Genetics and epigenetics of class II and class III malocclusions

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Abstract. Malocclusion is the major diagnosis in orthodontic field. According to Angle’s classification, malocclusion is divided into Class I, Class II, and Class III malocclusion. All of these classifications have etiologic factors that establish every specific characteristic of malocclusion. Genetic, is one of the most important etiology in malocclusion since it can be inherited congenitally, for instance many variations in transcription and translation of multifarious genes occurred in masseter muscles, which is the main mastication muscle in human. MYH/MyH-C, MMPs and IGF genes are frequently used to determine the expression of genes in masseter muscle using RT-PCR or cRT-PCR. On the other hand, polymorphism of ACTN3, which can influence fibre type proportions and also muscle performance is found in muscle and skeletal type of Class II malocclusion. MATN1, HSPG2, ALPL, and EPB41 genes are found linked to lp36 related to Class III malocclusion. However, genetic factor does not usually stand alone. It can be influenced by environment which called epigenetic factors. Increasing acetylation activity will initiate a chromatin domain formation that consists of genes for MyH-C fast type gene expression. In contrast, increased of deacetylation activity resulting in closed chromatin confirmation on the chromatin area to limit the access into transcription complexes for MyHC type I gene expression. In malocclusion cases, the most common way to study about heritability is using masseter muscles by analyzing their types of fibers related to every malocclusion’s phenotype. In this review, writer will explain more about genetic study in masseter, histone modification, and also genetic and epigenetic factors of Class II and Class III malocclusions, which involve gene mutation and polymorphism for genetic factors and histone acetylation and deacetylation for epigenetic factors.

1. Introduction
As we all already acknowledged, the main and most common diagnosis in orthodontic is malocclusion. Malocclusion is a developmental condition in which most cases are caused by moderate or severe distortions of normal development, not by pathologocial processes [1].
One is stated for having a malocclusion if there is a variation from an ideal occlusion that has dental health and/or psychosocial implications [2]. Malocclusion develops as a condition with complex trait, which is influenced by factors of transcription and factors of growth that acts on bone, teeth, and skeletal muscles [3]. Referring to Angle’s classification, malocclusion is divided into Class I, Class II, and Class III malocclusions. Malocclusion derived from many etiological factors. Profit examined malocclusion from three major etiological factors. There are specific causes, hereditary influences, and environmental influences. In this review, we emphasize etiological factor for malocclusions from hereditary influences [4].

Many studies have been carried out to find out about the relationship between malocclusions and hereditary factors. Twin studies, a study that compares between monozygotic and dizygotic twins, is the most common studies that have been used to find out about hereditary traits involved in malocclusions. For example, a study conducted by Horowitz, Osborne, and DeGeorge (1990) resulted that in adult twins that is monozygotic type, variations in mandibular length, anterior cranial base, facial height (total and lower) were exhibit significantly. There was also a study conducted by Hunter (1965), he did a linear measurement on lateral cephalograms and the result stated that genetic components of variability for vertical measurements are stronger than sagittal/anteroposterior dimension [5,6]. Thus, many scientists use this twin study method as a reference to measure hereditary influences in malocclusions.

Since the Class II and Class III malocclusions are considered more complex than the Class I malocclusion, this literature review will be observing more about hereditary influences in these two particular malocclusions only. We hope this review will help many clinicians, especially orthodontists, in determining the right diagnosis and proper treatment planning for the patients.

2. Genetic studies in masseter muscle
Masseter muscle is one of the most important components in stomatognathic system. Any changes in masseter muscle’s morphology or functional movements can affect craniofacial form. Also, many variations in transcription and translation of multifarious genes occurred in masseter muscles. Continuous force of masseter muscle on the mandible is considered to be almost nonstop, representing a low amplitude, high frequency source of force, thus it will affect bone morphology and composition. Therefore, this type of muscle is the most common to be used in the identification of malocclusion.

In maseter muscle studies for malocclusions, one of the most commonly used research methods is by using RT-PCR or cRT-PCR to analyze maseter muscle samples from orthodontic patients that treated with orthognathic surgery, especially when the used surgical technique was bilateral sagittal split osteotomy. From that technique, adequate maseter muscle samples can be obtained from the deep portion’s superficial area point, 1.5 centimeters from the angle of mandible’s lowest point. The maseter muscle samples obtained then stored on wet sterile gauze wraparound in ice. Each specimen sized about 0.5 cm² and weighed 60 to 120 milligrams. To avoid mRNA’s degradation of molecule, muscle tissues were kept frozen in isopentane, cooled by liquid form of nitrogen (approximately 196°C) few minutes after excision and then these sample was cut on a cryostat at approximately 10 µm. Anti-MHC antibodies was used afterwards to differentiate the four fiber types contained in the muscle by immunostaining the samples. Gene analysis was held afterward by isolating rNA with TRIzol reagent, treating them with DNAse 1, and reisolating them with RNAqueous. After gene analysis, microarray and statistical analysis were conducted by comparing all the data according to ages, sex, sagittal, and vertical malocclusion group.
When studying the expression of genes in masseter muscles, there are several kinds of genes that are frequently used:

a. Genes encoding the Myosin Heavy Chain (MyHC/MHC proteins of masseter muscle
b. Genes encoding several modulator proteins which considered to be important in masseter muscle regeneration (such as fibronectin) and remodeling (such as metalloprotenases/MMPs)

c. Regenerating muscle genes as a respond to the mechanic stimulus (for example, the Insulin-like Growth Factors/IGF)

d. Myostatin genes involved in the adaptive behavior of masseter muscle

Masseter muscle also consists four types of fibers which differs in compositions for every classification of malocclusions. These types of fibers are:

a. Type I MHC-protein which consists (MYH7).

b. Type IIA (MYH2) and/or type IIX , which consists MHC-protein.

c. Hybrid type I/II, which consists a mixture of both MHC protein types.

Neonatal/ atrial type, which consists α cardiac muscle isoforms combined with hybrid type I/II [3,7–9].

These fiber types have functional differences in response to muscle mechanic force mechanism that will affect mechano-transduction and bone modeling to produce jaw deformation phenotype. Although there are no evidence proved that variations in size or proportions of masseter muscle fiber types will affect condylar growth or mandibular length yet, fiber types are considered to be tightly related with facial vertical growth’s variations.

3. Histone modifications

Epigenetic is a term that defines a heritable change in phenotype without involving the underlying DNA sequence change. Acetylation, phosphorylation, methylation, ubiquination, and ADP ribosylation of the greatly preserved histones are parts of epigenetic modifications which influence the genetic potential of DNA. The greatly preserved histones are including H2A, H2B, H3, and H4. In a eukaryote cell’s nucleus, genomic DNA are located around octamers of these protein core to form nucleosome [10]. At cellular and molecular level, epigenetic may arise from three mechanisms. They are DNA methylation, micro RNA based mechanisms, and histone modifications. From these three mechanisms, histone modifications are known to be the epigenetic regulators of the chromatin. Histone modification comprises of histone acetylation and histone deacetylation.

![Histone octamer](image)

**Figure 1.** Histone octamer
Histone acetylation is regulated by the histone acetyltransferase enzyme which catalyze the addition of acetyl group to histone groups. On the contrary, histone deacetylation is a process of removing groups of acetyl from histone groups that catalyzed by histone deacetylase enzyme, resulting in chromatin compaction and gene transcription inhibition [11]. Martin and Zhang (2005) concluded that special histone modifications have the responsibility for the genome compartmentalization into different domains; and according to Kouzarides (2007), histone code is able to command the environment of chromatin, allowing it to manage some of nuclear processes, specifically replication, transcription, DNA repair, and condensation of chromosome [10].

![Figure 2. Epigenetic mechanisms. [11]](image)

4. **Class II malocclusion**

An occlusion considered as a Class II malocclusion is when the lower teeth occlude more distally from the normal occlusion [1]. This classification of malocclusion is often characterized with upper jaw is positioned more anteriorly than the lower jaw or the lower jaw is positioned more posteriorly than the upper jaw. Furthermore, Class II malocclusion are divided into Class II division 1 and Class II division 2 malocclusion. A Class II division 1 malocclusion is identified by a narrow maxilla, proclined upper anterior teeth, abnormal lip function, and some form of nasal obstruction and mouth breathing. Whereas the other division is characterized by a slightly narrow maxilla, crowding of the upper incisors which consists of overlapping and lingual inclination, and also a normal functioning of lip and nasal [12]. Class II division 1 and 2 have obviously different phenotypic characteristics, but that does not mean they both do not have the same polygenic inheritance [6].

4.1. **Genetics in Class II Malocclusion**

Heritability factor, which is closely associated to genetic components, is one of the etiologic cause of Class II malocclusions. Previously mentioned in the beginning of this review, masseter muscle fiber types are frequently used to study about gene association in malocclusion, including in Class III malocclusion.

There is one particular gene that influence fiber type proportions and also muscle performance. This gene is called ACTN3. There are also cytoskeletal proteins called α-actinins, binding actin filaments of
many types of cells. In skeletal muscle, there are two types of these proteins which commonly found, α-actinin-2 and α-actinin-3. These two skeletal muscle isoforms are encoded by ACTN2 and ACTN3 genes, and they are discovered on different chromosomes where ACTN2 is located in the long arm chromosome 1 while ACTN3 is located on chromosome 11. Their gene expressions are also different. Gene expression of ACTN2 is seen in all skeletal muscle fibers, but the expression of ACTN3 is only seen in a subset of type II fibers (fast type fibers) [13,14].

Genetic mechanisms of Class II malocclusion involved polymorphisms of those genes. An α-actinin-3 protein will increase skeletal muscle’s strength and contraction, this is happened because they were found in fast-type muscle fibers. Therefore, their contraction are considered to be fast and forceful. There are two single nucleotide polymorphisms (SNPs) in ACTN3, they are at rs18155739 and rs678397. These intronic SNPs have the possibility to alter level of proteins and have an effect on α-actinin-3 function [13].

R577X is a common gene polymorphism in ACTN3. This gene polymorphism will eliminate α-actinin-3 proteins which result in alterations of many aspects, such as muscle metabolism, bone mineralization, and fiber type proportions. Loss of α-actinin-3 is a result of a term called nonsense mutation in genetic studies. Mutation happened when DNA sequence is shifting permanently. A nonsense mutation is an alteration of DNA sequence which causes a premature stop codon in the transcribed mRNA. From real-time polymerase chain experiments, this loss of α-actinin-3 in masseter muscles were not compensate by ACTN2, because ACTN2 expression levels are adamantly unchanged while ACTN3 mRNA expression was undetectable. The absence of α-actinin-3 was commonly featured with type II fibers of masseter muscles in smaller diameters, but this action resulting in a significant genotype relationship, in which the decreasing fiber diameters will make the higher percentage of type II fiber. This is also causing an increase in ENPP1 expression. ENPP1 is a negative regulator of mineralization. Therefore, this phenomenon is closely associated with the development of Class II [13–15].

### Nonsense mutation

![Nonsense mutation](image)

**Figure 3.** Nonsense mutation

#### 4.2. Epigenetic in Class II Malocclusion

Epigenetic in Class II malocclusion occurs through histone modification. This process implicates Myosin Heavy Chain (MyHC) genes using the masseter muscle samples. It is an essential contractile protein in the skeletal muscle fibers that are encoded by genes in chromosome 14. Epigenetic regulation model increasing acetylation activity will initiate a chromatin domain formation that consists of genes for MyHC fast type gene expression. In contrast, increased of deacetylation activity resulting in closed
chromatin confirmation on the chromatin area to limit the access into transcription complexes for MyHC type I gene expression in malocclusion cases. Epigenetic mechanism, which also applied in malocclusion, consists of acetylation of lysine residue on the histone chromatin proteins by K-lysine Acetyltransferase 6B (KAT6B) enzyme and deacetylase of Histone Deacetylase 4 (HDAC4) enzyme. KAT6B is an enzyme of chromatin remodeling epigenetic and activated by MYO1C. This process results in HDAC4 gene expression negatively correlated with slow type I fibers and myosin type IIX fibers (fast type) positively. At the same time, KAT6B activity correlated with type IIX fibers negatively but not associated with type I myosin fibers (slow type) gene expression [3]. This phenomenon concludes that when there is an increase in type II muscle fibers population, it will decrease vertical dimension, which a decreased vertical dimension is one of the most familiar characteristics in Class II malocclusion.

5. Class III malocclusion

Contrary to Class II malocclusion, a Class III malocclusion occurred when the lower teeth occlude more mesially from the normal occlusion2. Usually, the lower jaw is positioned more protrusive than the upper jaw. This is why in Class II malocclusion the maxillary first molar’s mesiobuccal cusp of the is distally positioned [16]. The prevalence of Class III malocclusion are multifarious and may exhibit various anatomic characteristics among different ethnic groups [17]. The most widely known Class III malocclusion example is the pedigree of Hapsburg Jaw 18. Xue (2010) stated that this type of malocclusion is considered as a polygenic disorder and it is a result from interaction between susceptibleness of genes and environmental factors [18].

5.1. Genetics in Class III malocclusion

Many gene linkage studies have been done in Class III malocclusions. The results are also vary from one chromosome to another. Studies from Balkhande P B (2018) and Doraczynska-Kowalik A (2017) show that the gene bounds in Class III malocclusions that have been conducted in genetic studies are located in 1p36, 1p22.1, 2p13, 3q26.2, 6q25, 11q22, 12q1, 12q3, 12q13.13, 12q23, 14q24.3, 14q31.2, and 19p13.2. [19,20] But from all of those gene locations, the most frequently mentioned are genes in 1p36. There are four genes in 1p36 related to Class III malocclusion[19,20]:

a. Matrilin (MATN1); is a cartilage extracellular matrix protein secreted by chondrocytes to upregulate chondrogenesis.
b. Heparan Sulfate Proteoglycan 2 (HSPG2); is a molecule containing multidomain protein associated to cartilage formation and craniofacial abnormalities.
c. Alkaline Phosphatase (ALPL); plays a role in mineralization and vitamin B6 metabolism.
d. Erythrocyte Membrane Protein Band 4.1 (EPB41); consists of protein associated with the cytoskeleton.

Aside from those gene linkage analyses, there is also a missense mutation phenomenon occurs in relation with Class III malocclusion. Nikopensius, et al (2013) showed that missense mutation which is a point mutation in which single nucleotide results in a codon, coding for a different amino acid also appear in Class III malocclusion, a scarce missense mutation that is heterozygous at DUSP6 gene c.545C>T (p.Ser182Phe).[21] Here, a single nucleotide of cytosine was mutated into thymine. Therefore, the amino acid which supposedly produces Serine protein, produce Phenylalanine protein instead. p.Ser182Phe (rs139318648) is an uncommon variant of tri-allelic at dbSNP137 and 1000 genome database, with MAF of 0.00066. DUSP6 gene transcription activation allegedly regulated by Fibrous Growth Factor/Fibrous Growth Factor Receptor (FGF/FGFR) and MAPK/ERK signaling during the important process of skeletal growth's early stage. FGFR2 and FGFR3 allegedly associated to retrognathism and/or hypoplasia of maxilla for their implication in biology of cranial suture and craniosynostosis and they also indicated to modulate risk of general abnormal maxillomandibular discrepancies, which Class III malocclusion has the same characteristic with. On the other hand, growth factors like NGF, PDGF, EGF, and HGF, also have a role in adult tissue development. These growth
factors can generate ERK and initiate the transcription of other members of DUSP6 family to compensate if there is a lack of DUSP6 genes. Other studies also mentioned IGF1, located at 12q23, to be associated with Class III malocclusion in which represents a favorable biological interest candidate gene because their system has a notable role in the growth of skeletal complex and metabolism of normal bone [18,21,22].

![Missense mutation](image)

**Figure 4. Missense mutation**

### 5.2. Epigenetics in Class III Malocclusion

Other aspects that we must consider of having an association with Class III malocclusion, is epigenetic factor. Related to Class II malocclusion, epigenetic in Class III malocclusion is also involving the activity of KAT6B and HDAC4. In Class III malocclusions, the expression of these genes works vice versa. Correlation between the expression of KAT6B and HDAC4 and the expression of MYH1, MYH2, MYH6, MYH7, and MYH8, stated that HDAC4 expression has a negative correlation with MYH7 expression and in contrast, their correlation with MYH1 expression is positive. The amount of KAT6B mRNA expressed in Class III malocclusion was greater than in Class II malocclusion. This also leads to an osteogenic transcription factor RUNX2 activation, making KAT6B hold a strong association with mandibular prognathism [3](Huh et al. 2013, Heather Desha et al. 2015, Doraczynska-Kowalik et al. 2017).

### 6. Conclusions

Genetic and epigenetic are two important mechanisms that affect human life. Malocclusion, for example, is also influenced by these mechanisms. There are three classification of malocclusion according to Angle, and Class II and Class III have their own specific characteristic that were derived from genetic and epigenetic process. Recently, the most common way to study about heritability in malocclusion is using masseter muscles by analyzing their types of fibers related to every malocclusion’s phenotype. Genetic mechanism in these two types of malocclusions may arise from nonsense and missense mutation of DNA. Whilst the epigenetic mechanism occurs through histone modifications by histone acetylation and deacetylation.

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