A Preliminary Study on MYO1H Single Nucleotide Polymorphism (rs10850110) in Mandibular Prognathism in Malay population

Siti Nazirah Yahya[1], Nurul Syafiqah Abdul Razak[2], Khairani Idah Mokhtar[3], Azrul Fazwan Kharuddin[4], Noraini Abu Bakar[5]*

1. Kulliyyah Of Dentistry International Islamic University Malaysia.
2. Kulliyyah Of Dentistry, International Islamic University Malaysia.
3. Unit Of Oral Biology, Kulliyyah Of Dentistry, International Islamic University Malaysia.
4. Kulliyyah of Science, International Islamic University Malaysia.
5. Orthodontic Unit, Kulliyyah Of Dentistry, International Islamic University Malaysia.

Abstract

Evidence suggests that several genes; including Myo1H, play an important role in the etiology of Class III malocclusion. Single nucleotide polymorphism (SNP) in marker rs10850110 (locus 12q24.11) within Myo1H gene has been associated with the incidence of mandibular prognathism (MP). MYO1H is a class 1 myosin which has been implicated in various motile processes including cytoskeleton reorganization. Therefore, genetic alteration in genes responsible for muscle function will also affect the skeletal growth.

This study aimed to detect the presence of Myo1H (rs10850110) SNP and to determine its genotype and allele distribution in MP patient in the local population.

The sample comprises of 31 patients; 14 patients from class I malocclusion (control samples) and 17 patients from class III malocclusion (MP). Cephalometric measurements were performed prior to saliva samples collection. The DNA was amplified using the specific primers for the marker rs10850110 and the genotyping was done by sequencing. Chi-square test was used to determine the over-representation of marker allele ($p<0.05$).

Presence of Myo1H SNP (rs10850110) was detected in local population analysed and the distribution of its genotype and allele could be observed. There were significant differences between allele ($p=0.000$) and genotype ($p=0.000$) frequency within and between control (Class I) and Class III malocclusion.

Our findings are in agreement with previous studies suggesting positive influence of Myo1H (rs10850110) SNP in the incidence of MP. Further studies should be developed in order to understand the exact role and mechanism of Myo1H in different classes of malocclusions.

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Introduction

Malocclusion is defined as a deviation in intermaxillary and/or intermaxillary relations of teeth from normal occlusion and is often associated with other dentofacial deformities. Among the dental pathologies, malocclusions feature the third highest prevalence after tooth decay and periodontal diseases and therefore rank the third among world-wide dental public health priorities[1]. Malocclusions has resulted in various implications to the individual's life, such as psychosocial problems related to impaired dentofacial aesthetics, disturbances of oral functions, including mastication, swallowing and speech, and allow greater susceptibility to trauma and periodontal diseases[2]. The prevalence of Class III (also known as mandibular prognathism) varies with age[3,4] ethnic groups[5,6] and sex, being more frequent in females[7,8].

Few studies have shown that the incidence of mandibular prognathism is higher in Asian populations such as Korean, Chinese, and

Corresponding author:
Asst Prof Dr Noraini Abu Bakar
DDS(USM), MSc Orthodontics(London), MOrth RCS (Edinburgh)
Head of Paediatric Dentistry, Orthodontics and Dental Public Health Department Kulliyyah of Dentistry,
International Islamic University Malaysia
Kuantan Campus, 25200 Kuantan,
Pahang, MALAYSIA
E-mail: nor_aini@iium.edu.my

Japanese as compared to European populations[8,9,10].

Both genetic and environmental factors may affect craniofacial development, creating intricate and elaborate multifactorial causes for malocclusion[11]. This complexity explains in part why most treatment approaches of malocclusion are directed to the symptoms rather than to its fundamental cause[12]. Several evidences suggested that genetic factors contribute to the malocclusion susceptibility. Single Nucleotide Polymorphism (SNP) in certain genes has been associated with incidence of malocclusion. SNP are single-base pair change in DNA sequence that occur with high frequency in human genome, thus it is typically used as markers of a genomic region in genetic studies. Association studies have found positive correlations for mandibular prognathism and genes *EPB41, SSX21P*, and *PLXNA*, located within the locus 1p22-p36, while genes *COL2A1, MYO1H, TGFB3*, and *LTBP2* within the 12q13-q24 locus[12]. Additionally, previous study had demonstrated an association between a marker in *MYO1H* (rs10850110) at locus 12q24.11 and mandibular prognathism phenotype[13]. *MYO1H*, located at 12q24.11 is a class 1 myosin that is in a different protein grouping than the myosin heavy chain isoforms found in the skeletal muscle sacromeres, which are the basis of fiber typing. Myosins are superfamily of motor proteins that involve in generating force and movement along actin filaments[14]. Class 1 myosin is necessary for cell motility, phagocytosis and vesicle transport[15]. Myosin heavy chain isoforms was revealed to be found in the masseter muscle via immunohistochemical staining and gene expression studies[16]. Few studies suggest that muscle affect the skeletal growth during embryonic stage, postnatal stage, homeostatic relationship in adult and aging process[17]. Therefore, genetic alteration in genes responsible for muscle function will also affect the skeletal growth.

Previous studies done on population at different geographical locations managed to prove that polymorphism in the marker rs10850110 of *MYO1H* was a risk factor for Class III mandibular prognathism[13]. However, very limited data on genetic study of malocclusion is available from the local population in Malaysia. Thus, the main goal of this preliminary paper was to detect if similar polymorphism presents in the cases of malocclusion observed in the local Malay patients and to determine its genotype and allele distribution. The result obtained from this study could be used as foundation for future research; hence enable and clinicians to intercept by means of genetic approach and provide better treatment methods to decrease the severity of this condition in the future.

Materials and methods

This research was a case control study of active orthodontic patients from the Orthodontics Department, Kulliyyah of Dentistry, International Islamic University Malaysia (IIUM). Ethical approval was obtained from IIUM Research Ethics Committee (IREC) (REF NUMBER: IIUM/305/14/11/2/IREC 549)

Patient selection

The subjects were active orthodontic patients from the Orthodontic Specialist Clinic, Kulliyyah of Dentistry, IIUM. The assessment of eligible subjects involved clinical examination and review of subjects' clinical and radiographical records. Clinical record assessment includes a combination of study models, cephalometric tracings and photographs. Eastman and Wits Cephalometric analyses were performed.

This preliminary study involved a total of 31 Malay patients, whereby 17 patients from Class III malocclusion and 14 patients from Class I malocclusion (as control) were recruited. The sample selections were based on the criteria given as follows:

Inclusion criteria for Class III samples:

- Fit and healthy
- Cephalometric analysis with value indicative of Class III based on Eastman analysis (ANB should be <2˚ and SNB should be >81˚)
- SNA within normal range indicative of average maxilla (81˚±3˚)
- Negative Wits appraisal (AoBo) of < -2mm
- Concave facial profile

Inclusion criteria for Class I samples:

- Fit and healthy
- Cephalometric value indicative of Class I based on Eastman (ANB within 2˚ to 4˚, SNA within range of 81˚±3˚ and SNB within range of 78˚±3˚)

- Wits appraisal (AoBo) within Class I (-2mm to +2mm)
- Straight facial profile

Exclusion criteria:

- Craniofacial deformity including cleft lip and palate
- Endocrinological problem
- Anomalies in tooth number, morphology and eruption

Cephalometric analysis

Eastman Analysis

SNA angle is used to assess the position of the maxilla in relation to the cranial base. The mean value is 81˚ ± 3˚, value less than 78˚ indicate a maxillary retrognathism, while angle larger than 84˚ would indicate a maxillary prognathism. SNB angle is used to assess the position of the mandible in relation to the cranial base. The mean value is 78˚ ± 3˚. Value less than 75˚ indicate mandibular retrognathism, while value larger than 81˚ indicate prognathic mandible.

ANB can also be measured once SNA and SNB were obtained. It is used to determine the relative position of the jaws to each other, related to the cranial base. Mean value is 3˚ ± 2˚. An ANB angle greater than 5˚ indicates a Class II tendency and angle less than 1˚ indicate a Class III tendency. However, in this study, border line of 1˚ and 5˚ were eliminated as it does not represent a true prognathism or retrognathism of the mandible. Therefore, the ANB values of 2˚ to 4˚ were taken as Class I subject.

Wits Analysis

The Wits Appraisal is a linear measurement used to assess the antero-posterior jaw disharmony. A linear difference is measures between the projection of points A and B to the occlusal plane (AoBo). The average jaw relationship is -1mm in Males (AO is behind BO by 1mm) and 0mm in Females (AO and BO coincide)[19]. In skeletal Class III patient, BO is located ahead of AO.

Genetic analysis

For the genetic analysis, 5 ml of unstimulated saliva was collected from the subjects. The subjects were asked to spit into a container. The saliva samples were stored at -20˚C until being processed. DNA was extracted using GeneAll ® Exgene ™ Kit (GeneAll, China) following the protocol provided. The gene fragment (803 bp) of *MYO1H* containing the *rs10850110* SNP (12q24.11) marker was amplified using the primers as below: [18]

H1For 5'-AATTCTGTCTGCTCCGCATC-3'
H1Rev 5'-ATTTCCATCCAATGGTGCAG-3'

The PCR mixture (25µl) contained 0.5 µM of each primer (Sigma), 1 X PCR buffer (containing 2 mM $MgCl_2$), 0.2 mM of each *d*NTP, and 1.25 U of EasyTaq® DNA Polymerase (TransBionovo, China). The standard amplification conditions were; initial denaturation at 95˚C for 4 min, followed by 35 cycles of 94˚C for 30 sec, 56.8˚C for 30 sec, 72˚C for 1 min, with the final extension of 72˚C for 10 min. The PCR reaction protocol and the primer sequences were adapted from previous study[18].

The PCR product was electrophoresed on 1.5 % agarose (Transgen Biotech, China) in 1 X TBE buffer (100 V; 60 minutes) and was observed under UV light using gel documentation system. The band observed at 803 bp was excised and purified from agarose gel using the commercially available purification kit Expin ™ Gel SV (GeneAll, China) following the protocol provided. The purified PCR product was then sent for sequencing using the H1For primer.

Statistical analysis

The data collection was taken using quantitative primary data and Chi-square test was used to assess the significant in comparison and correlation of genotype and allele in each class. The genotype and allele frequency were compared within each class of malocclusion namely Class III mandibular prognathism and Class I control samples. *P*-value of less than 0.05 was considered statistically significant ($p<0.05$).

Results

Training on cephalometric analysis with specialist was done and subsequently inter-examiner calibration was carried out on two lateral cephalograms to achieve a synchronized agreement between the two examiners. There were significant differences between the two classes of malocclusions with the ANB ($p=0.003$) and Wits ($p=0.045$). All samples were taken from patients of Malay ethnicity.

The concentration of genomic DNA (gDNA) obtained from the extracted saliva was within 20 to 40 ng/µl and suitable to be subjected

for PCR analysis. Electrophoresis of the PCR product showed a specific band at approximately 803 bp was produced. The band was excised and purified for further sequencing analysis.

Sequencing result of DNA samples was observed using electropherogram to detect the presence of homozygous or heterozygous allele and polymorphic position of both nucleotides. Homozygous GG genotype is presented as single peak (Figure 1(a)) and heterozygous AG genotype is presented by double peaks Figure 1(b).However, homozygous AA genotype could not be found in this study.

	Patient	Age (y)	Gender	Ethnic	SNA (°)	ANB (°)	AO-BO (mm)	Genotype SNP (rs10850110)	Allele
CLASS I (control)	1	24	M	Malay	80	3	1	GG	G
	2	23	M	Malay	78	2	-1	GG	G
	3	23	M	Malay	80	3	1	GG	G
	4	24	M	Malay	84	3	0	GG	G
	5	24	F	Malay	81	4	2	GG	G
	6	24	F	Malay	80	4	2	GG	G
	7	23	M	Malay	83	2	2	AG	G
	8	22	F	Malay	79	4	1	GG	G
	9	24	M	Malay	84	2	-1	GG	G
	10	24	F	Malay	82	2	-1.5	GG	G
	11	25	F	Malay	84	4	0	GG	G
	12	23	F	Malay	81	2	-2	GG	G
	13	23	F	Malay	79	4	1	GG	G
	14	15	M	Malay	84	1	-2	GG	G
CLASS III	1	24	F	Malay	82	0	-6	GG	G
	2	23	F	Malay	80	-1	-10	GG	G
	3	25	F	Malay	81	-3	-8	GG	G
	4	24	F	Malay	84	-2	-9	AG	A
	5	23	F	Malay	84	0	-3	GG	G
	6	25	F	Malay	84	-1	-11	GG	G
	7	22	M	Malay	79	-3	-8	GG	G
	8	22	M	Malay	79	-2	-13	GG	G
	9	23	M	Malay	83	-2	-8	GG	G
	10	24	F	Malay	84	-1	-6	GG	G
	11	25	F	Malay	82	1	-3	GG	G
	12	24	F	Malay	81	1	-5	GG	G
	13	25	F	Malay	81	-1	-4	GG	G
	14	23	F	Malay	80	0	-4	GG	G
	15	24	M	Malay	80	-1	-5	GG	G
	16	23	F	Malay	82	0	-4	GG	G
	17	21	F	Malay	80	-1	-5	GG	G

Table 1. Cephalometric measurements and genotypes for rs10850110.

	Genotype			Allele	
Class III n=17	GG	AG	AA	G	A
	16	1	0	33	1
	*p=0.000**			*p=0.000**	
	HWE (p=0.900)				
Class I (control) n=14	GG	AG	AA	G	A
	13	1	0	27	1
	*p=0.001**			*p=0.000**	
	HWE (p=0.889)				
Class III, Class I (control)	29	2	0	60	2
	*p = 0.000**			*p = 0.000**	

Table 2. Genotype and allele distribution of the MYO1H gene (SNP rs10850110).

Based on Table 1 for cephalometric variables and their genotypes, a total of 17 samples from mandibular prognathism patients and 14 samples from control group were able to be sequenced and analyzed accordingly.

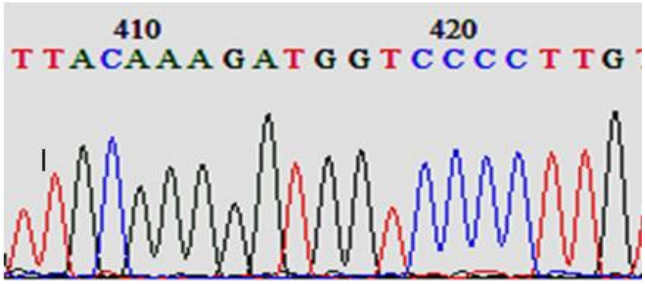

Figure 1 (a). Example of homozygous GG genotype as demonstrated by the sequencing result.

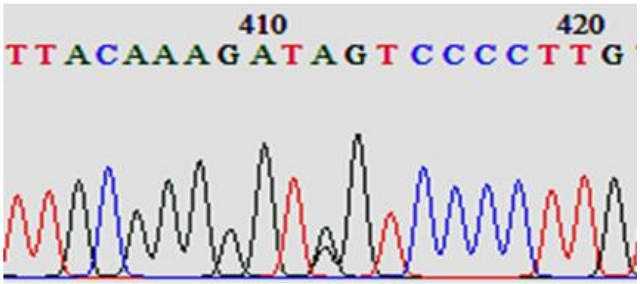

Figure 1 (b). Example of heterozygous AG genotype as demonstrated by the sequencing result

The distribution of genotype and allele in Class I and Class III malocclusion samples was statistically analyzed with Chi-square test as shown in Table 2. There was a significant difference between GG and AG frequency for genotype in Class I (p=0.001) and Class III (p=0.000) and between G and A frequency for allele in Class I (p=0.000) and Class III (p=0.000). From this table, we can see that G allele of marker rs10850110 was over-represented in both classes of malocclusion. There are significant differences between allele and genotypes frequencies in subjects with Class III versus Class I control samples. The distribution of genotype frequency in both subjects and control groups were according to Hardy-Weinberg equilibrium (p>0.5)

Discussion

Craniofacial anomalies, including mandibular prognathism is a multifactorial in nature, thus it has a complex trait. Genetic susceptibility to a certain trait is most commonly due to single nucleotide polymorphism (SNP) [20], where changes occur with a single base substitution and creates one nucleotide difference in two strands of DNA[21].

The frequency of minor alleles usually studied for their association with the trait in question that would indicate causality or elevated risk of developing or protecting a particular trait[22]. Since genomic association analysis are applied to unrelated individuals and have more power in the identification of common variants in a population[23], therefore this method has been used in this research.

This preliminary study performed on local Malay population in an attempt to detect the presence of *MYO1H* SNP (rs10850110) in Class III mandibular prognathism subjects as well as their genotype and allele distribution. As for patient selection, cephalometric measurement was done using Eastman analysis as it is commonly used in United Kingdom utilizing Caucasion Class I normal data. In Eastman, Nasion (N) tends to move anteriorly, thus, overestimating some of true value. Therefore, additional analysis independent of this region was used in this study to further support an existing Eastman analysis. Wits appraisal use functional occlusal plane instead of cranial base as reference point to reduce error associated with discrepancy of cranial base position[24]. Thus, it was used in this study as an additional measurement to support an existing Eastman analysis.

Among SNPs, marker *rs10850110* (promoter polymorphism) at locus 12q24.11 corresponds to 5' of myosin 1H. SNP within this region is believed to be responsible for mandibular prognathism[13]. In spite of small sample size, this current paper managed to detect the presence of both ancestral G allele and minor A allele in SNP *rs10850110* (locus 12q24.11) within *MYO1H* gene in both healthy Class I control subjects and mandibular prognathism Class III subjects.

Interestingly, the distribution of genotype and allele frequency was observed to be significantly present in the samples analysed from both classes of malocclusions. In both healthy and mandibular prognathism subjects, the majority of the genotype present was homozygous GG genotype compared to heterozygous AG genotype while homozygous AA genotype was not detected in this study. Additionally, the ancestral allele which is G allele of SNP *rs10850110* was over represented in both classes as compared to minor allele which is A allele. Our results also demonstrated significant difference in genotype and allele frequencies between Class III and Class I control samples. However, larger number of samples will be required in order to ascertain the presence of genetic association between *Myo1H* (SNP rs10850110) with mandibular prognathism Class III subjects in the local population.

Previous study using modest sample size (44 mandibular prognathism and 35 Class I) performed on different ethnics (24 Europeans, 15 African American, 2 Hispanic and 3 Asian) pointed out that the ancestral G allele of marker rs10850110 was over-represented in mandibular prognathism subjects (p=0.03). The frequency of minor allele which is A allele varies between ethnics, with the highest found in Europeans (0.275) and the least in African American (0.008) while Japanese Asian (0.089) and Han Chinese Asian (0.148)[13]. On the other hand, the population diversity data for rs10850110 available in The International HapMap project (halotype map) shown that the distribution of A allele is relatively high in European population (HapMap-CEU; A=0.29, G=0.71) as compared with Asian population (HapMap-HCB; A=0.08, G=0.91.,HapMap-JPT; A=0.16, G=0.83., HapMap-CHB; A=0.11, G=0.89). Among those three Asian population studied, homozygous AA genotype was detected with lowest frequency among the other genotype only in HapMap-JPT (AA=0.01, AG=0.30, GG=0.69), in addition to a relatively high frequency in European population (AA=0.06, AG=0.45, GG=0.4) [25]. Since the subject in the current study were Asian Malay and in addition to small sample size, this could explain why A allele was hardly to be detected in this study and thus, association analysis comparing allele frequencies between mandibular prognathism and healthy control subjects were not able to be concluded from the current analysis.

Problem in treating Class III cases is that clinician could not predict growth and severity of the malocclusion. Treatment such as functional appliances for Class III cases has mixed review and clinical application considered by most clinicians to be inconvenient with high relapse tendency. To date, due to unpredictable expression and wide spectrum of dentofacial variations present in malocclusion patients, most treatment approaches are directed to the symptoms rather than etiology[12]. Considerable evidences suggested that several genes influence the risk of developing mandibular

prognathism, however there were still limited animal and human functional studies done to elucidate the specific function and influence of those genes in this trait. From genetic perspective, intervention could be done by gene therapy to either counteract the gene expression by inhibit growth of mandible at earlier stage or directly target at the responsible gene.

To the best of our knowledge, this is the first study reporting the nucleotide changes in rs10850110 within *MYO1H* gene observed in mandibular prognathism of local Asian Malay population. Thus, this is regarded as the first step to support a more extensive genetic research in the future in order to expand further knowledge for better understanding of genetic influence in mandibular prognathism. Future study with bigger sample size and more strict variables could be performed to detect an association between *MYO1H* SNP within different classes of malocclusion and to better infer the exact role and mechanism of *MYO1H* SNP in determining malocclusions. Since genetic polymorphism may varies in severity of malocclusions, age groups, gender and ethnic background, these should be considered in future investigations so that it may provide useful information for clinician to provide better treatment modalities.

Conclusions

The presence of *MYO1H* SNP (rs10850110) in local Malay population was able to be detected and the distribution of genotype and allele could be observed and determined. This preliminary study will lay the foundation for future studies to identify the exact role and mechanism of *MYO1H* polymorphism affecting mandibular growth. The identification of genetic influences in malocclusions can aid in the prevention and improve treatment modalities of maxilla-mandibular discrepancies.

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Declaration of Interest

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