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MICRO-RNA REGULATORS OF CANDIDATE GENES INVOLVED IN CLASS II SKELETAL MALOCCLUSION - A DATA MINING APPROACH

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Abstract

Background:

Epigenetic regulators play a vital role in determining a complex phenotype. Skeletal Class II malocclusion is one such phenotype, which is a polygenic, complex disorder. The identification of epigenetic regulators would aid in understanding the complex relationship between the epigenetic marks and the phenotype. Also, these epigenetic marks can be considered for developing diagnostic leads upon validation for a specific disorder.

Materials and methods:

The present study follows an observational study design, which was performed using computational tools. The preliminary data about the genes associated with the Skeletal class II malocclusion was derived from DisGeNet, followed by identification of the protein-protein interaction networks. The microRNA targets were then identified using miRDB and the unique microRNA population common to all the five genes were further curated using the Venn plot.

Results:

The DisGeNet database provided information on the genes that were associated with skeletal Class II malocclusion. The five genes identified were *ACTN3, GH1, HDAC4, HMGA2* and *KAT6B.* One microRNA, *hsa-miR-892c-5p* was unique to *ACTN3, HDAC4* and *HMGA2.* The *hsa-miR-3925-5p* and *hsa-miR-590-3p* were found to be common to the genes *ACTN3, HDAC4* and *GH1 + HMGA2* respectively.

Discussion:

The identification of microRNAs targeting candidate genes could aid in defining the role of these microRNAs in establishing the phenotype. The future scope of this study lies in curating microRNAs that are common to class II malocclusion related candidate genes. This panel of differentially expressed microRNAs can further be developed as early diagnostic marker, for identifying the skeletal abnormality that they would be possibly associated with.

Keywords: Class II malocclusion, genetics, epigenetics, gene expression, regulators, microRNAs.

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Introduction

Skeletal Class II malocclusion comprises the misalignment of dental and skeletal components that affects the mandibular and maxillary fit to a larger extent. The misalignment is typically characterized by the overgrowth of the upper jaw or an undergrowth of the lower jaw. Although not very serious, the condition can bring about problems related to incorrect bite patterns and aesthetic issues, including difficulty in chewing of food, speech problems, increased risk of dental trauma etc. This discrepancy in the positioning of the jaws can result in various dental and skeletal discrepancies, such as increased overjet (excessive horizontal overlap of the upper front teeth over the lower front teeth) and a reduced chin prominence. In addition, it contributes to temporomandibular joint (TMJ) discomfort. [1] Angle proposed a classification system for skeletal Class II abnormality based on the relationship between the mandibular first molars and maxillary first molars. Based on the inclination of maxillary central incisors, Class II malocclusions are divided into 2 types: Class II Division 1 and Class II Division 2.

In Class II Division 1, the maxillary central incisors are typically inclined labially, and protrude outward toward the lips with or without a narrow maxillary arch. This inclination leads to an increased overjet. The incisors' vertical placement can vary from an open bite to deep overbite. Class II Division 1 malocclusions often involve a protruded or prominent upper jaw relative to the lower jaw. Patients with this type of malocclusion may exhibit a prominent upper lip and a more noticeable overjet. In contrast, Class II Division 2 malocclusion presents with the maxillary central incisors inclined lingually, indicating retroclination. This inclination can result in a deep overbite, where the upper front teeth significantly overlap the lower front teeth vertically. Patients with this malocclusion may have a retruded or flattened upper lip and a more severe overbite. [2] The current treatment options for Skeletal Class II malocclusion often require orthodontic and surgical interventions. The use of braces, headgear and appliances are used to improve the alignment of the teeth to ensure proper biting pattern. Orthognathic surgery is recommended in a few cases for repositioning of the jaws and to achieve a balance between facial and dental components. [3]

Skeletal Class II malocclusion has a complex etiology that involves both genetic and environmental factors. Understanding the interplay between these factors is essential in comprehending the development of this malocclusion. Early detection and prompt intervention are important in managing Skeletal Class II malocclusion, as early diagnosis can be effectively treated during growth and development. While the exact genetic basis can be intricate and multifactorial, there is evidence to suggest that genetics play a significant role in the development of skeletal malocclusions. Certain genetic traits can lead to variations in jaw size, shape, and position. These genetic factors can be inherited from one's parents and may contribute to the inherent structural characteristics of the jaw bones and dental arches. Variations in genes related to craniofacial development can increase the likelihood of developing a Class II malocclusion. In most cases, Skeletal Class II malocclusion results from the complex interaction between genetic and environmental factors.

Individuals with a genetic predisposition may be more susceptible to developing malocclusions, but the specific expression of these traits can be influenced by environmental circumstances. For example, a person with a genetic predisposition for Class II malocclusion might develop it if they engage in habits like thumb-sucking or experience trauma that further exacerbates the condition. Several studies have shown that skeletal malocclusion and craniofacial features are heritable. Family history and patterns of malocclusion within families support the idea that genetic factors contribute to the variation in jaw growth, tooth positioning, and facial proportions. [4] Candidate gene studies have provided substantial evidence on the genes associated with craniofacial development, jaw growth, and tooth eruption. Mutations or variations in these genes can influence the development of skeletal processes resulting in malocclusions. These variations can impact gene expression and regulatory mechanisms that control craniofacial development including the growth patterns of bones, cartilage, and muscles involved in facial and jaw development. Some candidate genes include those involved in bone and cartilage growth, hormone regulation, and facial morphogenesis. [5] Familial studies and twin studies provide strong evidence for a genetic component in skeletal malocclusion. The twin studies have shown a higher concordance rate for malocclusion among monozygotic (identical) twins compared to dizygotic (fraternal) twins, suggesting a genetic influence. [6]

Skeletal malocclusion is considered to be polygenic with the involvement of multiple genes that collectively contribute to the condition. Also, there exist environmental factors and habitual practices such as thumb-sucking, tongue thrusting, and prolonged use of pacifiers. Additionally, injuries or trauma to the jaw region during childhood or adolescence can impact the alignment of the jaws and contribute to malocclusions. Environmental factors can act in conjunction with genetic predisposition, exacerbating or mitigating the condition's severity. These interactions can contribute to the variability observed in malocclusion in relation to their severity and presentation. [7] In addition to the genetic components, epigenetic factors can also play a crucial role in craniofacial development. These epigenetic factors alter the gene expression by incorporation of modifications without any potential alterations in the DNA sequence. [8] Genetic predisposition lays the basis for future malocclusion development, but individual differences in how these elements interact and influence the particular malocclusion type and severity can be significant. Advances in Genetics and Genomics are assisting researchers in their understanding of these intricate interactions between genes and environment in the development of such diseases. [9] In this context, the present study was designed to identify one of the epigenetic components, the non-coding RNA associated with the reported candidate genes related to skeletal class II malocclusion.

When genetics are a significant factor in malocclusion, the efficacy of orthodontic and orthopedic treatments can be reduced. In these instances, orthognathic surgery may be necessary in the future. The ideal approach would be to target the specific gene responsible for either maxillary prognathism or mandibular retrognathism, depending on the underlying cause.

Although still just a proposed idea, the first stage in addressing skeletal irregularities is to pinpoint the key genes and their effects. Conducting genetic analyses is crucial to precisely identify the pertinent genetic indicators associated with a particular dental or skeletal misalignment. Although there has been extensive literature concerning the genetic basis of various dentofacial abnormalities and malocclusions, the literature on the genetic basis of class II malocclusion is minimal.

Identifying epigenetic regulators is crucial to fully comprehend the intricate correlation between epigenetic marks and phenotype. Moreover, these marks serve as potential leads for the diagnostics of specific disorders.

Materials and Methods

Database source:

The source of genes related to the phenotype of skeletal Class II malocclusion was derived from the DisGeNet database. The DisGeNET database is a comprehensive and publicly accessible resource that focuses on integrating information about human diseases and their associated genes. DisGeNET, commonly known as "Disease Gene Network," serves as a valuable tool for researchers, clinicians, and bioinformaticians who are interested in understanding the genetic basis of diseases and the relationships between genes and diseases. [10] The access to the DisGeNET database is freely available online, and the resource can be accessed at the following website: https://www.disgenet.org/.

Protein-Protein interaction analysis:

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a widely used online database and tool that provides information about protein-protein interactions (PPIs) and functional associations between proteins. It helps researchers understand the complex network of interactions that occur between proteins within cells, providing insights into cellular processes, pathways, and functions. The STRING database integrates the network data from various sources acquired from experiments, computational predictions and curated databases. The visualization of the protein interaction network presents nodes and edges, where nodes represent proteins and edges represent interactions. The interaction scores reflect the reliability of interaction. The STRING can be accessed through the web interface (https://string-db.org/). The five genes identified using the DisGeNet database were used as a query to retrieve the interaction network. [11]

Gene ontology analysis:

Gene ontology (GO) analysis is a tool that aids researchers in comprehending the biological processes, activities, and cellular components connected to a collection of genes or proteins. PANTHER (Protein ANalysis THrough Evolutionary Relationships) was employed for categorization of genes based on molecular functions, biological process, cellular component, protein class and pathway. [12,13] The STRING data for protein-protein interactions were used as an input to analyze the ontology data of the pooled genes.

miRNA analysis:

miRDB is a database that focuses on microRNA (miRNA) target prediction and functional annotation. miRNAs are small non-coding RNA molecules that play a crucial role in post-transcriptional gene regulation by binding to specific target messenger RNAs (mRNAs) and influencing their expression levels. miRDB provides researchers with tools to predict potential miRNA targets, understand their functions, and explore miRNA-related biological processes. [14,15] The database can be accessed at the following website: http://mirdb.org/.

Curation of miRNAs:

The identification of the unique microRNA population common for all the five genes were curated employing the Venn plot. A Venn diagram or a Venn plot, is a graphical representation which is used to illustrate the relationships and intersections between different sets or groups. The Venn plot consists of overlapping circles, each representing a separate set or category. The areas where the circles overlap indicate the elements that are common to multiple sets, while the non-overlapping areas represent elements that are unique to each set. The size of the circles and the overlap regions can provide insights into the relative sizes and relationships between the sets. [16]

Results

The DisGeNet database provided 5 different genes *viz., ACTN3, GH1, HDAC4, HMGA2* and *KAT6B* which were shown to have association with Skeletal Class II malocclusion. The information obtained spanned during the years 2013-2017. The cytogenetic location and the protein encoded by the genes are given in Table 1. The protein-protein interactions established by these genes were also demonstrated in Figure 1.

Table 1: The list of genes implicated in Skeletal Class II malocclusion as described by Disgenet

Gene name	Cytogenetic location	Protein
ACTN3	11q13.2	*Alpha Actinin 3*
GH1	17q23.3	*Growth Hormone 1*
HDAC4	2q37.3	*Histone deacetylase 4*
HMGA2	12q14.3	*High Mobility Group AT-Hook 2*
KAT6B	10q22.2	*Lysine Acetyltransferase 6B*

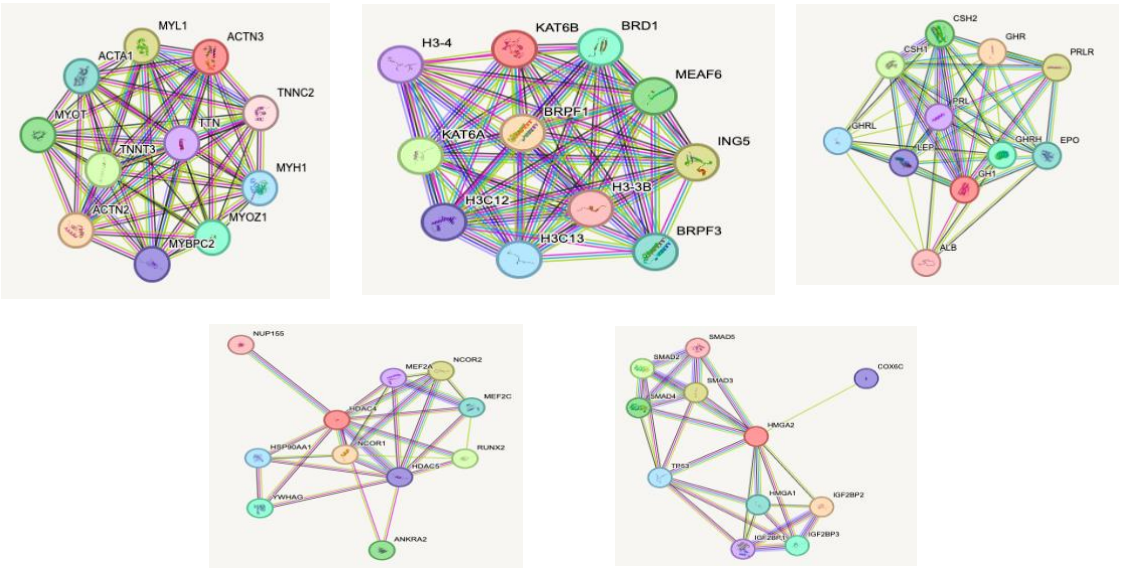

Figure 1: The protein-protein interaction network of genes (a) ACTN3, (b) GH1, (c) KAT6B, (d) HDAC4 and (e) HMGA2

The pool genes of all the networks were clustered into different groups using the Panther tool, for gene ontology analysis. Interestingly, there were 19 genes (*MEF2A, MEF2C, GHRH, SMAD2, SMAD3, SMAD4, SMAD5, ACTA1, SMAD3, IGF2BP1, IGF2BP2, IGFBP3, ACTN2, ACTN3, PRL, GH1, TNNT3, CSH1, RUNX2*) related to the developmental process and 4 genes related to growth process (*GHRH, CSH2, GH1, CSH1*) (Figure 2). The identification of microRNA population for each of the 5 genes revealed, 8, 283, 5, 214 and 163 microRNAs targeting GH1, *HMGA2, ACTN3, HDAC4* and *KAT6B* respectively. The Venn plot demonstrated, unique microRNA population such as hsa-miR-892c-5p, hsa-miR-3925-5p

and hsa-miR-590-3p for the gene combinations *ACTN3 + HDAC4 + HMGA2*, *ACTN3 + HDAC4 +KAT6B* and *GH1 + HMGA2* (Table 2) (Figure 3).

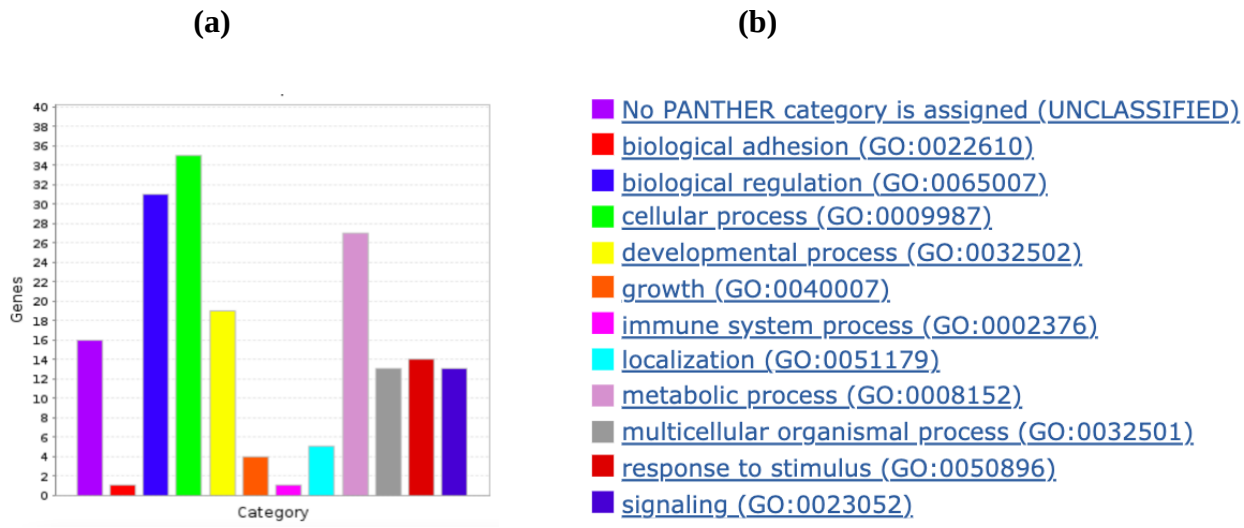

Figure 2: Gene ontology analysis demonstrating the biological processes cluster in which the candidate genes and their network genes are placed.

Table 2: List of microRNAs unique to specific combination of genes

Gene combinations	Unique microRNAs
ACTN3 + HDAC4 + HMGA2	hsa-miR-892c-5p
ACTN3 + HDAC4 +KAT6B	hsa-miR-3925-5p
GH1 + HMGA2	hsa-miR-590-3p
HMGA2 + KAT6B	hsa-miR-522-3p hsa-miR-9983-3p hsa-miR-224-3p hsa-miR-129-5p hsa-miR-548n hsa-miR-548t-5p hsa-miR-4311 hsa-miR-7515 hsa-miR-3085-5p hsa-miR-1283 hsa-miR-203a-3p hsa-miR-548az-5p hsa-miR-3678-3p hsa-miR-6885-3p hsa-miR-1288-5p hsa-miR-548at-5p hsa-miR-143-5p
ACTN3 + HDAC4	hsa-miR-153-3p hsa-miR-4691-3p hsa-miR-92b-5p

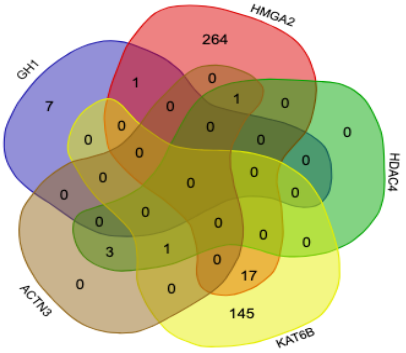

Figure 3: The Venn plot depicting the unique microRNAs associated with the genes related to Class II skeletal malocclusion.

Discussion

Skeletal class II malocclusion is a polygenic disorder, with the involvement of multiple genetic, epigenetic and environmental factors. These factors have profound impact on the growth and development of skeletal and oro-facial structures. The advancements in the field of Genomics, Transcriptomics, Proteomics and Epigenomics have unraveled the candidate genes involved in Class II malocclusion. The data for the present study was derived from the DisGeNet database, which provided us with 5 major genes studied in association with the phenotype. Several genetic variants in these genes have been found to influence the phenotype by modulating the gene expression process. Furthermore, epigenetic marks such as DNA methylation, histone modifications and non-coding RNAs have been widely studied to understand the role of these components in development of skeletal malocclusion. The present study is one of its kind which is designed to identify the epigenetic component, the microRNA, that can regulate the gene expression process by interfering with the mRNA transcripts at the post-transcriptional level, eventually leading to diminished protein synthesis. Since Skeletal Class II malocclusion is a polygenic complex disorder, deciphering the role of these miRNAs in the development of the phenotype becomes crucial.

ACTN3 (Alpha-actinin-3):

Alpha-actinin-3 is a protein that is encoded by the *ACTN3* gene, also known as alpha-actinin-3. The gene is largely expressed in fast-twitch muscle fibers, which are used in quick, strong activities like sprinting and weightlifting. The sarcomere, the structural component of muscle fibers, contains the alpha-actinin-3 protein, which is involved in muscle contraction. The genetic variations identified in this gene have been found to be associated with several phenotypes such as athletic performance, exercise adaptation, recovery, sporting injury risk, facial morphology, skeletal abnormalities etc. [17] One of the widely studied genetic variants of this gene is the *rs1815739* polymorphism that is characterized by two

common alleles: the R allele (for the amino acid arginine) and the X allele (for a premature stop codon). There are three genotypes for the *ACTN3* gene: RR (homozygous for the R allele), RX (heterozygous, one R allele and one X allele), and XX (homozygous for the X allele). Individuals inherit one allele from each of their parents. The ability of an individual's muscles to contract depends on whether they have the functioning R allele of the *ACTN3* gene. In line with this, the individuals with RR genotype may have an advantage in sports that call on explosive muscle power, such as weightlifting and sprinting. Individuals with the XX genotype, however, could be less able to produce powerful, explosive muscular contractions. In addition to the genetic component, training, diet, and other environmental factors also play major roles in athletic performance. [18] A recent study conducted by Alalim and team, demonstrated the genetic association of *rs1815739* polymorphism with various malocclusion phenotypes in a Turkish population. They demonstrated that the maxillary anteroposterior and maxillary incisor inclination was significantly associated with the polymorphism in the population studied. [19]

GH1 (Growth hormone):

Growth hormone, often referred to as somatotropin, is produced by the *GH1* gene, commonly referred to as Growth Hormone 1. The anterior pituitary gland, a little gland found at the base of the brain, produces and releases growth hormone, a protein hormone. It is essential for controlling how humans and other animals grow and develop. The GH1 gene is found on chromosome 17 in humans. Growth hormone deficit (GHD) disorder can be due to mutations or variants in the *GH1* gene, resulting in decreased production of growth hormone. A study reported that, the children presenting with growth hormone deficiency when treated using growth hormones for longer periods showed an intense influence on the craniofacial complex. This therapeutic strategy was more advantageous and was found to decrease the disproportion of jaw dimensions, thus preventing the gnathic malocclusions. [20] A systematic review analysis by Zhang and team revealed the importance of hormones and nutrition in the treatment outcome of patients with skeletal Class II malocclusion. The administration of hormones, such growth hormones, insulin, insulin-like growth factor, sex hormones, improved the effects of treatment using functional appliance on skeletal Class II malocclusion cases. [21]

HDAC4 and KAT6B:

Histone deacetylase 4, or HDAC4, is an enzyme that controls gene expression and remodeling of chromatin. It belongs to the family of enzymes known as histone deacetylases, which are important in altering the structure of chromatin. Gene transcription is normally repressed as a result of this deacetylation process, effectively silencing gene expression. [22] This gene plays a vital role in the development of bones and that the mice lacking HDAC4 presented with chondrocyte hypertrophy leading to abnormal bone formation. [23] Huh et al., conducted a study to determine the association of two genes *KAT6B* (Chromatin modifying histone acetyltransferase) and *HDAC4* (deacetylase), the proteins involved in the

epigenetic process of histone modification. The masseter muscle samples were obtained from patients who underwent orthognathic surgery for malocclusion. The muscle fibers were assessed for expression of the genes employing microarray and quantitative real time PCR. Interestingly, the team observed multiple fold increases in the expression of *KAT6B* and *HDAC4* in patients with deep bite in comparison to open bite cases. In addition, the expression of HDAC4 and KAT6B achieved statistical significance with sagittal class III and class II malocclusion phenotypes. The expression of HDAC4 showed a negative correlation with slow myosin type 1 and a positive correlation with myosin gene, type IIX. [24]

HMGA2:

The High Mobility Group AT-hook 2 protein, which is encoded by the *HMGA2* gene, has been linked to the development of the skeleton and the craniofacial region. Although mutations or dysregulation of the *HMGA2* gene are not a direct cause of skeletal malocclusion, they can indirectly contribute to craniofacial and skeletal abnormalities that may be related to malocclusion. [25] A recent study demonstrated the role of *HMGA2* gene in skeletal malformations. The facial anomalies in Hmga2-/- knockout mice were assessed parallelly with the osteoblast differentiation of pre-osteoblast MC3T3-E1 cells with Hmga2-/- gene knockout. The study provided evidence on the involvement of Hmga2 in the differentiation of osteoblast and bone growth. [26]

The present study identified a pooled population of genes interacting with the key genes chosen for the analysis. Of the total 55 pooled genes, 19 genes formed a cluster which was associated with development and 4 were associated with growth. Thus, the analysis of the preliminary interaction network has revealed several other genes that could act in consonance with the key genes to exhibit the phenotype. Studies conducted by Fontoura revealed numerous genes being associated with different phenotypes of skeletal malocclusion. Genetic variation in the genes *FGFR2, EDN1, SNAI3, MYO1H, TWIST1, COL1A1* and *TBX5* were found to be associated with craniofacial skeletal abnormalities. [27] The polymorphism studies related to facial variations in the genes *viz., ADK (rs7924176), HMGA2 (rs17101923)* and *AJUBA (rs997154)* were analyzed in the Caucasian population. The polymorphic markers assessed the *rs997154*, of AJUBA were found to influence the phenotype, Class II malocclusion. [28] Among, the genes studied *HDAC4* and *KAT6B* were found to be exclusively connected to epigenetic mechanisms. The genes code for histone deacetylase and histone acetyltransferase respectively, thereby exerting a strong influence over the chromatin packaging.

MicroRNAs are a class of non-coding RNA that essentially controls the expression of genes in eukaryotic organisms. They act as post-transcriptional regulators of gene expression and are typically 21–25 nucleotides long. The miRNAs produced can function in two ways: (a) the miRNAs halt the process of translation by attaching to mRNA targets and prevent ribosomes from attaching to the target RNA, and (b) miRNAs can initiate the degradation of target RNA, eventually resulting in degradation of mRNA. [29] A single miRNA can have multiple gene targets and is potentially

involved in the fine-tuning of the gene expression process. Any form of dysregulation in the production or function of miRNAs results in diseases. Since these miRNAs are involved in the pathogenesis of the disease, exploring their expression in tissues, quantification of the same in diseased and normal tissues can provide evidence on the regulation of target gene expression in a cell or tissue-specific manner. [30]

The experimental evidence on the microRNAs targeting the key genes involved in skeletal class II malocclusion was found to be scarce. The microRNA, hsa-miR-892c-5p, was found to be the only epigenetic marker common to the genes *ACTN3 + HDAC4 + HMGA2.* Zhao and colleagues demonstrated that hsa-miR-892c-5p was associated with osteoarthritis (OA), employing a computational approach. It was revealed that cyclin D1 and D2 were the potential targets of miR-892b. [31] Integrated *in silico* analysis followed by validation showed miRNA hsa-miR-892c-5p as a promising diagnostic marker for active tuberculosis. [32] The miRNA, hsa-miR-3925-5p, was found to target *ACTN3 + HDAC4 + KAT6B* genes. This miRNA was shown to preferentially target the SARS-CoV-2 genome. [33] There was not much information about this microRNA related to infectious, communicable or genetic disorders. The other microRNA, hsa-miR-590-3p, has been implicated in the epithelial-mesenchymal transition process in several cancer types. [34] The role of this miRNA was also elucidated in autoimmune myocarditis, where it was found to target the nuclear factor kappa-B. [35] All the microRNA populations identified to be associated with the key genes controlling the skeletal abnormalities have to be experimentally validated with respect to Skeletal class II malocclusion.

It should be considered that genetic associations may be influenced by racial and ethnic differences. When studying the genetic basis of complex traits and conditions, including Skeletal Class II malocclusion, it's essential to recognize that genetic associations can vary among different racial and ethnic groups. Human populations around the world exhibit genetic diversity, with distinct genetic variations and allele frequencies. These genetic differences can lead to variations in how genes related to craniofacial development and malocclusions are expressed within different racial and ethnic groups. Certain genetic variations associated with malocclusions may be more prevalent or unique to specific populations. The historical migration and isolation of human populations have led to the accumulation of genetic differences over time. As a result, some genetic predispositions to malocclusions or variations in craniofacial structures may be more pronounced in certain racial or ethnic groups due to their unique population histories and genetic backgrounds. Racial and ethnic groups often have distinct cultural practices, diets, and environmental exposures. These factors can influence how genetic predispositions are expressed. For example, dietary habits may impact jaw growth and dental development, potentially affecting the severity or presentation of malocclusions differently in various populations. Access to healthcare and orthodontic treatment can also vary among racial and ethnic groups. Disparities in healthcare access and utilization can influence the diagnosis and management of malocclusions within different populations. Research findings suggest that there is indeed more genetic diversity within continents than between them. To be more specific, the level of genetic variation within a continent can range from 85% to 90%. Meanwhile, the genetic variation between continents is only

between 10% and 15%. This emphasizes the importance of understanding and studying genetic diversity within populations on a regional level.[36] With the rapid development of next-generation DNA sequencing technologies, it is expected that hundreds of thousands of novel human SNPs will be discovered in the coming years. Genome-Wide Association Study (GWAS) has also been utilized to identify disease-related SNPs that play a crucial role in comprehending the molecular mechanisms of evolution.

A significant amount of more than a billion humans worldwide are found to be completely deficient in the fast muscle fiber protein alpha-actinin-3 due to polymorphism in the ACTN3 Gene which results in deficient mRNA expression. This gene enhances forceful, fast skeletal muscle contraction and the loss of alpha-actinin-3 was accompanied by significantly smaller type II fiber diameter in masseter muscle.Human masseter muscle fibre properties in different sagittal and vertical malocclusions and proposed that both Type I and Type II fibres are closely associated with variations in vertical facial growth rather than the sagittal growth. A common ACTN3 polymorphism, R577X, results in alpha-actinin-3 protein absence, changes in fibre type proportions, muscle metabolism and bone mineralization. Zebrik et al concluded that ACTN3 577XX is overrepresented in skeletal class II malocclusion, compared to the control group, suggesting biological influence during bone growth. [37]

Recent research has explored the effects of the ACTN3 gene on the craniofacial area, specifically its role in determining muscle fiber type in the masseter muscle. This discovery has implications for the development of skeletal issues. The studies conducted by Zebrick et al [37] were conducted on patients undergoing orthognathic correction and showed that open bite malocclusion is linked to a decrease in fiber diameter, while deep bite malocclusion is associated with an increase. This could be due to a higher presence of Type II fibers in the masseter muscle, despite their smaller size. They have also investigated the prevalence of two SNP's at rsl8l5739 and rs678397 (both C-T transitions) in ACTN3. Their findings suggest that in individuals with deep bite malocclusion, the ACTN3 577XX variant is less common, indicating that muscle fiber variation can impact facial height. RT-PCR (Reverse Transcription Polymerase Chain Reaction) have demonstrated that the levels of ACTN2 remain constant in the 577XX genotype, despite the significant reduction of ACTN3 levels. This suggests that ACTN2 may not be able to compensate for the lack of ACTN3 gene in the masseter. Significant differences in the SNPs have been studied between Class II skeletal problems and controls.

Understanding the genetic factors involved in malocclusion can help determine the best treatment plan and retention protocol. Recent research has shown a clear correlation between specific genes and skeletal class II malocclusion. Genes such as FGFR2, MSX1, MATN1, MYOH1, ACTN3, GHR, KAT6B, HDAC4, and AJUBA have been identified as positively linked to this condition. **FGFR2** (Fibroblast Growth Factor Receptor 2) is involved in craniofacial development and has been associated with variations in jaw size and shape that can contribute to malocclusion. Variations in FGFR2 have been linked to changes in jaw size and shape, which can contribute to the development of Skeletal Class II

malocclusion. Anomalies in this gene may lead to a protruded or retruded jaw, influencing the positioning of the upper and lower jaws. **MSX1**: MSX1 (Muscle Segment Homeobox 1) is a transcription factor involved in dental and craniofacial development. Variations in this gene can influence tooth and jaw development, potentially leading to malocclusion. Genetic variations in MSX1 have been associated with abnormal tooth and jaw development. These variations can lead to dental anomalies and contribute to the development of malocclusions. **MATN1**: MATN1 (Matrilin 1) is involved in extracellular matrix formation in cartilage and may play a role in craniofacial growth and development. Alterations in MATN1 may affect the extracellular matrix in the jaw region, potentially influencing craniofacial growth and development. **MYOH1**: MYOH1 (Myosin Heavy Chain 1) is involved in muscle function and may have implications for the muscular aspects of jaw movement and alignment. Variations in MYOH1 may have implications for the muscles involved in jaw movement and bite alignment. Changes in muscle function can affect the positioning of the jaws and contribute to malocclusion. **ACTN3**: ACTN3 (Alpha-actinin-3) is associated with muscle function, and variations in this gene may impact the muscles involved in jaw movement and bite alignment. Variations in ACTN3 can impact the muscles responsible for jaw movement. Dysfunctional or altered muscle function may influence bite alignment and jaw positioning. **GHR**: GHR (Growth Hormone Receptor) is involved in the regulation of growth and development, including craniofacial growth. Genetic variations in GHR may influence the growth patterns of craniofacial structures, potentially contributing to the development of Skeletal Class II malocclusion. **KAT6B**: KAT6B (Lysine Acetyltransferase 6B) plays a role in craniofacial development and gene regulation. **HDAC4**: HDAC4 (Histone Deacetylase 4) is involved in gene regulation and may influence the development of craniofacial structures. **AJUBA**: AJUBA (Jub, Ajuba LIM protein) plays a role in cell adhesion and signalling, which can impact tissue development in the craniofacial region. Alterations in AJUBA can affect cell interactions and signalling in the craniofacial region, potentially influencing tissue development and jaw alignment. The identification of these specific genes associated with Skeletal Class II malocclusion has the potential to inform personalized treatment plans. Orthodontists and healthcare professionals can use genetic information to tailor interventions, monitor progress, and develop retention protocols that consider the patient's unique genetic predispositions.

The limitations of the present study are, (a) the data derived from the DisGeNet database was based only on the information accumulated from the original database BEFREE through the years 2013-2017, (b) new genes have been identified to be associated with Class II malocclusion, which has to be assessed for their potential microRNA targets. The process of covering all the genes linked to the phenotype is out of the scope of this study, hence the investigation was restricted only to those genes that have been elaborately studied with respect to malocclusion (c) the microRNA data provided for the candidate genes have to be further validated for ascertaining their functional role in establishment of the phenotypes, and (d) identification of differentially expressed microRNAs from the pool should be curated to obtain the most appropriate microRNAs related to Skeletal Class II malocclusion phenotype. Taken together, the present study

provides insight into the epigenetic involvement mediated through non-coding RNAs, in class II malocclusion. More exploration in this field of epigenetics would add to our current understanding of the role of candidate gene expression and the establishment of malocclusion phenotypes.

Conclusion

The present study is the first of its kind to identify the microRNA population associated with the candidate genes for Skeletal Class II malocclusion. The preliminary data provided here can be further validated using experimental approaches to narrow down the vital microRNAs that are differentially expressed in the class II malocclusion phenotype. Also, these microRNAs can serve as potential diagnostic markers, thereby aiding in early detection of the disorder, which can be treated using less invasive therapeutic modalities, such as hormone therapy, with not much surgical interventions.

Conflict of interest

The authors have no conflict of interests to declare.

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