# Polymorphism in 4'-UTR Region of *PITX2* in Vertical Mandibular Symmetry

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**ABSTRACT**

Study on specific genetic pathways of condylar phenotype variation related to vertical mandibular asymmetry remain rare. *PITX2*, a gene active in the Nodal Pathway that determines the left-right symmetry during embryogenesis, has been reported in expression and regulation of skeletal-muscle development as well as differentiation of satellite cells in adult muscle. The aim of this study is to analyze the phenotypes of expressed *PITX2* and its polymorphisms in vertical mandibular asymmetry based on skeletal and dental analysis. Pre-treatment panoramic radiographs from selected 62 orthodontics patients (20.7 ± 3.2 year old) were analyzed using Kjellberg symmetry Index. Subdivision of malocclusions that are limited to Angle's classification was recorded. DNA material was obtained using buccal swabs, followed by Polymerase Chain Reaction (PCR) and Sanger sequencing with ChromasPro 2.13 software (Technelysium, Queensland, Australia) and then compared to archival data from gene bank number ENSG00000164093 (www.ensembl.org).Genotype analysis showed 3 polymorphisms (rs72554076, rs761511445, rs372257787) in 4'-UTR of 16 subjects (25.8%) with various vertical mandibular asymmetry causing a C>A change at 47-105 in 13 patients, G>A change at 47-9 in 1 patient and G>T change at 46+100 in 2 patients, respectively. The characteristics of vertical mandibular asymmetry and canine subdivision dominated in these subjects. Our findings suggest that complex polygenic trait of vertical mandibular asymmetry should consider *PITX2* polymorphisms that related to muscular disorder.

***Keywords**: PITX2, vertical mandibular asymmetry, subdivision malocclusion*

Mandibular asymmetry is a common craniofacial deformity related to asymmetrical muscular function of jaw movement which results from asymmetric growth of mandibular condyle or certain diseases affecting the facial growth.(1)(2) There are vertical and rotational growth patterns in mandibular asymmetry related to mandibular undergrowth or overgrowth. The deformity often worsens with time as the imbalance between affected and unaffected sides is progressing.(3) As the primary center of mandibular growth, the condyle undergoes a remodeling process, responding to continuous stimuli during jaw movements.

There were some documentations about

**Abbreviations:** PITX2, Paired-like homeodomain transcription factor2; NSP, Nodal Signalling Pathway

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specific growth factors or other signaling molecules expressions in the regulation of mandibular morphogenesis related to condylar growth. However, asymmetrical jaw function could alter the intra-articular mechanical dynamics which could persist or renew the muscular activities in one or both of the condyles.(4) The major focus of skeletal muscle regeneration research will help the clinician to understand the developmental of muscle program at the genetic, cellular, and molecular levels.(6)

The muscular compensatory mechanism might be responsible for asymmetrical ramus height found on both sides of the subjects with malocclusions. The expression of *PITX2* as a mandibular compensatory mechanism to the asymmetry of the middle third of the face in subjects with posterior asymmetry in the vertical dimension of facial growth was studied.(7) There was suggestive association ($p<0.05$) with *PITX2* identification in 3-dimensional dentoalveolar phenotypes in subjects with malocclusion analysis.(8) The Angle classification of class II division 1 has significantly higher condylar asymmetry index compared to class II division 2, class III, and normal occlusion group. The malocclusions could be a predisposing factor of asymmetric condyles if left untreated.(9)(2)

Genetic factor, as well as environmental factors, plays an important role in the etiology of skeletal anomalies. A better understanding of the genetic variables contributing to the skeletal anomaly should be developed as a new preventive strategy in personal orthodontics treatment.(10) Based on twin studies conducted to show the genetic effects in dentofacial skeletal characteristics, the heritability of skeletal characteristics appears stronger than dental characteristics. Genetic components have stronger influence on variability in vertical dimension compared to sagittal dimension.(11)(12)

The development of molecular biology can help the clinician to recognize various genes contributing to the shape, size, and acceleration of mandibular growth. Determination of candidate genes in particular skeletal variability is related to the polygenic nature of craniofacial traits, which is involved in the formation of any malocclusion with mandibular asymmetry.(3)(11)(13) Single nucleotide polymorphisms are valuable genetic markers to reveal the evolutionary history, while common genetic polymorphisms could explain the heritable risk of common disease or anomaly,such as mandibular asymmetry.(14)

Panoramic radiograph is a routine radiograph for diagnosing mandibular asymmetry and is relatively more reliable in vertical measurements.(15)(5) The phenotype of variation in skeletal asymmetry was obtained by Kjellberg's method as it was easier to perform in terms of identifying and measuring points in vertical mandibular asymmetry.(16)(17) The extended period of mandibular growth and its rigid attachment to the maxillary located in cartilage of the condyle-fossa relationship are responsive in any biophysical environmental changes, including orthodontics treatment.

Previous study reported that differences in the NSP of *PITX2* promoted the development of left-right patterning of mesoderm and endoderm during embryogenesis and remained in masseter muscle in adults.(18)(7) Therefore, phenotype-genotype correlation studies of vertical mandibular asymmetry are greatly needed as the fundamental concept to understand the mechanisms responsible for malocclusions and craniofacial anomalies. This research was conducted to analyze the phenotypes of expressed *PITX2* and its polymorphisms in vertical mandibular symmetry based on skeletal and dental analysis.

## MATERIALS AND METHODS

This is a cross-sectional study conducted on a selected 62 orthodontics patients ($20.7 \pm 3.2$ years old) of Dental Hospital Faculty of Dentistry, Universitas Sumatera Utara from July 2016 to March 2017. The study protocol was reviewed and approved by the ethics committee of Medical Faculty, Universitas Sumatera Utara (100/DATE/KEPK FK USU-RSUP HAM/2017). All pre-treatment panoramic radiographs taken by the same X-ray machine (AUGE series, Asahi Roentgen, Japan) were measured by using an x-ray reviewer. The following contours (mandibular line, angle, ramus and notch, and condylar process) were marked on transparent paper on the x-ray film with a pencil and measured according to the Kjellberg's symmetry index.(15) PCR analysis was done in the Integrated

Laboratorium of Medical Faculty, Universitas Sumatera Utara and sequencing was performed by the 1st base Laboratories, Selangor-Malaysia.

**Experimental inclusion**

The subjects with poor quality panoramic radiographs and dental casts were excluded. Based on medical records, the exclusion criteria includes previous orthodontic treatment or prosthodontics history, congenital history, facial trauma, incomplete denture except third molar, and oral bad habits history. The 62 subjects, regardless of temporomandibular joint status, were recalled for genotyping analysis. Samples of DNA material were collected by buccal swab with wooden stick (Nesco®).

**Problem formulation**

Selection of samples for vertical mandibular symmetry was based on Kjellberg's symmetry index that less than 93% difference categorized as asymmetry (Figure 1). The symmetry between the right and left condylar was estimated using the following formula:

$$\text{Kjellberg Symmetry Index (SI)} = \frac{\left(\frac{CH}{RH_A}\right)}{\left(\frac{CH}{RH_B}\right)} \times 100$$

Molar and Canine classification was verified by asymmetric malocclusions in sagital based on Angle's subdivision classification on pre-treatment dental cast analyze (Figure 2). To assure validity and reliability of intra-rater digitized panoramic radiograph measurements, *Cohen's Kappa* was performed. There is no requirement to assure consistency in scoring of the occlusal parameters since dental cast was used as indicator.

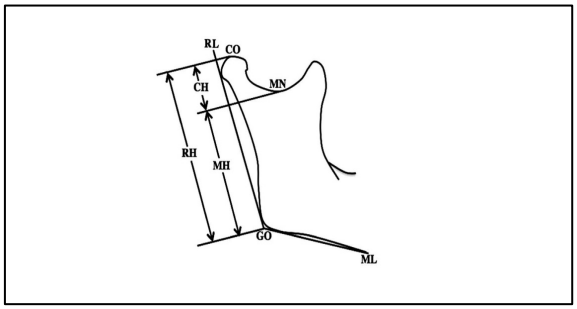


**Fig. 1.** Measurement of vertical mandibular symmetry with Kjellberg's technique

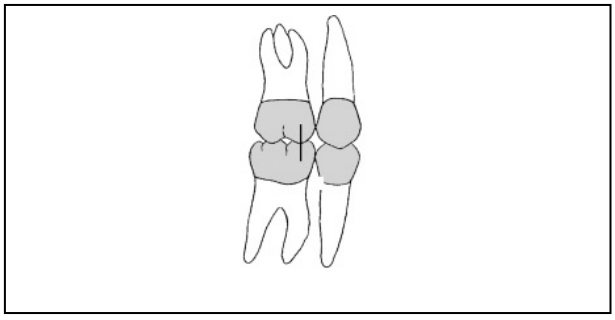

**Fig. 2.** Classification of Angle's Molar and Canine subdivision

Genomic analysis to obtain Patients' DNA using Presto TM (Geneaid, Taiwan) with buccal swab method. The following primers were used: Region A forward 5'-AATCTCTGCTGACGTCACGT and reverse CCAGACTCGCATTATCTCAC-3'; Region B forward 5'-TAGTCTCATCTGAGCCCTGC and reverse TTCTTGCGCTTTCGCCCGA-3'; Region C forward 5'-CTTGACACTTCTCTGTCAGG and reverse AAGCGGGAATGTCTGCAGG-3'; Region D forward 5'-CAGCTCTTCCACGGCTTCT and reverse TTCTCTCCTGGTCTACTTGG-3'; Region E forward 5'-GTAATCTGCACTGTGGCATC and reverse AGTCTTTCAAGGGCGGAGTT-3'. PCR was performed at 55°C annealing temperature. Amplified products were separated on

agarose gels, reamplified, separated on 3% agarose gels, purified with KAPATaq™ Extra Hotstart readymix with dye (Kapa Biosystem) and sequenced directly with CLUSTAL 2.1 multiple sequence alignment. Chromatograms were visualized with Chromas Pro 2.13 software (Technelysium, Queensland, Australia) and compared to archival data from gene bank number ENSG00000164093 (www.ensembl.org).(19)

## RESULTS

In this study, the author sequenced the *PITX2* coding region and intron–exon boundaries of 62 selected multi-ethnic patients who came for orthodontics treatment and received no prior orthodontics treatment. The finding showed 16 subjects with PITX2 polymorphisms. The study identified 3 polymorphisms (rs72554076, rs761511445, rs372257787) in 4'-UTR with various vertical mandibular asymmetry, both near (in close proximity of the) splice junctions. The SNPs rs72554076 caused a C>A change at 47-105 in 13 patients (Figure 4). The SNPs rs761511445 caused a G>A change at 47-9 in 1 patient (Table 1) and SNPs rs372257787 caused a G>T change at 46+100 in 2 patients (Table 1). The clinical data of phenotype including vertical mandibular asymmetry and subdivision of molar and canine classification can be observed on Table 1. The higher score of phenotype characteristics were found in subjects with compound mutation and new rs number that have never been reported before.

Table 1. Clinical data of SNPs of *PITX2* and phenotype vertical mandibular symmetry

| Subject | Base | SNPs | Sequence | Age | Vertical mandibular symmetry (0=Symmetry; 1=Asymmetry) | Molar Symmetry (0=Symmetry; 1=Asymmetry) | Canine Symmetry (0=Symmetry; 1=Asymmetry) |
|---|---|---|---|---|---|---|---|
| **3** | 46+100 | G>T | CAGCGAGGGGCGCTTCCC | 22 | 1 | 0 | 1 |
| **10** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 23 | 1 | 0 | 0 |
| **15** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 15 | 1 | 0 | 1 |
| **20** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 24 | 0 | 1 | 1 |
| **24** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 22 | 1 | 1 | 1 |
| **29** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 11 | 1 | 0 | 0 |
| **30** | 47-9 | G>A | CCTCTTTCTCCTCCGGCC | 25 | 1 | 0 | 1 |
| **51** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 24 | 1 | 0 | 0 |
| **59** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 16 | 1 | 0 | 0 |
| **62** | 46+100 | G>T | CAGCGAGGGGCGCTTCCC | 24 | 1 | 0 | 0 |
| **85** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 23 | 1 | 0 | 1 |
| **105** | 47-105<br>184+11<br>184+23 | C>A<br>G>A<br>G>A | CCTCTTTCTCCTCCGGCC<br>TAAGGCCGGGAGGGAAGC<br>GGAAGCGCAGGCCGCGCG | 21 | 1 | 1 | 1 |
| **112** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 19 | 0 | 0 | 1 |
| **122** | 47-105<br>46+80 | C>A<br>C>A | CCTCTTTCTCCTCCGGCC<br>CTGGCCCTGCGGCGAGGC | 23 | 1 | 1 | 1 |
| **126** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 18 | 1 | 1 | 1 |
| **142** | 47-105 | C>A | CCTCTTTCTCCTCCGGCC | 16 | 1 | 1 | 0 |
| **TOTAL** | | | | | **14** | **6** | **10** |

## DISCUSSION

The complexity of mandibular asymmetry explains, in part, the reason of why most treatment approaches for malocclusion are directed to the symptoms rather than to its etiology. In our study, we hypothesized that an asymmetric malocclusion may be the result of dental arch asymmetry development due to skeletal asymmetry in the maxillofacial skeletal complex. Remodelling the condyle and the glenoid fossa in mandibular deviation suggests that the asymmetry may be an adaptive response to functional demands. However, the study of genetics is fundamental to understand the underlying biology of craniofacial growth and dental relations.(11)(20)

Bio-molecular explanation of the original DNA coding (A, T, C or G) for certain locus in a genome showed that vertical mandibular variances had specific genotype locations. There are natural variations in DNA sequence, which means a specific gene on a

locus can vary even within homologous chromosomes in the same individual. The mode of inheritance describes how the genetic information is passed down through "allele" which forms homozygotes or heterozygotes from one generation to another. Inspite of these specialized cell types, most cells within an individual's differentiate into muscle, nerve, skin cell, etc, or become an organ based on the pattern of genes that are turned "on" or "off" within each cell. The activation process of the gene is referred as "gene expression" which mostly lead to production of protein or a set of related proteins. (21)(10)

Previous studies have shown that malocclusions have a remarkable effect on condyle morphology in mandibular asymmetry and vice versa. The high prevalence of moderate and severe asymmetries were reported in 327 Caucasian subjects aging between 8 to 12 years old when comparing condylar and gonial angle on both sides of the mandible based on panoramic radiograph.(22) A clinically significant prevalence of molar and canine subdivision relationships was reported in Kuwait and hungarians adolescents.(23)(13)

Based on previous studies regarding vertical mandibular asymmetry, the measurement of asymmetry were various and no specific 'gold standard' for the threshold norm value in each population was found. This research was a basic novel research combining skeletal and dental phenotype with wide genome analysis of vertical symmetrical zone that has never been conducted on Indonesian population with its diverse demographic background with similarity in seeking orthodontics treatment.

The clinical data of SNPs (rs72554076, rs761511445, rs372257787) of *PITX2* and phenotype reported that vertical mandibular asymmetry asymmetry and canine asymmetry dominated in vertically. Asymmetries between both sides of the mandible may be due to an adaptive response of the mandible to deviations during functions, which may cause modelling of the condyle and glenoid fossa, as well as remodeling and modelling of the mandibular bone. The process would then lead to dimensional differences of size or shape between the right and left sides of the mandible.(4) The *PITX2* is a master regulator gene that regulates the production of a protein that binds to specific region of DNA and regulates other genes expressed in the eyes, teeth, heart, and abdominal organs.(12)

In this study, the asymmetry of condylar height was dominated by subjects with polymorphism, except in polymorphism SNPs rs372257787 which caused a G>T change at 46+100 in 2 patients. There was no Molar asymmetry in subjects with G>T change, except in C>A. Moreover, the presence of gene-gene and/or gene-environment interaction in mechanisms of the trait's etiology, partial or incomplete phenotypic approaches and the combined effect of genetic variants in non-coding regulatory regions could also result in missing heritability.(20) In this study, a new mechanism of the regulation of *PITX2* transcriptional activation through the action of *PITX2* isoform was found. This can explain the craniofacial abnormality in non-syndromic patients related to *PITX2* gene expression in muscle function, especially masseter muscle which might influence the development of malocclusion in asymmetrical dental relationship. These new *PITX2* functions on satellite-cell biology might be considered in developing therapeutic strategies for muscular disorders in mandibular asymmetry.(8)

Since the subjects consists of multiethnic patients, further studies regarding ethnicity are required. Determining the effect of different diets on individuals with and without *PITX2* polymorphisms would be of interest as a means to reveal the interaction of genetic and environmental factors. According to previous studies of genetic basis and its relation to orthodontics, orthodontists should be aware of the etiological factors of vertical mandibular asymmetries to determine the best treatment plan for each patient in the era of truly personalized orthodontics.(10)(11)

This data provided by the human genome project have made it feasible to map inherited vertical mandibular asymmetry related to Polygenic "complex" trait since this *PITX2* with its multiple proteins. Identification of *PITX2* polymorphisms might influence dental compensation, especially in the symmetrical zone. Our findings suggest that complex polygenic trait of vertical mandibular asymmetry should consider *PITX2* polymorphisms that are related to

muscular disorder in order to develop interceptive orthodontics.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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