IGF-1 Rs5742632 Snps in Mandibular Asymmetry Adult Patients at Dental Hospital Universitas Sumatera Utara

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Abstract

The aimed of study to analysis the IGF-1 rs5742632 Single-nucleotide polymorphisms (SNPs) based on the severity of mandibular asymmetry. This is a cross-sectional study of genotype-fenotype study of IGF-1 rs5742632 SNPs and mandibular asymmetry. There were 60 pre-treatment adult orthodontic medical record that was included in this study. The mandibular asymmetry was measured in panoramic radiographs with Lemos method. The SNPs of IGF-1 rs5742632 analysis was performed based on polymerase chain reaction – restriction fragment polymorphisms (PCR-RFLP). The test of genotype distribution and allele frequency for significance by using the Chi square- test followed by Hardy-Weinberg Equilibrium calculation.

There were twenty subjects (33.3%) of TT, eleven subjects (18.33%) of CC, and twenty nine subjects (48.33%) of TC with the meant age of 21.71 ± 2.31 year old. There was a significant difference ($p<0.05$) IGF-1 rs5742632 polymorphism among light, moderate, and severe mandibular asymmetry. The analysis framework of modeling mandibular asymmetry severity from DNA polymorphism can contribute the assessment of feasibility and psychological acceptability the genetic screening to change clinician's management mindset in the orthodontics primary care setting.

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Introduction

Previous study provided novelty information that some effected genes could estimate facial shape in some populations that might influence the normal-range facial features.[1] There were comprehensive assessments of underlying etiology combined with imaging studies mentioned about the genetic potential in mandibular development.[2,3] Panoramic radiograph has been widely used for asymmetry screening of lower third face with various timmer indexes assessment of mandibular asymmetry that have been developed such as: Habets, Kjellberg, Levandoski and Lemos method.[4–7]

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The advantages of Lemos method are that it allows differential diagnosis between functional and morphological asymmetry and simultaneously assessed the horizontal, vertical and angular mandibular measurements in patients with and without posterior crossbite.[4] The etiology of skeletal mandibular asymmetry in previous report on the dizygomatic twins showed that genetic factor is more dominant than environment.[8] The severity of mandibular asymmetry is correlated to the condyle height and disc status in caucasian young adult population with asymptomatic unilateral disc displacement (non-syndromic and non-pathological).[9,10]

Modern techniques in advanced human genetics had created tremendous opportunities in developing treatment methods and prevented the severity of medical issues, such as computerized processing, mechanical sequencing, DNA

amplification, and hybridization, those of which have provided knowledge about genomes at DNA sequence level.[11] As one of growth factors act in cellular proliferation and differentiation in mandibular condyle, IGF-1 is mainly located in chromosome 12q23.2.12 In general, IGF-1 is the protein that mainly produced by the liver in response to growth hormone in pituitary gland,[12] which correspond to the growth hormone mediators and influence both growth and differentiation of bone and skeletal muscles.[13,14]

The signalling of IGF-1 could regulate the cell division in a group of polypeptide molecules with the autocrine or paracrine mechanisms that occur in the bone growth of human study.[15] Besides, IGF-1 can increase lipid, glycogen and protein synthesis through metabolic regulation, triggered proliferation and induced myoblastic or osteoblastic tissue that affect muscles and bones.[16] An animal study reported that the increasing sequence of IGF-1 expression in mandibular condylar cartilage was found in unilateral anterior crossbite.[17] In human study, the histopathological tests of cartilage condylar hyperplasia specimen showed positive stains of insulin-like growth factor-1 (IGF-1) in each layer of surface cartilages.[18] The presence of IGF-1 in longitudinal bone growth plates was also reported as the main growth factor (GF) which stimulated the proliferation and differentiation of chondrocytes in temporomandibular joint, endocrine and paracrine or autocrine mechanisms.[19] The early benefits of IGF-1 gene detection has been reported as one of the main mediators in promoting muscle and bone growth to optimize the timing of orthodontic treatment as well as in orthopedic treatment.[20]

In regulating the metabolic demands of bone remodelling process, IGF-1 via it's receptor has novel potential in therapies because it is involved in various aspects of skeletal physiology. IGF-I was a significant growth factor for muscle repair and potential option for stem-cell therapy.[21] The precise biological and functional characterization of IGF-1 isoforms in skeletal muscle were particularly essential in elucidating the specific signaling pathways that promote both competing processes of cellular proliferation and differentiation in muscle regeneration.[14,21] The IGF-1 level in conjuction with skeletal classification, cervical stage, and gender analysis could predict the timing and intensity of individual variation in mandibular length and total anterior facial height growth rate.[21] The IGF-1 level demonstrated a statistically significant difference between early and late adolescent [p=0.033] among orthodontics and orthopedics patients. The IGF-1 level and polymorphism IGF-1 rs5742632 showed no statistically significant difference in scoliosis and vertical mandibular asymmetry.[22] As an additional tool to optimize orthodontic treatment timing in the skeletal maturation stage, the IGF level serum was used as an indicator from third to fifth cervical stage.[23] Accordingly, this study aims to analysis the IGF-1 rs5742632 polymorphism based on the severity of mandibular asymmetry.

Materials and methods

Sample study

This is a cross-sectional study of genotype-fenotype study of IGF-1 polymoprhism and mandibular asymmetry. The pre-treatment panoramic radiograph of orthodontic patients which were taken using the same X-ray equipment OC200D1-4-1 and had chronological age above 18 year old. There were 60 pre-treatment panoramic radiographs which also had complete teeth or missing a similar-type tooth on the bilateral side according to patients' medical record. Afterwards, the panoramic radiographs were measured digitally based on the Lemos method with the CliniView software version 10.1.2. The IGF-1 genotype analysis was obtained from the DNA isolation that was kept in integrated laboratory, Universitas Sumatera Utara that most of which was Sofyanti's data.[22]

Mandibular asymmetry

In contrast to previous study that used the Kjellberg technique, the classification of mandibular asymmetry was performed based on Lemos methods (Figure 1),[4] as follows: A difference between 2.0 mm to 3.0 mm was categorized as light asymmetry, 3.1 mm to 5.0 mm as moderate asymmetry and more than >5.0 mm as severe asymmetry. The distance between pogonion (Pg) and gonion (Go) were referred as the corpus length (CL). The difference between both sides of condyle height was referred as the condylar height difference (CHD), and the corpus length is shown as ∑corpus length (∑CL).[4]

Figure 1. Mandibular Asymmetry Index with Lemos Analysis.

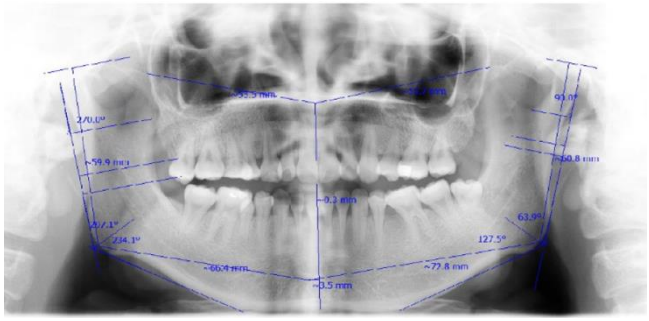

Figure 1. Mandibular Asymmetry Index with Lemos Analysis.

Isolation of DNA and IGF-1 variant's analysis

The primary data of DNA was isolated with the polymerase chain Reaction (PCR) – RFLP methods, forward 5'-GATGGCACTTCTTTTTATTTCTTG-3' and reverse 5'-TGGCAGTGCATCTTTCAGA-3'.23 The PCR cycling conditions consisted of an initial denaturation at 95 ^{o}C for 5 min, followed by 35 cycles of denaturation at 94^{o}C for 30 s, annealing at 54 ^{o}C for 45 s, extension at 72 ^{o}C for 1 min, and final extension at 72 ^{o}C for 5 min. Restriction enzymes and PCR products (223bp) were digested with *EcoR1* (RFLP) enzymes to show IGF-1 gene polymorphisms and analyzed with agarose gel electrophoresis 4% at 37 oC for 1 min, which is in accordance to the standard procedure (New England Bio Labs, Beverly, MA). The figure 2 showed PCR products was separated in 4% agarose gel stained with ethidium bromide and visualized under ultraviolet light with gel documentation (Uvitec Reader).[23,24]

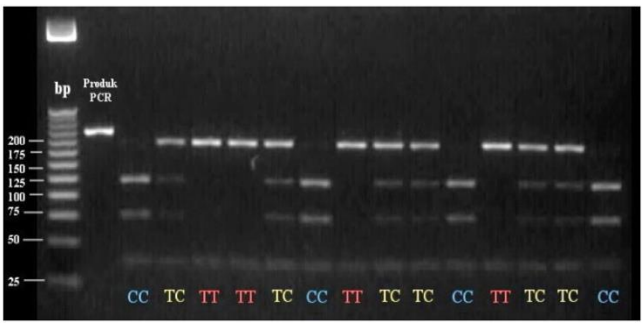

Figure 2. Polymorphism variations IGF-1 rs5742632 and PCR product of RFLP (TT: 191 32 bp; TC: 191,116,75,32 bp; CC: 116-,75,32-bp).

Data Analysis

Initially, intraclass consistency coefficient Cronbach's alpha was used to measure reliability scale of digital mandibular asymmetry analysis. All statistical analyses were performed using statistical software STATCAL. Several categories of variables related to mandibular asymmetry and polymoprhism IGF-1 were used to determine the distribution of frequency, variation, and significant correlation of the relationship. The test of genotype distribution and allele frequency for significance was done using the Chi square-test followed by Hardy-Weinberg Equilibrium calculation.

Results

The intraclass consistency coefficient with digital radiograph measurement in CHD (r=0.868) and ∑CL (r=0.889) were good. Figure 2 shows the PCR products with the enzyme RLFP EcoR1 rs5742632 (Agarose 4%; DNA ladder 25-1000) with the polymorphism variations of IGF-1 rs5742632 (TT: 191-32 bp; TC: 191,116,75,32bp; CC: 116-,75,32bp). Figure 3 showed distribution frequency of polymorphism IGF-1 rs5742632 according to age (years old) and gender.

There were twenty subjects (33.3%) of TT, eleven subjects (18.33%) of CC, and twenty-nine subjects (48.33%) of TC with the meant age of 21.71 ± 2.31 year old. There was a significant difference ($p<0.05$) of polymorphism IGF-1 among light, moderate, and severe mandibular asymmetry was shown in Table 1.

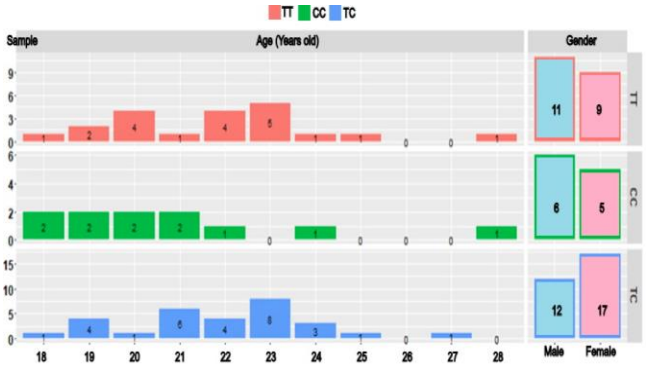

Figure 3. Distribution frequency of polymorphism IGF-1 rs5742632 according to age (years old) and gender.

Polymorphism IGF-1 rs5742632	n (%)	*P*
TT	20 (33.33%)	0.002*
CC	11 (18.33%)	
TC	29 (48.33%)	

Table 1. Genotype distribution of IGF-1 rs5742632 SNPs in mandibular asymmetry patients.
*) p<0.05: significant difference.

Discussion

A 21-year analysis of dentofacial deformities in the Indonesian population reported that mandibular asymmetry correction had been considered and become priority in treatment strategy during skeletal correction with orthognatic surgery.[25] The embryology of condylar cartilage is unique and different with cartilage of the cranial base synchondroses or the epiphyses of the long bones. Classification of mandibular asymmetry severity in this study was referred to the cross-sectional study of 327 children with mandibular asymmetry based on Lemos method, where digital panoramic radiograph showed that more than 50 % had moderate to severe mandibular asymmetry based on mandibular severity analysis.[26,27]

The phenotype of mandibular and postural asymmetry in orthodontic and orthopaedic sub-population were also correlated to the polymorphism of IGF-1 rs5742632.[22] There was a significant difference of polymorphism IGF-1 at various mandibular asymmetries in adult orthodontics patients as summarized in Table 1. The isoform of IGF-1 and the IGF-1/PI3K/Akt signaling could stimulate the pathway and skeletal muscle atrophy which might lead to the differences in condylar growth and significant change of IGF-1 level for diagnosing and determining the modality orthodontic.[22,28]

While 2D radiograph in diagnosing the mandibular asymmetry is limited, the qualitative research in understanding the complexity of mandibular asymmetry can estimate the normal range of skeletal analysis in emerging panoramic radiograph at condylar mandibular fenotype level. These findings support clinicians to establish a threshold value in the diagnostic level as a simple approach in orthodontic treatment with mandibular asymmetry interference.[29]

Even though previous study reported that the risk factors index and model of dentocraniofacial morphological pattern could be used to predict the occurrence of asymmetry mandibulofacial in Indonesian subpopulation,[30] this study of IGF-1 in DNA level can provide information about magnitude, timing of human growth and development was basically deposited in gene and locally in growth factor. Meanwhile, a significant difference of mandibular asymmetry severity based on Lemos method, the phenotype of skeletal malocclusion and mandibular growth pattern should be considered in the further study. The combination of IGF-1, cervical stage, skeletal classification, and gender could predict the mandibular length and total anterior facial height in orthodontics subjects.[31]

Conclusions

In summary, our findings revealed that there was a significant difference of polymorphism IGF-1 among light, moderate, and severe mandibular asymmetry. This analysis framework can change clinician's management mindset in the orthodontics primary care setting although genetic-phenotype studies of mandibular asymmetry should be studied further.

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Declaration of Interest

The authors report no conflict of interest.

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