the cephalometric analysis software. Total of 50 patients with class II skeletal malocclusion with $ANB \geq 4^\circ$; $Co-Gn \leq 125^\circ$.

DNA was extracted from the patient's peripheral blood. DNA extraction was done using QiaAmp Blood Mini Kit (Qiagen), as instructed by standard blood-DNA extraction protocol. DNA amplification was completed using the PCR technique with a pair of primers. Primers COL1A1_F: 5'-GCCTCCCAGAACATCACCTA -3' and COL1A1_R: 5'-TGGGATGGAGGGAGTTTACA -3' were used for COL1A1, both with a target of 456 bp. Primers COL11A1_F: 5'-TGTGTGCCTGTATTCCCAGA -3' and COL11A1_R: 5'-GAGGAC CCTGCATGAGAA-3' were used for COL11A1, both with a target of 415 bp. Sequencing was performed by Sanger sequencing method (Genetics Science, Jakarta, Indonesia). Then, the data were again analyzed using BLAST to find any homology and BioEdit-Win software version 7.2.3 to find their alignment.

They confirmed that there are SNPs in COL1A1 and COL11A1 in class II skeletal malocclusion in Javanese ethnic group patients with mandibular micrognathism. In Caucasians, rs2249492 has an increased risk of class III skeletal malocclusion, whereas it is found in 70% of class II skeletal malocclusion cases in Javanese patients with mandibular micrognathia. This SNP affects the synthesis of matrix metalloproteinase 2 (MMP2) enzymes, which cause an abnormal facial appearance, short stature and osteogenesis disorders. According to Baumert, SNP rs224942 in muscles shows decreased muscle strength. In this study, rs224942G>A or rs224942G/A is found; therefore, there is a probability of a relationship between class II skeletal malocclusion and muscle tension.

Based on this study, they found that in the COL11A1 gene sequence, SNPs at c.134373A>C or c.134373C/A occur in 36% of the samples. Moreover, SNPs at both c.134373M and c.134555Y are seen in the same patients, which is 16% of the samples. In addition, there is a difference in DNA sequences found in this study compared to those at Gene Bank, which is deletion at c.[134227delA]. This deletion is in NCBI RefSeq: NG_008033.1. This is suspected to be a characteristic allele of the Javanese ethnic group (Ardani et al., 2020).

In another study, by Da Fontoura aimed to evaluate the associations between craniofacial candidate genes and skeletal variation in patients with malocclusion. The sample consisted of 269 Caucasian and untreated individuals aged 15 to 68 years with malocclusion who were seeking orthodontic treatment at the University of Iowa and private practices in eastern Iowa.

Digital, analog, and cone beam computed tomography (CBCT)–derived pretreatment lateral cephalometric radiographs were utilized for phenotyping. Skeletal classification was based on cephalometric and dental criteria ANB, Overjet, Wits, Angle classification, soft tissue profile. Skeletal Class II where considered if 2 of the following; $ANB > 4.8$, $Overjet > 5.0$, $Wits > 2.4$, Angle Class II molar/canine in one side, Convex profile < 170 . Skeletal Class I where considered if 2 of the following; $0.3 < ANB < 4.8$, $0 < Overjet < 5$, $-1.3 < Wits < 2.4$, Angle Class I molar/canine, $170 < Profile < 178$. Skeletal Class III if 2 of the following; $ANB < 0.3$, $Overjet < 0$, $Wits < -1.3$, Angle Class III molar/canine in one side, Concave profile > 178 .

Genomic DNA was extracted from buccal mucosal samples collected using Oragene kits. Selection criteria for genes/loci were based on at least 1 of the following: 1) evidence of genetic association or linkage to malocclusion phenotypes in previous studies, 2) known expression patterns and/or biological functions in the craniofacial complex, 3) known role in etiology of craniofacial conditions with phenotypic spectra that include skeletal malocclusion, and 4) previous GWAS indicating association with craniofacial variation.

They observed that the multinomial logistic regression for type of skeletal malocclusion showed that SNPs within COL1A1 were associated with developing class II or III of skeletal malocclusion relative to class I. COL1A1 encodes the proalpha chain of type I collagen. In human midterm fetuses, COL1A1 is found in the perichondrium of Meckel's cartilage; the periosteum of the mandibular bone; the mandibular condylar cartilage; and the perichondrium, periosteum, and bone collar of the cranial base cartilage. Mutations in COL1A1 have been found in patients with osteogenesis imperfecta and Ehlers-Danlos syndrome, both of which commonly involve facial anomalies, including micrognathia, frontal bossing, and midface hypoplasia (Da Fontoura et al., 2015).

5. MATERIALS AND METHODS

5.1 Ethical Approval

The Ethical Committee of the Institute of Health Sciences, Uskudar University approved of this study. These patients had already been selected to be treated at the Department of Orthodontics, School of Dentistry at Marmara University and were about to start or started orthodontic treatment.

5.2 Patient Selection

The study will be carried out on swab samples that will be taken from individuals who have different skeletal sagittal and vertical facial types and who have applied for treatment in the Department of Orthodontics, Marmara University Faculty of Dentistry. Our study sample consisted of 49 patients comprising of 32 females and 17 males.

In accordance with the criteria determined clinically and radiologically, the individuals who applied to our clinic as of January 2019 will be screened and the adult individuals who are found appropriate will be invited to the study.

After obtaining informed consent forms from individuals participating in the study, buccal cells will be provided for DNA isolation with the purpose of DNA isolation, and the samples obtained will be forwarded to the Laboratory of Basic Medical Sciences Medical Biology and Genetics. It is planned to perform the isolation on the same day.

Real-time PCR method will be used in genotyping. In this system, genotyping is performed with the help of primers designed specifically for the region where the

variation is located. Commercial genotyping kits will also be used in this process (Applied Biosystems Foster City, CA, USA, # 4351379).

5.2.1 Selection Criteria

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 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 swab 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) Write name of patient and date of collection on the tube.
- 2) Rinse the patient's mouth with water
- 3) Remove the swabs from its package. Open the tube and take out the swabs. Hold it by its plastic shaft
- 4) Ask the patient to open their mouth and vigorously rub the inside of each cheek and over the gums of the test subject twenty times. Hold the swab as close to its head as is comfortable for the test subject. While you rub the cotton swab, turn the plastic shaft. This will ensure the entire tip is covered with cells from the cheek
- 5) carefully place it back into its original tube.

5.3.2 DNA extraction

DNA extraction or DNA isolation is a routine procedure to collect DNA for subsequent molecular or forensic analysis. There are three basic and two optional steps in a DNA extraction:

- i. Breaking the cells open; commonly referred to as cell disruption or cell lysis, to expose the DNA within. This is commonly achieved by chemical and physical methods-blending, grinding or sonicating the sample.
- ii. Removing membrane lipids by adding a detergent or surfactants.
- iii. Removing proteins by adding a protease(optional but almost always done).
- iv. Removing RNA by adding an RNase (often done).
- v. Precipitating the DNA with an alcohol — usually ice-cold ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a *pellet* upon centrifugation. This step also removes alcohol-soluble salt.

5.3.2.1 Procedure for DNA extraction

DNA extraction from swab samples was done following steps in the 'Manual purification protocol handbook' of DNA genotek. The manual purification of DNA from whole swab sample is as follows:

- i. Sample is incubated at 50°C in a water incubator for a minimum of 1 hour. This heat-treatment is to maximize DNA yield and ensure that nucleases are permanently inactivated.

- ii. The entire sample is transferred to a 15ml centrifuge tube, by pouring or by pipetting with a glass or plastic pipette. The volume of the sample is recorded.
- iii. 1/25th volume of PT-L2P is added and is mixed by vortexing for a few seconds. The samples then become turbid as impurities and inhibitors are precipitated.
- iv. Samples then is incubated on ice for 10 minutes to allow effective removal of impurities.
- v. After ice incubation, samples are then centrifuged at room temperature for 10 minutes at as high a speed as is possible with minimum 3,500 X g.
- vi. The majority of the clear supernatant is transferred with a pipette to a fresh 15 ml centrifuge tube. A small volume of the supernatant was left behind while transferring the clear supernatant to avoid disturbing the pellet which contained impurities. The pellet was then discarded.
- vii. Room temperature 95% to 100% ethanol was added to the clear supernatant. It was gently mixed by inversion 10 times. During mixing with ethanol, the DNA is precipitated and appeared as a clot of DNA fibers.
- viii. The sample was left to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.
- ix. Samples are again centrifuged at room temperature for 10 minutes at as high a speed as possible with minimum 3,500 X g.
- x. The supernatant was carefully removed completely with a glass or plastic pipette then it was discarded. The DNA pellet was not disturbed. Precipitated DNA is found as a pellet at the bottom of the tube and possibly as a smear down the side of the tube, facing away from the center of the centrifuge.
- xi. Ethanol wash: 1 ml of 70% ethanol was added to the tube without disturbing the smear or the pellet. It was then left to stand at room temperature for 1 minute. The ethanol was gently swirl and removed carefully, so the smear was not disturbed. A short centrifugation (less

than 1 minute) is performed to facilitate complete removal of the supernatant.

- xii. The DNA was rehydrated by adding 0.5 – 1 ml of TE solution and by vertexing the sample for 30 seconds. By vertexing, the DNA smeared on the side of the tube is ensured to be recovered.
- xiii. The DNA was completely rehydrated (pellet and smear on the side of the tube) by incubating at room temperature followed by vertexing or pipette mixing, or incubation for 1 hour at 50°C with occasional vertexing. Incomplete rehydration of the DNA will cause inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.
- xiv. The rehydrated DNA was transferred to a 1.5 ml microcentrifuge tube for storage. Storage of the fully rehydrated DNA is done in TE at 4°C for up to 2 months.

5.3.2.2 Quantification of DNA

A diphenylamine (DPA) indicator will confirm the presence of DNA. This procedure involves chemical hydrolysis of DNA: when heated (e.g. $\geq 95^{\circ}\text{C}$) in acid, the reaction requires a deoxyribose sugar and therefore is specific for DNA. Under these conditions, the 2-deoxyribose is converted to w-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue colored compound. DNA concentration can be determined measuring the intensity of absorbance of the solution at the 600nm with a spectrophotometer and comparing to a standard curve of known DNA concentrations.

Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm is used as a measure of DNA purity. DNA absorbs UV light at 260 and 280 nanometers, and aromatic proteins absorb UV light at 280 nm, a pure sample of DNA has the 260/280 ratio at 1.8 and is relatively free from protein contamination. A DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8.

DNA can be quantified by cutting the DNA with a restriction enzyme, running it on an agarose gel, staining with ethidium bromide or a different stain and comparing the intensity of the DNA with a DNA marker of known concentration.

Using the Southern blot technique, this quantified DNA can be isolated and examined further using PCR and RLFP analysis.

In this study, quantification of DNA is done by absorbance method. The purified sample is first treated with RNase to digest contaminating RNA fragments by ethanol precipitation of the DNA. The detailed protocol is described below:

A 10 μ l aliquot of purified RNase treated DNA with 90 μ l of TE (1/10 dilution) was diluted. It was then mixed by gently pipetting up and down, bubbles are left to clear.

- i. TE was used in the reference (blank) cell.
- ii. Absorbance was measured at 320 nm, 280 nm and 260 nm.
- iii. Corrected A280 and A260 values were calculated by subtracting the absorbance at 320 nm (A320) from the A280 and A260 values.
- iv. DNA concentration in ng/ μ l = corrected A260 X 10 (dilution factor) X 50 (conversion factor).
- v. A260 / A280 ratio: Divide corrected A260 by corrected A280.

5.3.2.3 Genetic sequencing

Due to the lack of certainty about the causative genes or mutations in these genes in the patient sample, and also the low number of patient samples being examined, the screening for the presence of all of the known mutations in the known genes by SNP analysis was impractical. So, in order to look for mutations causing DI, it was decided to screen the COL1A1 gene as mutations in this gene are most commonly associated with DI type I. The method selected for this is based on the alignment and comparison of the fluorescent traces produced by Sanger DNA

sequencing of PCR amplified of genomic DNA. The DNA sequences obtained from patient samples are compared to standard reference sequences found on the NCBI genome data base (www.ncbi.nlm.nih.gov/genome). Figure 4-7 shows an example of the alignment of exon 11 of the BRCA1 gene in a patient versus reference sequence showing a point mutation.

Using this technology will enable any mutations (variations from the standard sequence) that are to be found in the patient samples and then derive an idea about the effect of these mutations might have on the protein being coded for.

For the sequencing reactions the area covering the first 8 exons of the Col1A1 gene were amplified by polymerase chain reaction (PCR) using the following primer pair:-

Forward primer TACTGACAACGCCCCTCTTC

Reverse primer TGGGAGTTCTTCTATAGGAG

Following this amplification step Sanger sequencing reactions were performed using the primer sequences as follows:

PrimerF2 GCCTTGTGTGTCCACTCTCC

PrimerF3 AGGGATGCATCTTTGCAGGAAT

PrimerR2 GAATTGAAAGGCAGAAGACGGC

PrimerR3 AAGGCCTCTCCACTTACTCCT

The sequencing reactions were run using the ABI 3730XL DNA analyzer (Life Technologies Corporation, 5791 Van Allen Way, PO Box 6482 Carlsbad, California 92008) using a 50cm array and running POP7 polymer. The chemistry we use is ABI Big Dye Terminator v 3.1 (ABI PRISM® BigDye™ Terminator v3.1 Cycle Sequencing Kit. Protocol Foster City, CA, USA: Applied Biosystems, 2002; Part number 4337035 Rev. A) (Kieleczawal and Mazaika, 2010). All the sequencing reaction was performed by Source (BioScience Dublin, Ireland).

The sequence data generated was compared to the published DNA sequences using VISTA - VISualization Tools for Alignments (Ernest Orlando Lawrence Berkeley National Laboratory, U.S.A.) - this URL allows the alignment of multiple sequences for comparison in order to identify any sequence variations.

5.4 Cephalometric Analysis

In our study cephalometric radiographs was used to evaluate the malocclusion. All cephalometric radiographs were traced using NemoTech Cephalometric tracing software and in order to avoid bias all cephalometric radiographs were traced by single examiner.

5.4.1 Skeletal Points

- 1) Nasion (N): The most anterior point on the frontonasal suture in the midline.
- 2) Sella (S): The midpoint of the sella turcica (pituitary fossa).
- 3) Orbitale (Or): The most inferior and anterior point on the orbital margin.
- 4) Basion (Ba): The most posterior and inferior part of foramen magnum.
- 5) Porion (Po): The upper- and outer-most point on the external auditory meatus.
- 6) Anterior nasal spine (ANS): The most anterior extension of the maxilla.
- 7) Posterior nasal spine(PNS): The most posterior extent of the hard palate.
- 8) Pogonion (Pg): The most anterior point on the bony chin.
- 9) Menton (Me): The most inferior point of the mandibular symphysis in the midline.

- 10) Gonion (Go): The most posterior and inferior point on the angle of the mandible.
- 11) Gnathion (Gn): The most anterior and inferior point on the bony chin.
- 12) Point A (subspinale): the deepest point on the curved profile of the maxilla between the anterior nasal spine and alveolar crest.
- 13) Point B (supramentale): the deepest point on the curved profile of the mandible between the chin and alveolar crest.

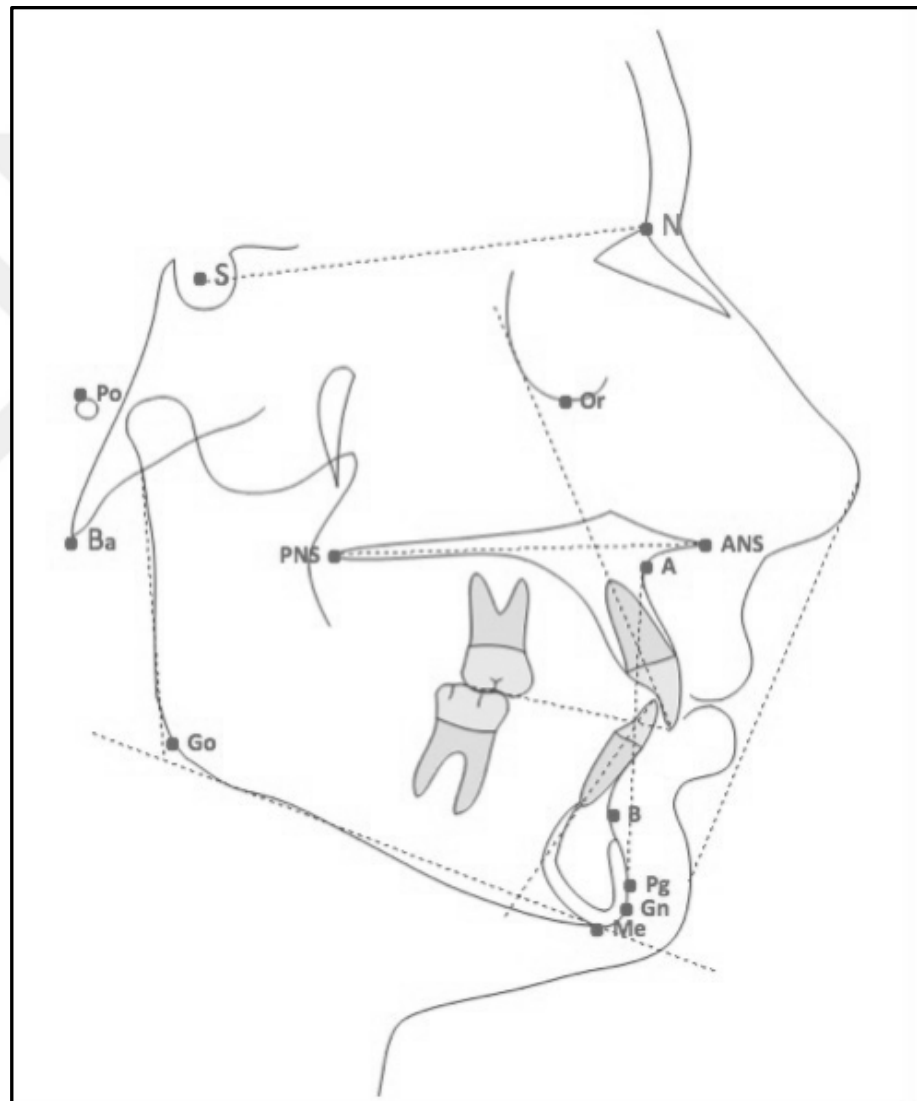


Figure 5.1 : Cephalometric Skeletal Points

5.4.2 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

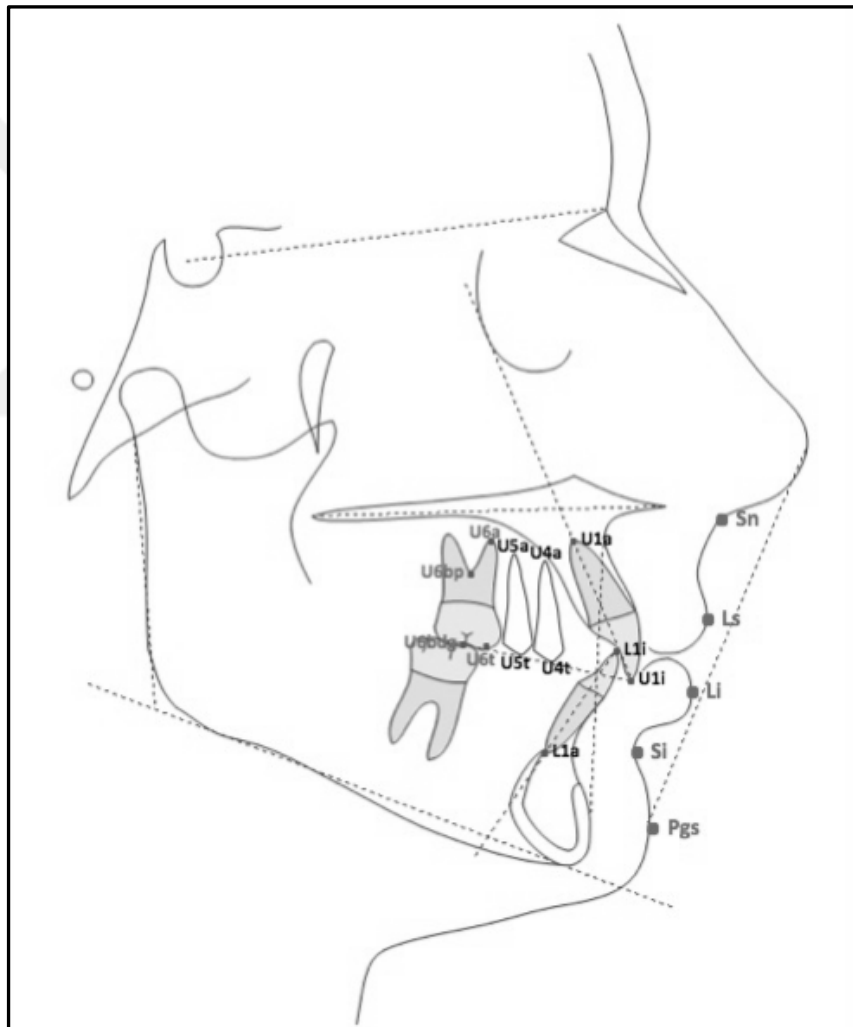


Figure 5.2 : Cephalometric Dental points

5.4.3 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 connecting the midpoint of sella turcica with nasion.
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 Horizontal Plane (FHP): The plane crossing from the superior point of Porion to the most inferior point of the Orbitale.

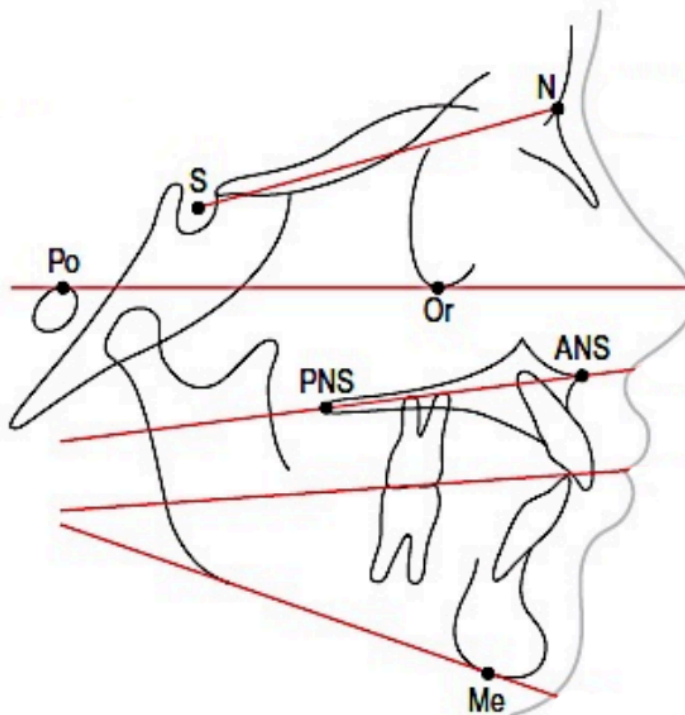


Figure 5.3 : Cephalometric reference planes

5.5 Cephalometric Measurements Used in this Study

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

Vertical

1. Sum of Inner Angles: The collective sum of the saddle angle, articular 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.

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.

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 5.1: Normal values of cephalometric analysis

Analysis	Normal	Standard deviation
Total inner angles	396°	+/- 3
GoMe-SN	32°	+/- 7
Jarabak	60%	+/- 1
ANSMc/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 were performed using SPSS software version 25. Descriptive analyses were presented using means, standard deviations, median, minimum and maximum values for continues data and frequencies 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

Phenotypic variability

The Marmara analysis resulted in 3 deferent categories sagittal, vertical, and dental, each expressed by deferent criteria. Vertical discrepancies expressed by total inner angles, GoMe-Sn, ANSMe/NMe, Jarabak analysis, Frankfort mandibular angle, and Maxillary height, vertical discrepancies ranging from skeletal open bites to skeletal deep bites for individuals.

Sagittal discrepancies explained by SNA, SNB, ANB, Wits analysis, N perpendicular A, and Maxillary depth, sagittal maxillomandibular discrepancies ranging from extremely convex profiles and class II skeletal malocclusions to extreme concave profiles and class III skeletal malocclusions in individuals.

Finally, dental discrepancies expressed by Incisor SN, Maxillary incisor to occlusal plane, Incisor Mandibular plane angle, and Mandibular incisor to occlusal plane.

Genotype-phenotype Associations

49 buccal swab samples were collected in this study which successfully analyzed for COL1A1 rs1800012 polymorphism. 5 (10,2%) of the patients were genotyped as AA, 18 (36,7%) as AC, and 26 (53,1%) as CC.

A few interesting associations were observed between the SNPs and type of skeletal malocclusion for class II or III skeletal malocclusion compared to skeletal class I, but none were significant. Interestingly, COL1A1 SNPs rs1800012 alleles AC and CC show probable association with vertical discrepancies, they were not significant.

7.1 Prevalence of evaluation criteria

Table 7.1: 2

		Frequency	Percent
<i>COLIA1</i>	AA	5	10,2
	AC	18	36,7
	CC	26	53,1
	Total	49	100,0

Table 7.2: Gender frequency

		Frequency	Percent
<i>Gender</i>	f	31	63,3
	m	18	36,7
	Total	49	100,0

Table 7.3: Total inner angles frequency

		Frequency	Percent
<i>Total inner angles</i>	low	29	59,2
	normal	11	22,4
	high	9	18,4
	Total	49	100,0

Table 7.4: GoMe-SN frequency

		Frequency	Percent
<i>GoMe-SN</i>	low	3	6,1
	normal	35	71,4
	high	11	22,4
	Total	49	100,0

Table 7.5: ANS Me/NMe Frequency

		Frequency	Percent
<i>ANS Me/NMe</i>	low	18	36,7
	normal	12	24,5
	high	19	38,8
	Total	49	100,0

Table 7.6: Jarabak frequency

		Frequency	Percent
<i>Jarabak</i>	low	36	73,5
	normal	7	14,3
	high	6	12,2
	Total	49	100,0

Table 7.7: FMA frequency

		Frequency	Percent
<i>FMA</i>	low	17	34,7
	normal	7	14,3
	high	25	51,0
	Total	49	100,0

Table 7.8: Maxillary Height frequency

		Frequency	Percent
<i>Max Height</i>	normal	2	4,1
	high	47	95,9
	Total	49	100,0

Table 7.9: Vertical pattern frequency

		Frequency	Percent
<i>Vertical</i> Pattern	low	21	42,9
	normal	9	18,4
	high	19	38,8
	Total	49	100,0

Table 7.10: SNA frequency

		Frequency	Percent
<i>SNA</i>	normal	23	46,9
	prognathic max	7	14,3
	retrognathic max	19	38,8
	Total	49	100,0

Table 7.11: SNB frequency

		Frequency	Percent
<i>SNB</i>	normal mand.	12	24,5
	prognathic mand.	9	18,4
	retrognathic mand.	28	57,1
	Total	49	100,0

Table 7.12: ANB frequency

		Frequency	Percent
<i>ANB</i>	Class I	17	34,7
	Class II	15	30,6
	Class III	17	34,7
	Total	49	100,0

Table 7.13: Wits frequency

		Frequency	Percent
<i>wits</i>	Class I	15	30,6
	Class II	11	22,4
	Class III	23	46,9
	Total	49	100,0

Table 7.14: N per A frequency

		Frequency	Percent
<i>N per A</i>	normal max	17	34,7
	prognathic max	12	24,5
	retrognathic max	20	40,8
	Total	49	100,0

Table 7.15: Maxillary depth frequency

		Frequency	Percent
<i>Max depth</i>	normal max	12	24,5
	prognathic max	9	18,4
	retrognathic max	28	57,1
	Total	49	100,0

Table 7.16: Maxillary position frequency

		Frequency	Percent
<i>max. position</i>	normal	17	34,7
	prognathic	9	18,4
	retrognathic	23	46,9
	Total	49	100,0

Table 7.17: I-SN frequency

		Frequency	Percent
<i>I-SN</i>	normal incisor angle	9	18,4
	Proclined Upper Incisor	28	57,1
	Retroclined Upper Incisor	12	24,5
	Total	49	100,0

Table 7.18: IMPA frequency

		Frequency	Percent
<i>IMPA</i>	normal incisor angle	5	10,2
	Proclined Upper Incisor	24	49,0
	Retroclined Upper Incisor	20	40,8
	Total	49	100,0

Table 7.19: UI-OP frequency

		Frequency	Percent
<i>UI-OP</i>	normal incisor angle	15	30,6
	Proclined Upper Incisor	18	36,7
	Retroclined Upper Incisor	16	32,7
	Total	49	100,0

Table 7.20: LI-OP frequency

				Frequency	Percent
<i>LI-OP</i>	normal	lower	incisor 8		16,3
	angle				
	Proclined lower Incisor		22		44,9
	Retroclined		lower 19		38,8
	Incisor				
	Total			49	100,0

7.2 Vertical Analysis

Table 7.21: Total inner angle analysis

			<u>genetic results COL1</u>			P value
			AA	AC	CC	
total inner angles	low	Count	2 _a	11 _a	16 _a	29
		% within total	6,9%	37,9%	55,2%	100,0%
		inner angles				
		% within genetic results COL1	40,0%	61,1%	61,5%	59,2%
normal		Count	2 _a	5 _a	4 _a	11
		% within total	18,2%	45,5%	36,4%	100,0%
		inner angles				
		% within genetic results COL1	40,0%	27,8%	15,4%	22,4%
high		Count	1 _a	2 _a	6 _a	9
		% within total	11,1%	22,2%	66,7%	100,0%
		inner angles				
		% within genetic results COL1	20,0%	11,1%	23,1%	18,4%
Total		Count	5	18	26	49
		% within total	10,2%	36,7%	53,1%	100,0%
		inner angles				
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0%

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and total inner angles $p > 0,05$.

Table 7.22: GoMe-SN analysis

		genetic results COL1				P value	
		AA	AC	CC	Total		
GoMe-SN	low	Count	0 _a	2 _a	1 _a	3	0,765
		% within GoMe-SN	0,0%	66,7%	33,3%	100,0%	
		% within genetic results COL1	0,0%	11,1%	3,8%	6,1%	
	normal	Count	4 _a	13 _a	18 _a	35	
		% within GoMe-SN	11,4%	37,1%	51,4%	100,0%	
		% within genetic results COL1	80,0%	72,2%	69,2%	71,4%	
	high	Count	1 _a	3 _a	7 _a	11	
		% within GoMe-SN	9,1%	27,3%	63,6%	100,0%	
		% within genetic results COL1	20,0%	16,7%	26,9%	22,4%	
Total	Count	5	18	26	49		
	% within GoMe-SN	10,2%	36,7%	53,1%	100,0%		
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%		

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and GoMe-SN $p > 0,05$.

Table 7.23: ANSMe/NMe analysis

			genetic results COL1				P value
			AA	AC	CC	Total	
ANS Me/NMe	low	Count	1 _a	9 _a	8 _a	18	0,537
		% within ANS Me/NMe	5,6%	50,0%	44,4%	100,0%	
		% within genetic results COL1	20,0%	50,0%	30,8%	36,7%	
	normal	Count	1 _a	3 _a	8 _a	12	
		% within ANS Me/NMe	8,3%	25,0%	66,7%	100,0%	
		% within genetic results COL1	20,0%	16,7%	30,8%	24,5%	
	high	Count	3 _a	6 _a	10 _a	19	
		% within ANS Me/NMe	15,8%	31,6%	52,6%	100,0%	
		% within genetic results COL1	60,0%	33,3%	38,5%	38,8%	
Total	Count		5	18	26	49	
	% within ANS Me/NMe		10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1		100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and ANS Me/NMe $p > 0,05$.

Table 7.24: Jarabak analysis

		genetic results COL1				P value	
		AA	AC	CC	Total		
Jarabak	low	Count	4 _a	14 _a	18 _a	36	0,871
		% within Jarabak	11,1%	38,9%	50,0%	100,0%	
		% within genetic results COL1	80,0%	77,8%	69,2%	73,5%	
	normal	Count	1 _a	2 _a	4 _a	7	
		% within Jarabak	14,3%	28,6%	57,1%	100,0%	
		% within genetic results COL1	20,0%	11,1%	15,4%	14,3%	
	high	Count	0 _a	2 _a	4 _a	6	
		% within Jarabak	0,0%	33,3%	66,7%	100,0%	
		% within genetic results COL1	0,0%	11,1%	15,4%	12,2%	
Total	Count	5	18	26	49		
	% within Jarabak	10,2%	36,7%	53,1%	100,0%		
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%		
	Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.						

There is no statistically significant frequency distribution difference between COL1 results and Jarabak $p > 0,05$.

Table 7.25: Frankfort mandibular angle analysis

		<u>genetic results COL1</u>				P value	
		AA	AC	CC	Total		
FMA	low	Count	2 _a	5 _a	10 _a	17	0,347
		% within FMA	11,8%	29,4%	58,8%	100,0 %	
		% within genetic results COL1	40,0%	27,8%	38,5%	34,7%	
	normal	Count	0 _a	5 _a	2 _a	7	
		% within FMA	0,0%	71,4%	28,6%	100,0 %	
		% within genetic results COL1	0,0%	27,8%	7,7%	14,3%	
	high	Count	3 _a	8 _a	14 _a	25	
		% within FMA	12,0%	32,0%	56,0%	100,0 %	
		% within genetic results COL1	60,0%	44,4%	53,8%	51,0%	
Total		Count	5	18	26	49	
		% within FMA	10,2%	36,7%	53,1%	100,0 %	
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0 %	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and FMA $p>0,05$.

Table 7.26: Maxillary height analysis

		genetic results COL1					P value
			AA	AC	CC	Total	
Max Height	normal	Count	0 _a	1 _a	1 _a	2	0,854
		% within Max Height	0,0%	50,0%	50,0%	100,0%	
		% within genetic results COL1	0,0%	5,6%	3,8%	4,1%	
	high	Count	5 _a	17 _a	25 _a	47	
		% within Max Height	10,6%	36,2%	53,2%	100,0%	
		% within genetic results COL1	100,0%	94,4%	96,2%	95,9%	
Total	Count		5	18	26	49	
	% within Max Height		10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1		100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the, 05 level.

There is no statistically significant frequency distribution difference between COL1 results and Max Height $p > 0,05$.

Table 7.27: Vertical pattern analysis

		<u>genetic results COL1</u>					P
			AA	AC	CC	Total	value
Vertical pattern analysis	low	Count	2 _a	9 _a	10 _a	21	0,619
		% within patient vertical	9,5%	42,9%	47,6%	100,0%	
		% within genetic results COL1	40,0%	50,0%	38,5%	42,9%	
	normal	Count	0 _a	4 _a	5 _a	9	
		% within patient vertical	0,0%	44,4%	55,6%	100,0%	
		% within genetic results COL1	0,0%	22,2%	19,2%	18,4%	
	high	Count	3 _a	5 _a	11 _a	19	
		% within patient vertical	15,8%	26,3%	57,9%	100,0%	
		% within genetic results COL1	60,0%	27,8%	42,3%	38,8%	
Total	Count	5	18	26	49		
	% within patient vertical	10,2%	36,7%	53,1%	100,0%		
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%		

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and patient vertical pattern $p > 0,05$.

7.3 Sagittal Analysis

Table 7.28: ANB analysis

			genetic results COL1				P value
			AA	AC	CC	Total	
ANB	Class I	Count	3 _a	7 _a	7 _a	17	0,467
		% within ANB	17,6%	41,2%	41,2%	100,0%	
		% within genetic results COL1	60,0%	38,9%	26,9%	34,7%	
	Class II	Count	0 _a	6 _a	9 _a	15	
		% within ANB	0,0%	40,0%	60,0%	100,0%	
		% within genetic results COL1	0,0%	33,3%	34,6%	30,6%	
	Class III	Count	2 _a	5 _a	10 _a	17	
		% within ANB	11,8%	29,4%	58,8%	100,0%	
		% within genetic results COL1	40,0%	27,8%	38,5%	34,7%	
Total	Count		5	18	26	49	
	% within ANB		10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1		100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and ANB $p > 0,05$.

Table 7.29: Wits analysis

			<u>genetic results COL1</u>				P value	
			AA	AC	CC	Total		
wits	Class I	Count	1 _a	6 _a	8 _a	15	0,361	
		% within wits	6,7%	40,0%	53,3%	100,0%		
		% within genetic results COL1	20,0%	33,3%	30,8%	30,6%		
		Class II	Count	0 _a	6 _a	5 _a		11
			% within wits	0,0%	54,5%	45,5%		100,0%
			% within genetic results COL1	0,0%	33,3%	19,2%		22,4%
	Class III	Count	4 _a	6 _a	13 _a	23		
		% within wits	17,4%	26,1%	56,5%	100,0%		
		% within genetic results COL1	80,0%	33,3%	50,0%	46,9%		
	Total	Count	5	18	26	49		
		% within wits	10,2%	36,7%	53,1%	100,0%		
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0%		

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and wits $p > 0,05$.

7.4 Maxillary Position Analysis

Table 7.30: SNA analysis

		genetic results COL1				P value
		AA	AC	CC	Total	
SNA normal	Count	2 _a	6 _a	13 _a	23	0,646
	% within SNA	8,7%	31,6%	56,5%	100,0%	
	% within genetic results COL1	40,0%	33,3%	50,0%	46,9%	
	prognathic max Count	0 _a	4 _a	3 _a	7	
	% within SNA	0,0%	57,1%	42,9%	100,0%	
	% within genetic results COL1	0,0%	22,2%	11,5%	14,3%	
	retrognathic max Count	3 _a	8 _a	10 _a	19	
	% within SNA	15,8%	34,8%	52,6%	100,0%	
	% within genetic results COL1	60,0%	44,4%	38,5%	38,8%	
Total	Count	5	18	26	49	
	% within SNA	10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and SNA $p > 0,05$.

Table 7.31: N perpendicular A analysis

		<u>genetic results COL1</u>				P value
		AA	AC	CC	Total	
N per normal max A	Count	1 _a	6 _a	10 _a	17	0,373
	% within N per A	5,9%	35,3%	58,8%	100,0%	
	% within genetic results COL1	20,0%	33,3%	38,5%	34,7%	
prognathic max A	Count	2 _a	2 _a	8 _a	12	
	% within N per A	16,7%	16,7%	66,7%	100,0%	
	% within genetic results COL1	40,0%	11,1%	30,8%	24,5%	
retrognathic max A	Count	2 _a	10 _a	8 _a	20	
	% within N per A	10,0%	50,0%	40,0%	100,0%	
	% within genetic results COL1	40,0%	55,6%	30,8%	40,8%	
Total	Count	5	18	26	49	
	% within N per A	10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and N per A $p>0,05$.

Table 7.32: Maxillary depth analysis

		<u>genetic results COL1</u>					P value
		AA	AC	CC	Total		
Max depth	normal max	Count	0 _a	4 _a	8 _a	12	0,432
		% within Max depth	0,0%	33,3%	66,7%	100,0%	
		% within genetic results COL1	0,0%	22,2%	30,8%	24,5%	
	prognathic max	Count	1 _a	2 _a	6 _a	9	
		% within Max depth	11,1%	22,2%	66,7%	100,0%	
		% within genetic results COL1	20,0%	11,1%	23,1%	18,4%	
	retrognathic max	Count	4 _a	12 _a	12 _a	28	
		% within Max depth	14,3%	42,9%	42,9%	100,0%	
		% within genetic results COL1	80,0%	66,7%	46,2%	57,1%	
Total	Count	5	18	26	49		
	% within Max depth	10,2%	36,7%	53,1%	100,0%		
	% within genetic results COL1	100,0%	100,0%	100,0%	100,0%		

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and Max depth $p>0,05$.

Table 7.33: Maxillary position analysis

			<u>genetic results COL1</u>				P value	
			AA	AC	CC	Total		
max. position	normal	Count	1 _a	5 _a	11 _a	17	0,459	
		% within max. position	5,9%	29,4%	64,7%	100,0%		
		% within genetic results COL1	20,0%	27,8%	42,3%	34,7%		
	prognathic	Count	1 _a	2 _a	6 _a	9		
		% within max. position	11,1%	22,2%	66,7%	100,0%		
		% within genetic results COL1	20,0%	11,1%	23,1%	18,4%		
	retrognathic	Count	3 _a	11 _a	9 _a	23		
		% within max. position	13,0%	47,8%	39,1%	100,0%		
		% within genetic results COL1	60,0%	61,1%	34,6%	46,9%		
Total			Count	5	18	26	49	
			% within max. position	10,2%	36,7%	53,1%	100,0%	
			% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and max position $p > 0,05$.

7.5 Mandibular Position Analysis

Table 7.34: SNB analysis

		genetic results COL1			Total	P value
		AA	AC	CC		
SNB	normal mand.	Count	1 _{a, b}	1 _b	10 _a	12
		% within SNB	8,3%	8,3%	83,3%	100,0%
		% within genetic results COL1	20,0%	5,6%	38,5%	24,5%
	prognathic mand.	Count	1 _a	5 _a	3 _a	9
		% within SNB	11,1%	55,6%	33,3%	100,0%
		% within genetic results COL1	20,0%	27,8%	11,5%	18,4%
	retrognathic mand.	Count	3 _a	12 _a	13 _a	28
		% within SNB	10,7%	42,9%	46,4%	100,0%
		% within genetic results COL1	60,0%	66,7%	50,0%	57,1%
Total		Count	5	18	26	49
		% within SNB	10,2%	36,7%	53,1%	100,0%
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0%

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and SNB $p > 0,05$. Only the people who have normal mandibular position have significant proportion difference between CC and AA and between CC and AC.

7.6 Upper Incisor Inclination Analysis

Table 7.35: Incisor SN analysis

			genetic results COL1				P value
			AA	AC	CC	Total	
ISN	normal angle	incisor Count	3 _a	2 _a	4 _a	9	0,099
		% within I-SN	33,3%	22,2%	44,4%	100,0%	
		% within genetic results COL1	60,0%	11,1%	15,4%	18,4%	
	Proclined Incisor	Upper Count	1 _a	10 _a	17 _a	28	
		% within I-SN	3,6%	35,7%	60,7%	100,0%	
		% within genetic results COL1	20,0%	55,6%	65,4%	57,1%	
	Retroclined Upper Incisor	Count	1 _a	6 _a	5 _a	12	
		% within I-SN	8,3%	50,0%	41,7%	100,0%	
		% within genetic results COL1	20,0%	33,3%	19,2%	24,5%	
Total	Count		5	18	26	49	
	% within I-SN		10,2%	36,7%	53,1%	100,0%	
	% within genetic results COL1		100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and I-SN $p > 0,05$.

Table 7.36: Maxillary incisor occlusal plane analysis

			genetic results COL1				P value
			AA	AC	CC	Total	
UI-OP	normal angle	incisor Count	0 _a	4 _a	11 _a	15	0,072
		% within UI-OP	0,0%	26,7%	73,3%	100,0%	
		% within genetic results COL1	0,0%	22,2%	42,3%	30,6%	
Proclined Incisor	Upper	Count	1 _a	7 _a	10 _a	18	
		% within UI-OP	5,6%	38,9%	55,6%	100,0%	
		% within genetic results COL1	20,0%	38,9%	38,5%	36,7%	
Retroclined Upper Incisor		Count	4 _a	7 _{a, b}	5 _b	16	
		% within UI-OP	25,0%	43,8%	31,3%	100,0%	
		% within genetic results COL1	80,0%	38,9%	19,2%	32,7%	
Total		Count	5	18	26	49	
		% within UI-OP	10,2%	36,7%	53,1%	100,0%	
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and UI-OP $p > 0,05$. Only the people who have Retroclined Upper Incisor have significant proportion difference between AC and AA and between AC and CC.

7.7 Lower Incisor Inclination Analysis

Table 7.37: Incisor Mandibular plane angle analysis

			<u>genetic results COL1</u>				P value	
			AA	AC	CC	Total		
IMPA	normal angle	incisor	Count	2 _a	2 _{a, b}	1 _b	5	0,145
			% within IMPA	40,0%	40,0%	20,0%	100,0%	
			% within genetic results COL1	40,0%	11,1%	3,8%	10,2%	
Proclined Incisor	Upper	Incisor	Count	1 _a	8 _a	15 _a	24	
			% within IMPA	4,2%	33,3%	62,5%	100,0%	
			% within genetic results COL1	20,0%	44,4%	57,7%	49,0%	
Retroclined Upper Incisor		Upper Incisor	Count	2 _a	8 _a	10 _a	20	
			% within IMPA	10,0%	40,0%	50,0%	100,0%	
			% within genetic results COL1	40,0%	44,4%	38,5%	40,8%	
Total			Count	5	18	26	49	
			% within IMPA	10,2%	36,7%	53,1%	100,0%	
			% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	
Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.								

There is no statistically significant frequency distribution difference between COL1 results and IMPA $p>0,05$.

Table 7.38: Mandibular incisor occlusal plane analysis

			genetic results COL1			Total	P value
			AA	AC	CC		
LI- normal	lower	Count	1 _a	4 _a	3 _a	8	0,672
OP incisor angle		% within LI-OP	12,5%	50,0%	37,5%	100,0%	
		% within genetic results COL1	20,0%	22,2%	11,5%	16,3%	
Proclined Incisor	lower	Count	1 _a	8 _a	13 _a	22	
		% within LI-OP	4,5%	36,4%	59,1%	100,0%	
		% within genetic results COL1	20,0%	44,4%	50,0%	44,9%	
Retroclined Incisor	lower	Count	3 _a	6 _a	10 _a	19	
		% within LI-OP	15,8%	31,6%	52,6%	100,0%	
		% within genetic results COL1	60,0%	33,3%	38,5%	38,8%	
Total		Count	5	18	26	49	
		% within LI-OP	10,2%	36,7%	53,1%	100,0%	
		% within genetic results COL1	100,0%	100,0%	100,0%	100,0%	

Each subscript letter denotes a subset of genetic results COL1 categories whose column proportions do not differ significantly from each other at the ,05 level.

There is no statistically significant frequency distribution difference between COL1 results and LI-OP $p>0,05$

8. DISCUSSION

8.1 Discussion of Aims

The purpose of this study is to draw a relation between skeletal malocclusion and *COL1A1* rs1800012 polymorphism in order to determine whether collagen 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.

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).

For many years, studies have been conducted to determine the prevalence of malocclusion in different populations. A comparison of these results is almost impossible, and the results of studies, even those studies conducted in a population of the same origin, may show great variability. Variables such as the differences in classification of occlusal relationships, the developmental period of the study sample, examiner differences in determining the bounds of normal, and differences in sample sizes can affect the results. Instead of differentiating normal and abnormal in a population, determining frequencies of different types of malocclusions in a referred population may also give valuable information (M. Özgür and Türkkahraman, 2004).

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 et al., 2015).

The biggest concern shared among orthodontists and patients is the treatment outcome and stability of conventional orthodontic treatment and the impact on life. For patients, it has psychological and social impact although they agree to having treatment, it does have an impact on their daily life and want to be permanent and stable. Johal et al. showed that self-esteem of patients does not improve in the first few months of orthodontic treatment, but slightly improves as treatment continues.

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 et al., 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 buccal mucosa. Buccal swab is an excellent source of DNA for many types of genetic studies. Research has revealed that buccal mucosal DNA is equivalent in quantity and purity to DNA obtained from blood (Rylander-Rudqvist et al., 2006)(Quinque et al., 2006) and that the stability of buccal mucosal DNA is good when proper methods of handling are employed (Quinque et al., 2006).

Buccal mucosa swab 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 venipuncture, it does not quite meet the definition of 'non-invasive'. Taken all these into consideration, buccal swabs 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

When comparing the genotype and the general vertical pattern, it showed that patient with AC genotype were associated with higher frequency of low angle vertical pattern and patient with CC genotypes were associated with higher frequency of high vertical pattern patients.

When the total Inner angle was compared with genotypes, the AC and CC genotypes were also associated with higher frequency of patients with low vertical pattern. The same results were found when the genotypes were compared to the Jarabak results, both comparisons were statistically insignificant.

When maxillary height and Frankfort mandibular angle were used for comparing the vertical pattern with the genotypes, it was found out that the patients with CC genotype were 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.

When comparing the genotype and ANS Me/NMe, it showed that patient with AC genotype were associated with higher frequency of low angle vertical pattern and patient with CC genotypes were associated with higher frequency of high vertical pattern patients. which coincident the finding of the previous results where the general

vertical pattern where used to determine the skeletal vertical pattern of the patient. All the results were statistically not significant.

On the other hand, The CC and AC genotypes were associated with higher frequency of patient with normal vertical pattern when GoMe-Sn were used to determine the vertical pattern of the patients. When total Inner angle and Jarabak were used it showed high frequency of high angle vertical pattern with patients having CC and AC genotypes.

Meanwhile, ANB and Wits were used for determining skeletal sagittal relationship, it was found that patients with CC genotype were associated with developing class II or III of skeletal malocclusion relative to class I this relation was statistically not significant, in the study conducted by Da Fontoura he suggested a relationship between patient with Class III and Class II phenotypes and COL1A1 (Da Fontoura et al., 2015).

When comparing the genotype and SNA and N per A, it showed that patient with AC genotype were associated with higher frequency of retrognathic maxillary position and patients with CC genotypes were associated with higher frequency of normal maxillary position.

In contrast, when comparing genotype and maxillary depth, AC and CC genotype shows higher frequency of retrognathic maxillary position. which contradict the finding of the previous results where SNA and N per A where used to determine the maxillary position of the patient. All the results were statistically not significant.

For the mandibular position AC,CC genotypes were associate with higher frequencies of retrognathic mandible phenotype, which coincides with the finding of Ardani that there are SNPs in COL1A1 in class II skeletal malocclusion in Javanese ethnic group patients with mandibular micrognathism (Ardani et al., 2020). The relationship was statistically not significant.

CC genotype were associated with increased incisor inclination phenotype when both incisor SN was used to determine incisor inclination. While if incisor-occlusal plane was used to determine the phenotype CC genotype was associated with

higher frequency of normal incisor angle. The relationship was statistically not significant.

For the lower incisor inclination when incisor mandibular plane angle and incisor occlusal plane were used the CC genotype was associated with higher frequency of Proclined mandibular incisor phenotype. The relationship was statistically not significant.



9. CONCLUSION

- ◆ This study shows that there is a possible relationship between upper and lower incisors inclination and skeletal sagittal and vertical relationship phenotypes and the CC,AC and AA genotypes.
- ◆ There is a possible relationship between CC genotype and patient with high vertical growth pattern phenotype, and also AC genotype and patient with low vertical growth pattern phenotype.
- ◆ COL1A1 allele AC suspected to be a characteristic allele for Class III patients as AC genotype were associated with higher frequency of retrognathic maxillary position.
- ◆ COL1A1 allele CC suspected to be a characteristic allele for Class II patients as CC genotypes were associate with higher frequencies of retrognathic mandible phenotype.
- ◆ There is a possible relationship between CC genotype and patient with Proclined mandibular incisor phenotype.

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