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ACE and TPA25 *Alu* insertion polymorphisms in Minang Malays subethnic groups in Peninsular Malaysia

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ABSTRACT

Minangkabau Malays or Minang Malays (Melayu Minangkabau) is one of the Malay subethnic groups in Peninsular Malaysia. Migration of the Minangs from West Sumatra, Indonesia to the state of Negeri Sembilan Darul Khusus in Peninsular Malaysia took place during the late 17th and early 18th centuries, and their descendants now form the main sub ethnic group in this state. For the first time, an analysis of two *Alu* insertion loci (ACE and TPA25) has been carried out in Minang Malays subethnic group in Peninsular Malaysia. The epithelial buccal cells were collected from fifty unrelated healthy individuals by using indicating FTA card and were typed for ACE and TPA25 *Alu* locus. The PCR amplicons were separated by using 2% (w/v) agarose gel and visualized under UV light. In this study Minang Malays shows trend toward lower ACE and TPA25 *Alu* insertion, 0.320 and 0.330 respectively. F-stastistic value of 0.2935 suggested that vast genetic differentiation of two different loci existed within Minang Malays in Peninsular Malaysia.

KEYWORDS

Alu insertion polymorphism;
FTA;
PCR;
Minang malays;
Peninsular Malaysia

INTRODUCTION

Alu insertion consist over one million copies making them the most abundant class of short interspersed elements (SINEs) in human genome[1]. *Alu* element is dimeric 300bp sequences that mobilize via retroposition into new chromosomal location and their insertion at specific location is polymorphic[2]. The insertion of *Alu* element can be classified as a unique event, because once inserted these element are rarely removed[3]. This element also identical by descent (IBD) and inherited from common ancestor as compare to several DNA marker such as Human Leukocyte Antigen (HLA), mitochondrial DNA (mDNA) and Single Nucleotide Polymorphism (SNP) that identical by state (IBS)[4]. In addition, *Alu* element also has homoplasy free characteristic. This means the probability of two independent *Alu* insertions exist in the same genomic region in human population is essentially zero. All these nature of *Alu* element make them ideal for studying different aspects

of evolutionary biology and genetics ancestry.

Peninsular Malaysia is at the tip of mainland Southeast Asia whereas the states of Sabah and Sarawak are in the north east coastal area of Borneo Island. These two regions are separated by about 531 km of the South China Sea. The land area of Peninsular Malaysia covers 131 598 km^2, which consist of the following states: Perlis, Kedah, Pulau Pinang Perak, Selangor, Negeri Sembilan, Melaka, Johor, Kelantan, Terengganu, and Pahang. Negeri Sembilan is one of Malaysia's most unique and tiniest states which are the seat of the Minang Malay subethnic group, whom their descendent migrated across the Strait of Malacca from west Sumatra in the 15 century[5]. Negeri Sembilan is prominent with Minangkabau influences and this is proved by their home architecture which built with sweeping roof peaks shaped like buffalo horns[6]. This unique architecture only can be observed within this subethnic group. In addition, Minang Malays also practice the matrilineal social system known as the "*adat perpatih*" where the mother line or mother genealogy acts as the main lineage of family. In this system, the children are owned by the mother and her sisters, not to the father and his sisters or brother. Besides, the father has no right to claim the heritages of the family and acts only as the guard for the whole heritages and heirlooms of the family[7]. Minang Malays also can be distinguished from other Malays sub-ethinc groups by the dialect they spoken. Till date, the study of Malaysian Malays subethnic groups based on DNA marker especially using *Alu* insertion polymorphisms is very limited. Here, we report the data for ACE and TPA25 *Alu* insertion polymorphism in Minang Malays subethnic group in Peninsular Malaysia.

EXPERIMENTAL

Subpopulation samples

The ethical approval was obtained from Research Ethics Committee of Universiti Teknologi MARA (UiTM). Formal consent was attained from 50 healthy and unrelated individual Minangkabau Malays (Minang) subethnic groups. The Minagkabau Malays was recruited from Kampung Gagu, Jelebu and Kampung Daching, Beranang from state of Negeri Sembilan Darul Khursus (Figure 1). Three generations of ethnic origin was sought back for each subject to ensure genetic purity (Figure 2). All of them were interviewed to confirm their birthplace and family history. Those with mixed marriage were excluded from this study. The ethnicity of each representative was defined by the Malaysian Identity Card (MyCard) as registered with National Registration Department.

Figure 1 : Map of the geographical distribution of Minangkabau Malays individual used in this study

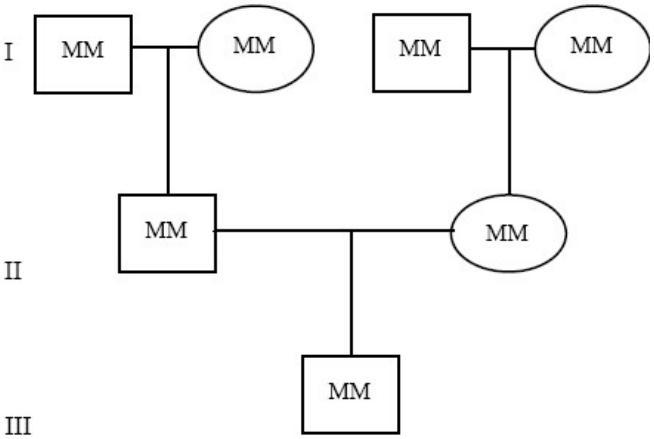

Figure 2 : A pedigree charts for ethnicity of an example of male representative through inclusion factor of three generation pure of Minang Malays (MM) subethnic groups. Squares designate males and circles represent females. Parents are connected by a horizontal line, and vertical lines lead to their offspring

Samples collection

Sample collection began with vigorously swabbing

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the inside a respondent's mouth and rub side by side on the inside cheek for about one minute. The foam tip was transferred to Fast Technology for Analysis (FTA) card and the colour of FTA card changed from pink to white which indicated the presence of samples. The FTA cards were stored at room temperature and ready for further used.

PCR amplification

Two human specific *Alu* insertion polymorphisms (1) ACE:located on chromosome 12 and (2) TPA25: located on chromosome 8, were typed in this study. There were commonly used in worldwide study of human population genetic[1-4]. A complete list of the specific oligonucleotide primers are shown in TABLE 1. Amplification of each *Alu* insertion polymorphism was performed in 20 µl reaction using 2mm FTA cards, 2x Phusion Human Specimen PCR Buffer, 10mM oligonucleotide primer, Phusion Human Specimen DNA polymerase and sterile H_2O. Each sample was subjected to an initial denaturation of 5 min at 98ºC followed by 35 amplification cycles of denaturation at 98ºC for 1 sec, optimized annealing temperature for 30 sec and followed by extension at 72ºC for 15 sec. The final extension temperature was set at 72ºC for 1 min, followed by the holding temperature at 4ºC and the final product was stored at 4ºC. A 10 µl DNA was electrophoresed on 2% agarose gel containing 1.3 µl Gold View™ Nucleic Acid stain. PCR product was directly visualized using fluorescence and molecular weight was determined using 100bp DNA ladder. All DNA samples will be kept until they are used up and will be destroyed after completion of the study.

Data Analysis

Allele frequencies were calculated using the gene counting method according to the formula given below;

$$\text{Frequency of an allele (p)} = \frac{2 \times \text{number of Homozygotes (+, +)} + \text{number of heterozygotes}}{2 \times \text{total number of individuals (N)}}$$

$$\text{Frequency of an allele (q)} = \frac{2 \times \text{number of Homozygotes (-, -)} + \text{number of heterozygotes}}{2 \times \text{total number of individuals (N)}}$$

Using these allele frequencies, heterozygosities at individual loci were estimated according to the formula H=2pq, where p and q are the frequency of an allele (+) and allele (–) respectively. Finally, F-stastistic was calculated to analyzed variation in the gene frequencies.

RESULTS & DISCUSSION

Amplification of *Alu* ACE and TPA25

The lengths of PCR products, including the priming sites and flanking DNA, ranged from approximately 400 bp to 500 bp. The fragments were about 300 bp longer than the PCR products without an *Alu* insertion. The occurrence of an *Alu* insert was complete by cycle sequencing for all the PCR products. There are three expected categories of *Alu* insertion polymorphism that can be observed from a particular population. They are well known as individual homozygous insertion for the presence of *Alu* insertion polymorphism (+, +), heterozygous individual (+, -) and individual homozygous deletion for the absence of *Alu* insertion (-, -).

Figure 3 illustrated the photograph of 2% (w/v) agarose gel containing the PCR product of Minang Malays for ACE *Alu* insertion polymorphism. Lane one and 20 possessed the 100bp DNA marker was used to estimated size of the PCR product by comparing the migration of the band distance with the size of DNA marker. The absence of a negative control band at lane two indicates that PCR is free from contamination meanwhile the presence of positive control band at lane 19

TABLE 1 : Primer sets for PCR amplification of *Alu* loci

ALU LOCUS	SEQUENCE (5'-3')	EXPECTED PRODUCT SIZE (bp)	ADAPTED FROM
ACE For	CTGGAGACCACTCCCATCCTTTCT	490-190	[2]
ACE Rev	GATGTGGCCATCACATTCGTCAGAT		
TPA25 For	GTAAGAGTTCCGTAACAGGACAGCT	457-134	
TPA25 Rev	CCCCACCCTAGGAGAACTTCTCTTT		

indicated that PCR had succeeded. Lane three and 17 showed a homozygous insertion individual indicated by presence of single band at 490bp. Lane seven and nine showed a heterozygous individual indicated by the presence of two bands at 490bp and 190bp. Lane four, five, six, eight, 10 until 16 and 18 showed a homozygous deletion individual indicated by presence of single band at 190bp.

20 possessed the 100bp DNA marker was used to estimated size of the PCR product by comparing the migration of the band distance with the size of DNA marker. The presence of positive control band at lane two indicated that PCR had succeeded meanwhile the absence of a negative control band at lane 19 indicates that PCR is free from contamination. Lane four showed a homozygous insertion individual indicated by pres-

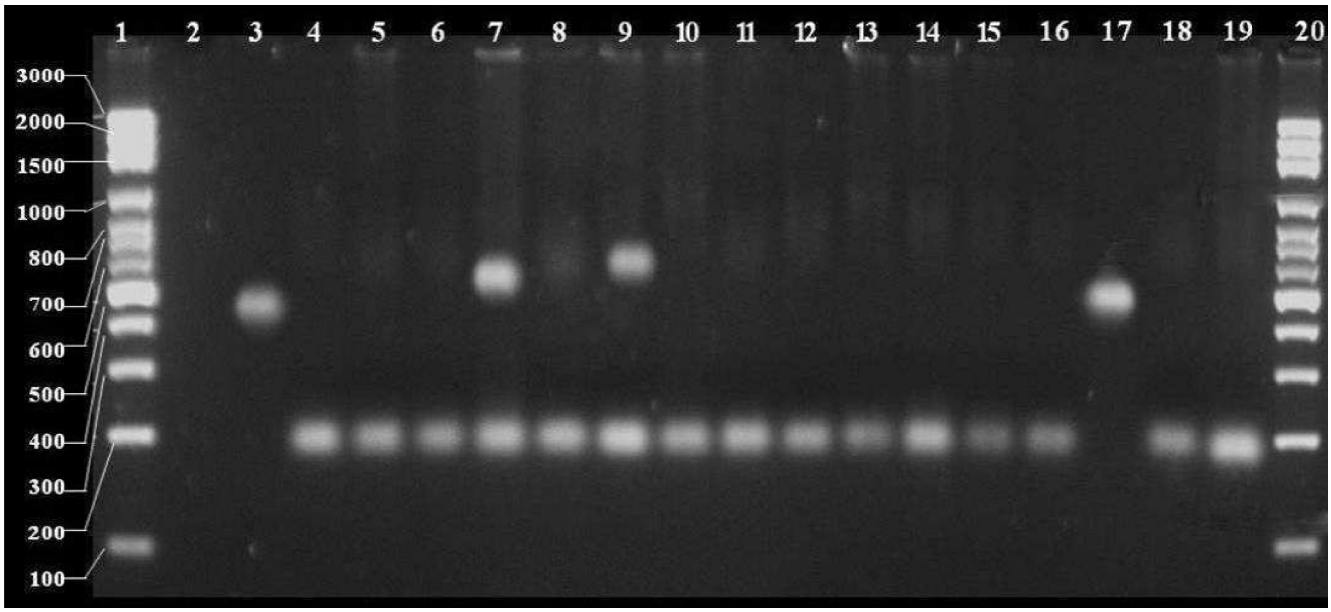

Figure 3 : Of a 2% agarose gel containing the PCR product of *Alu* ACE

Figure 4 illustrated the photograph of 2% (w/v) agarose gel containing the PCR product of Minang Malays for TPA25 *Alu* insertion polymorphism. Lane one and

ence of single band at 424bp. Lane 10 until 12 and 14 showed a heterozygous individual indicated by the presence of two bands at 424bp and 134bp. The rest

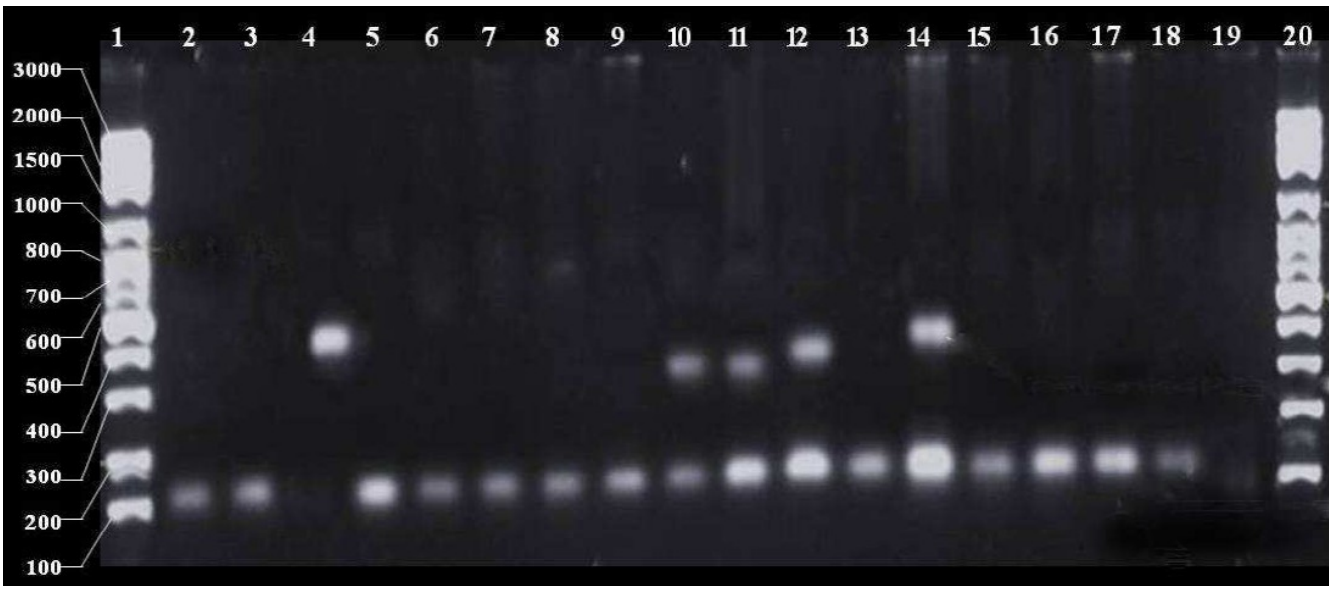

Figure 4 : Photograph of 2% gel containing the PCR product of *Alu* TPA25

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showed a homozygous deletion individual indicated by presence of single band at 134bp.

Lane from extremely left: 1: 100bp DNA marker, 2: PCR negative control, 3 and 17: Homozygous individuals with *Alu* insertion, 4-6: Homozygous individuals without *Alu* insertion, 7 and 9: Heterozygous individuals, 8, 10-16 and 18: Homozygous individuals without *Alu* insertion, 19: PCR positive control, 20: 100bp DNA marker

Lane from extremely left: 1: 100bp DNA marker, 2: PCR positive control, 3: Homozygous individuals without *Alu* insertion, 4: Homozygous individuals with *Alu* insertion, 5-9: Homozygous individuals without *Alu* insertion, 10, 11, 12 and 14: Heterozygous individuals, 13, 15-18: Homozygous individuals without *Alu* insertion, 19: PCR negative control, 20: 100bp DNA marker

The genotype data of ACE and TPA25 *Alu* insertion for all 50 samples are shown in TABLE 2. Inclusion factor of three generation pure used in this study provide a genetic code control thus gave the pattern to the allele frequency of *Alu* insertion in the populations study.

TABLE 2 : Allele frequencies and heterozygosities of ACE and TPA25 in Minang Malay sub-ethnic group

Alu locus	n	Frequency of the insertion	Genotype	Observed number	Expected number	Observed heterozygosity	Expected heterozygosity
ACE	50	0.320	(+,+)	8	5.120	0.320	0.435
			(+,-)	16	21.760		
			(-,-)	26	23.120		
TPA25	50	0.330	(+,+)	9	5.445	0.300	0.442
			(+,-)	15	21.44		
			(-,-)	26	22.445		

n= number of individual

Two *Alu* loci were highly variable and polymorphic in Minangkabau Malays, As for ACE and TPA25, Minangkabau Malays show a trend toward low and the number is slightly almost same for *Alu* insertion frequency. The heterozygosity value of ACE and TPA25 *Alu* loci in Minangkabau Malays was found to be 0.3200 and 0.300.

TABLE 3 represent the F-statistic across loci for Minang sub-ethnic group. It is presented with the heterozygosity indices. There are three elements belong to F-stastistic which are (1) inbreeding coefficient, (2) overall inbreeding coefficient, F_{IT} and (3) F_{IS} fixation index, F_{ST}.

F_{ST} is among the most widely used measures for genetic differentiation and plays a central role in ecological and evolutionary genetic studies[9]. From the table F_{ST} value for ACE and TPA25 are 0.264 and 0.300 repectively. As these values are above than 0.25, it's indicating a great genetic differentation of these two *Alu* locus within Minang sub-ethnic group[10]. In addition F_{IS} in a population study used to estimate the reduction in heterozygosity of an individual due to nonramdom mating within its population[11]. Positive value for loci ACE and TPA5 represent that there was a plenty of homozygotes at each of two polymorphic loci. Besides it also suggest that Minang sub-ethnic group in this study may inbreed. This is supported with high number of individuals of Homozygous for presence (+, +) and absence (-, -) for both loci that have been observed in this sub-ethnic group.

TABLE 3 : The F-stastistic across ACE and TPA *Alu* loci

Index	Value
H_I	0.3100
H_S	0.4387
H_T	0.4388
$F_{IS} = 1-(H_I/ H_S)$	0.2934
$F_{IT} = 1- (H_S/ H_T)$	0.0002
$F_{ST}=1-(H_I/ H_T)$	0.2935

CONCLUSIONS

Our study provide essential information to fill in the gap of knowledge regarding the ACE and TPA25 *Alu* insertion polymorphism variation in Minang Malay subethnic group. Further study will be conducted using more *Alu* loci to extent the understanding of genetic differentiation within Minang Malay subethnic group.

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