Polymorphism analysis of myosin 1H (G/A) and P561T (C/A) genes on class I, class II, and class III malocclusion

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Abstract:

CONTEXT: Besides environmental factors, genetic factors play an important role in the etiology of malocclusion. Polymorphisms of the Myosin 1H gene in orofacial muscle fibers are thought to influence the growth and development of the mandible. Growth hormone receptors are present on the growth of cartilage, especially the condyle of the mandible. The polymorphisms of the growth hormone receptor have an effect on the growth and development of the mandible. The potential of the Myosin 1H and P561T genes as bioindicators in aiding diagnosis of malocclusion is quite good based on the available literature. However, until now there has been no research that has observed genetic analysis on polymorphism-based malocclusion of the Myosin 1H and P561T genes in the Indonesian population.

AIMS: To determine the relationship between polymorphisms of Myosin 1H and P561T genes, towards the growth and development of the mandible in malocclusion cases.

SETTINGS AND DESIGN: Subjects were patients aged 17--45 years old with skeletal malocclusions who were undergoing or were about to undergo orthodontic treatment at RSGM-FKG UI (Universitas Indonesia's Dental Hospital), with 50 people in each group.

METHODS AND MATERIAL: Malocclusions were determined based on radiographic analysis of the initial cephalometry using the Stainer method. DNA samples were extracted from buccal swabs and blood cells in Class I and II malocclusion while nail clippings and hair follicles extracts were used in Class III malocclusion. DNA sequence amplification was carried out using Polymerase Chain Reaction, while Genetic Polymorphism Analysis of Myosin 1H and P561T genes was performed with Restriction Fragment Length Polymorphism.

STATISTICAL ANALYSIS USED: Pearson Chi-Square was used to analyze the Myosin 1H gene, while the Fisher Exact Test was used to analyze the P561T gene.

RESULTS: A relationship between Myosin 1H gene polymorphism and Class I, II, and III skeletal malocclusion was found. There was no correlation between P561T gene polymorphism and Class I, II, and III skeletal malocclusion.

CONCLUSIONS: Myosin 1H gene polymorphism is one of the risk factors for Class I, II, and III malocclusion. Extraction of DNA from hair follicles gave good results in terms of DNA quality and was a relatively easier sampling method compared to blood cell purification and buccal swabs.

Keywords: Polymorphism of myosin 1H gene, polymorphism of P561T gene, skeletal malocclusion

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Introduction

The growth and development of the maxilla and mandible is influenced by various factors, including genetic factors, age, gender, race, bad habits, diseases, nutrition, trauma, and environmental factors.\textsuperscript{[1‑5]} Genetic factors are one of the main factors that influence dentocraniofacial growth and development. Disharmony of the size of the maxilla with the size of the mandible will result in malocclusion.\textsuperscript{[1‑3,6]} This disharmony can occur due to the genetic crossover between one individual and another individual, in which the child obtains DNA from the cell nucleus of both their parents, 50% from the mother and 50% from the father, resulting in the child inheriting several traits from their parents. Genetic factors have a role in the development of skeletal discrepancies, through the inheritance of genes with specific characteristics. An understanding of the specific genes that have a role in the etiology of skeletal malocclusions, can help orthodontists determine their diagnosis and appropriate treatment plan for malocclusion cases.\textsuperscript{[3,7,8]}

The contractile activity of masticatory muscles affect skeletal craniofacial morphology.\textsuperscript{[6,10]} The function of masticatory muscles is highly dependent on the structure of the orofacial skeletal. Orofacial muscles are known to have wide reciprocal effect on the bone. Genetic changes which affects muscles will also affect areas of the skeleton. The Polymorphism of the Myosin 1H gene in orofacial muscle fibers are thought to influence the growth and development of the mandible.\textsuperscript{[5,11‑13]}

Growth hormones are peptide hormones that play an important role in regulating the growth and development of the craniofacial complex.\textsuperscript{[14‑17]} Growth Hormones need to bind to their specific cell surface receptors, in order to activate various intracellular signaling pathways.\textsuperscript{[18]} Growth Hormone receptors are present in the development of the cartilage, particularly the mandibular condyle. The polymorphism of the Growth Hormone receptors have an effect on the growth and development of the mandible.\textsuperscript{[3,19,20]}

Based on the available literature, Myosin 1H and P561T genes are potentially good bioindicators that can help determine the diagnosis of malocclusion.\textsuperscript{[21]} However, until now there has been no research which has observed genetic analysis on polymorphism-based malocclusions (Class I, II, and III) of the Myosin 1H and P561T genes in the Indonesian population. Therefore, this study is done to determine the relationship between the polymorphism of the Myosin 1H and P561T genes, towards the growth and development of the mandible in class I, II, and III malocclusions.

Subjects and Methods

This study used an analytic cross-sectional design. Subjects were patients aged 17–45 years with class I, II, and III skeletal malocclusions, who underwent or were about to undergo orthodontic treatment at The Faculty of Dentistry Universitas Indonesia’s Dental Teaching Hospital. The research was carried out after obtaining ethical clearance from the ethical commission of the Faculty of Dentistry Universitas Indonesia (Ethical Approval number 46/FKG/UI/X/202) and after obtaining research permission from The Faculty of Dentistry Universitas Indonesia’s Dental Teaching Hospital (Permission number ND-1835/UN2.F2.D/PDP. 04.02/Thesis/2020). The research was conducted from October to December 2020.

There were a total of 150 subjects, with 50 subjects in each malocclusion group. Class I malocclusion was determined as the control group. Class I, II, and III malocclusions were determined based on initial cephalometry radiographic analysis using the Stainer method. Patients with syndromes and other abnormalities in their growth and development, patients who had underwent orthognathic surgical treatment or used orthopaedic and myofunctional instruments, and patients with prognathic or retrognathic maxillas based on cephalometric measurements were excluded from this study.

DNA samples were extracted from nail clippings and hair follicles for the class III skeletal malocclusion group, and from buccal swabs and blood cells for the class I and II skeletal malocclusion groups. DNA sequence amplification was done with the PCR (Polymerase Chain Reaction) method. Myosin 1H (rs 10850110) Primers Forward (5’-ACTTTGCTTCCCCCTGTTA-3’) and Reverse (5’-CTGAGCCAGAGGATTGCT-3’); P 5 6 1 T ( r s 6 1 8 4 ) : P r i m e r s Forward (5’-GGGAAGCAGATCTCTTATGC-3’) and Reverse (5’-CTAGGAGAGTACTCTTATGC-3’). Genetic Polymorphism analysis of the Myosin 1H and P561T genes were done with the RLF (Restriction Fragment Length Polymorphism) technique. PCR-amplified DNA was digested with Cfr13I (Sau96) for Myosin 1H (rs 10850110) and Eco 1471 (Stul) for P561T (rs 6184). The data was analyzed statistically using SPSS (IBM Corp). A Pearson Chi-Squared test was conducted to analyze the relationship between polymorphism and craniofacial measurements in the Myosin 1H gene, and the Fisher Exact test was used to analyze the relationship between polymorphism and craniofacial measurements in the P561T gene.

Results

The visualization of the PCR result of the Myosin 1H gene at 291 bp can be seen in Figure 1.
Figure 2 shows that there is a genotype variation of the Myosin 1H gene, which has three genotypes: The GG, GA, and AA genotypes. In the homozygous GG wildtype genotype, 2 bands can be seen at 28 bp and 263 bp. The heterozygous GA genotype contained three bands at 28 bp, 263 bp, and 291 bp. No homozygous AA genotypes were found in this study.

The genotype distribution of the Myosin 1H gene in the three malocclusion groups: Class II, class III and class I as the control group, showed a distribution which is in accordance with the Hardy Weinberg Equilibrium law ($P = 0.187$; consistent $P > 0.05$).

The distribution of genotypes and alleles of the RFLP results in the class I, class II, and class III malocclusion groups can be seen in Table 1. The distribution of GG, GA, and AA genotypes in the class I malocclusion group were $62\%$, $38\%$, and $0\%$, in the class II malocclusion group $66\%$, $34\%$, and $0\%$, while in the class III malocclusion group were $34\%$, $66\%$, and $0\%$. The proportion of the GG genotype and G allele were the largest in the class I and II malocclusion groups, while in the Class III malocclusion group the GA genotype and the G allele had the largest proportion. The Chi-Square tests showed a significant difference between the three groups. A $P$ value of $<0.05$ for both the genotype and allele tests indicates a relationship between the polymorphism of the Myosin 1H gene with class II and class III malocclusion cases, and a difference in the proportion distribution of genotypes and alleles between the malocclusion groups [Table 1].

Analysis of risk estimation with the continuity correction Chi-square test in this study can be seen in Table 2, which shows an Odds Ratio of 1.145 for class II and 2.1 for class III. This is thought to indicate a risk factor for allele G compared to allele A in class II malocclusion cases, and a risk factor for allele A compared to allele G in class III malocclusion cases.

Identification of the P561T gene through PCR procedures were carried out on 150 samples that met the inclusion criteria. However, only 61 samples gave positive results.
had the largest proportion. Chi-square testing showed no significant difference among the three groups [Table 3].

Analysis of risk estimation with the with the continuity correction Chi-square test in this study can be seen in Table 4, which shows an Odds Ratio of 1.145 for class II and 2.02 for class III. This is thought to indicate a risk factor for allele C compared to allele A in cases of class II malocclusion and a risk factor for allele A compared to allele C in cases of class III malocclusion [Table 4].

**Discussion**

Identification of DNA samples from groups of orthodontic patients with class I, class II, and class III malocclusion using the PCR-RFLP method was done to observe the polymorphism of the Myosin 1H and P561T genes. 150 samples (for each gene) which met the inclusion criteria were obtained, with 50 samples in each malocclusion group. After the process of DNA extraction from buccal swabs, nails and hair follicles from 150 research subjects, DNA amplification was done with a PCR machine, eventually the polymorphism of the positive PCR results for the two genes were examined.[7,22‑27] For the Myosin 1H gene 150 PCR samples were positive, and for the P561T gene 61 PCR samples were positive.

There are three major steps involved in the PCR technique: Denaturation, annealing, and extension. In the first step; the DNA is denatured at high temperatures (from 90 to 97°C). In the second step, primers anneal to the DNA template strands to prime extension. In the third step, extension occurs at the end of the annealed primers to create a complimentary copy strand of DNA.[20,22,24‑26] The inability to obtain maximum results from the PCR amplification of the P561T gene was possibly due to the method of DNA extraction from buccal swabs, nail clippings, and hair follicles. Blood cells could have given better quality DNA, however this research was done during the ongoing COVID-19 pandemic, in which taking blood samples was risky. Despite that, the results of DNA extraction from hair follicles gave pretty good results in terms of DNA quality, which is in line with the research of Ghatak et al.[24] 2013. With the development of science and technology, DNA extraction can be obtained quickly through simple procedures via buccal swabs of the mouth, clippings of nails and hair using instant DNA extraction solutions, however the concentration and purity of the DNA aren’t fully optimal.[23,25] From the 150 samples which consisted of 100 samples from buccal swabs, 25 samples from nail clippings and 25 samples from hair follicles that were extracted with instant DNA extraction solutions, the PCR results were not the same, namely 150 samples of PCR products for the Myosin 1H gene could be read completely, however, only 61 samples of PCR products could be read for the P561T gene.

After DNA amplification with PCR in the Myosin 1H and P561T gene groups, an RFLP procedure was carried out via the administration of the restriction enzyme Cfr13I (Sau96I) for the Myosin 1H gene and Eco 147I (StuI) for the P561T gene.[29,30] Visualization of the RFLP product was done with a gel doc, which identified the genotypes of these samples. The GG and GA genotypes were identified in the Myosin 1H gene and the CC, CA, and AA genotypes were identified
in the P561T gene, this is in accordance the study of Tassopoulou-Fishel et al., 2012.[10,11,20,29‑34]

The Hardy–Weinberg test of the genotype distribution of the Myosin 1H gene polymorphism in this study showed a P value of 0.187 with X² = 1.739. Mathematically, this indicates the assumption that the genotypes and alleles in this study and the general population will generally be constant from generation to generation. In Table 1, it could be seen that the frequency of the GG genotype was higher in patients with class II malocclusion groups compared with patients in the class I malocclusion group, this is in accordance with the study of Arun et al.[29] 2016. The frequency of the GA genotype was found to be higher in patients in the class III malocclusion compared with the class I malocclusion group, this is in accordance with the study of Cruz et al.[31] 2017. The G allele had a 1.145 times higher risk compared with the A allele for class II malocclusion. Therefore, in class II malocclusion, the A allele was the allele with the lowest frequency or the allele with the protective effect. The G allele which has the highest frequency is considered the susceptible allele in class II malocclusion. The A allele had a 2.1 times higher risk compared with the G allele for class III malocclusion. Therefore, in class III malocclusion, the G allele was the allele with the lowest frequency or the allele with the protective effect. The A allele which has the highest frequency is considered the susceptible allele in class III malocclusion. Based on the results of the statistical tests, it can be concluded that there is no relationship between the P561T gene polymorphism with Class I, II, and III skeletal malocclusions.

For the P561T gene identification, a total of 61 samples had a positive PCR, in which the forward and reverse primers fused with the nucleotide bases in the 1,037 bp band.[33,35] The P561T gene samples consisted of 20 class I malocclusion samples, 14 class II malocclusion samples, and 27 class III malocclusion samples. The Hardy–Weinberg test in these two malocclusion groups showed a P value of 0.001, which is smaller than 0.05. In addition, the genotype test between class II and class III groups compared to class I showed a P value of 0.689, greater than 0.05, which indicates that there is no significant difference between class II and class III compared to class I, in other words there is no difference in the distribution of the proportion of genotypes between the malocclusion groups. A similar result was found in the Chi-square test between the C and A alleles, with a P value of 0.102, which also showed that there was no significant difference in the distribution of the C and A alleles between the malocclusion groups. Therefore, based on the available data, it can be concluded that the P561T gene polymorphisms, both genotypes and alleles, didn’t show any significant difference for class II malocclusion with class I malocclusion, and also no difference for class III with class I malocclusion. The A allele had a 2.02 times higher risk compared with the C allele for class III malocclusion. Therefore, in class III malocclusion, the C allele was the allele with the lowest frequency or the allele with the protective effect. The A allele which has the highest frequency, is considered the susceptible allele in class III malocclusion. Based on the results of the statistical tests, it can be concluded that there is no relationship between the P561T gene polymorphism with Class I, II, and III skeletal malocclusions.

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Conflicts of interest
There are no conflicts of interest.

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