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The Frequency of Y *Alu* Polymorphism (YAP) Indel in the Minangkabau Malays in Peninsular Malaysia

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Abstract: Background: Minangkabau Malays (Melayu Minangkabau) is one of the Malay sub ethnic groups in Peninsular Malaysia. During the late 17th and early 18th centuries, migration of the Minangs from West Sumatra to the state of Negeri Sembilan Darul Khusus in Peninsular Malaysia took place and their descendants now form the main sub ethnic group in this state. The genetic polymorphisms of Y chromosome at DYS 287 locus were analyzed in Minangkabau Malays. Methods: A total of 41 buccal cells from healthy unrelated individual's males from Minangkabau Malays were typed for the DYS 287. The PCR products were separated on 2% (w/v) agarose gel followed by visualization under UV light. Results: Three out of 41 samples (7.32%) showed insertion (YAP+) polymorphism, while the rest of the samples (92.68%) showed deletion (YAP-) polymorphism. This is the first report concerning the YAP in Malay population at Peninsular Malysia. Conclusion: The v*Alu*able data obtained in this study will contribute to fill in the gap in the knowledge of YAP distribution in Malaysian population and will allow continuous interpretation of the evolution of YAP.

Key words: Y *Alu* insertion polymorphism (YAP), DYS 287, FTA, PCR, Minangkabau Malays.

1. Introduction

The human Y chromosome is a useful marker for studies of human population genetic and has been recognized [1-3]. The non-recombining portion of human Y chromosomes has special features where it is a single haploid and involve father to son transmission only. As a consequence, the DNA sequence on the Y chromosome preserves a unique record of mutational events that occurred in previous generations. Therefore, polymorphisms in this region have thus been proposed as tools for investigated male-specific gene flow and for reconstructing paternal history [4].

One of the most useful and widely studied is Y-linked polymorphisms or another name is Y *Alu* Polymorphism (YAP) element. YAP element (DYS 287 locus) is referred to the *Alu* insertion (~ 300 bp) that is present at a specific site on the long arm of the Y chromosome, Yq11 [5]. This element is stable and originated almost 65 years ago as a component in human DNA [6]. This type of marker has been shown to be v*Alu*able for human population studies.

The people of Minangkabau or Minang make up majority of the population Negeri Sembilan Darul Khusus in Peninsular Malaysia and descendents came over from West Sumatera Province with the capital city of Padang [7]. The Minangkabau are known for the world's largest matrilineal social system, in which properties such as houses and land are inherited through female lineage. The Minangkabau have a history migrating to oversea such as Peninsular Malay during the late 17th century and 18th century ago. They migrated from West Sumatera to the state of Negeri Sembilan Darul Khusus, especially in Naning, Sg. Ujong and Rembau after the fall of the Malacca

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Sultanate [8]. Nowadays Minangkabau features are still visible in traditional architecture and the dialect of Malay spoken in Negeri Sembilan.

This study was conducted on the insertion and deletion of the DYS 287 element in Minangkabau Malays in Peninsular Malaysia. There is still no research carried out in Peninsular Malaysia involving Malay sub-ethnic group especially in Minangkabau Malays as inferred from DYS 287 YAP. Recent study only focused on Bidayuh ethnics of Sarawak population which was conducted by [9] and also research done by [10] where the study focused on Kadazan-Dusun population from East Malaysia. Through this study, it shows that Bidayuh and Kadazan-Dusun show a small percentage of DYS 287 YAP insertion.

2. Materials and Methods

2.1 Subpopulation Samples

Ethical approval and written permission from each volunteer was obtained from the Research Ethics Committee, UiTM Shah Alam. Buccal cells were collected from 41 healthy volunteers, from non-related individuals and randomly selected for this study. The Minagkabau Malays was recruited from Kampung Gagu, Jelebu and Kampung Daching, Beranang from state of Negeri Sembilan Darul Khusus (Fig. 1).

The volunteers were interviewed to ensure their family history and their family must at least from three generations were selected (Fig. 2). Those with unknown family history, mixed marriages and consanguineous and marriage were excluded from this

Fig. 1 Map of the geographical distribution of Minangkabau Malays individual.

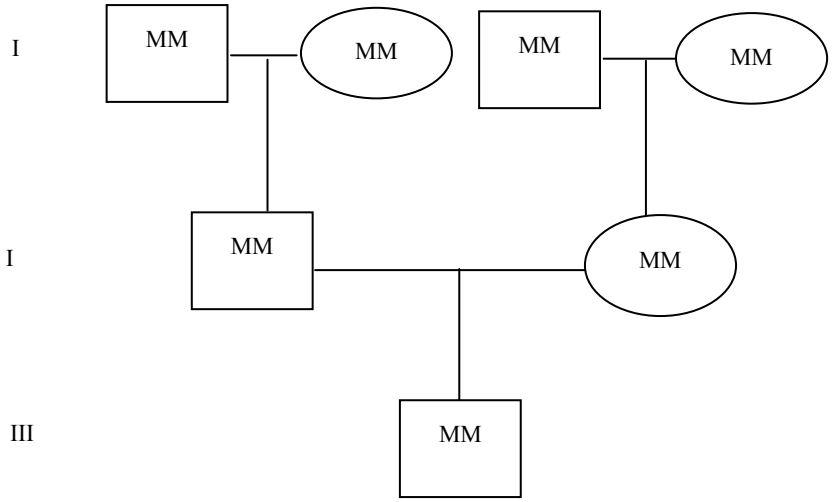

Fig. 2 A pedigree charts for ethnicity of an example of male representative through inclusion factor of three generation pure of Minangkabau Malays (MM) sub-ethnic groups. Squares designate males and circles represent females. Parents are connected by a horizontal line, and vertical lines lead to their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

study. Before donating a sample, each volunteer read and signed the consent form if they are part of this study. In favor of precise data acquisition, each volunteer must filled out the questionnaire, which asks detail regarding information on place of birth, ancestry of parents and grandparents and also current addresses.

The nationalities of each volunteer were defined by the Malaysian Identity Card (MyCard) as registered with National Registration Department. Volunteers were photographed with digital camera in front of the volunteer. All of the photographs were stored as JPEG files.

2.2 Samples Collection

Sample collection began with vigorously swabbing Sterile Foam Tipped Applicator (WHATMAN, Germany) inside a volunteer's mouth and rub side by side on the inside cheek for about one minute. The foam tip was transferred to FTA card (WHATMAN, Germany) and the color of FTA card changed from pink to white which indicated the presence of samples. FTA cards with whole buccal cell deposits were dried and stored at room temperature. The FTA cards were ready for further used.

2.3 Washing FTA Cards

A 2 mm (1/8 inch) disc the desire sample spot was punched out by using the 2.00 mm Harris Micro-Punch® Tool (WHATMAN, Germany). The sample disc was place into the PCR tube. Add 200 µL of FTA Purification Reagent (WHATMAN, UK) to each PCR tube and incubate for 5 min at room temperature. Next, all spent FTA purification was removed and discarded using a pipette. After that, the PCR tube was added with 200 µL of TE (10 mM Tris, 0.1 mM EDTA) buffer and incubated again for 5 min at room temperature. All TE buffer spent was removed and discarded using a pipette. Finally, the FTA disc was allowed to dry at room temperature for about one hour and the FTA card disc was ready for PCR amplification.

2.4 PCR Amplification

A complete list of the specific oligonucleotide primers are shown in Table 1. PCR was performed in the total volume of 25-µL reaction which contain 2.0 mm disk Whatman® FTA card, 2.0 µL $MgCl_2$ (Solis Biodyne, Estonia), 0.08 mM dNTPs (Solis Biodyne, Estonia), 10 × Reaction Buffer BD (Solis Biodyne, Estonia), 10 mM oligonucleotide primer (AITBIOTECH PTE LTD, Singapore) one unit of *Taq* DNA Polymerase (Solis Biodyne, Estonia) and double distilled water. The PCR cycling conditions were carried out on Thermal cycler machine (Cleaver).

Each sample was subjected to an initial denaturation of 1 min at 94 ºC followed by 35 amplification cycles of denaturation at 94 ºC for 15 s, annealing temperature for 30 s, and followed by extension at 72 ºC for 1 min. After the final extension at 72 ºC for 5 min, the samples were kept at 4 ºC until the end of electrophoresis set.

An amount of 8 µL DNA was electrophoresed on 2% agarose gel containing 1.3 µL Gold View™ Nucleic Acid Stain (Lonza, USA) at 100 V for about 70 min. PCR product was directly visualized using Gel Documentation System according to the manufacturer's instruction and molecular weight was determined using 100 bp DNA ladder (Solis BioDyne, Estonia).

3. Results and Analysis

Fig. 3 illustrated the photograph of 2% agarose gel

Table 1 Primer sets for PCR amplification of DYS 287 locus.

Name	Sequence (5'-3')	Expected Product Size (bp)	Adapted From
DYS 287 For	CA,GG,GG,AA,GA,TA,AA,GA,AA,TA	YAP+ 455	[5]
DYS 287 Rev	AC,TG,CT,AA,AA,GG.GG.AT,GG,AT	YAP- 150	

containing the PCR product YAP- of Minangkabau Malays while Fig. 4 illustrated the photograph of 2% agarose gel containing the PCR product YAP+ of Minangkabau Malays. The result was successfully amplified as a 150 bp for YAP deletion and 450 bp for YAP insertion.

The presence of positive control band was used to determine whether the PCR had succeeded meanwhile the absence of a negative control band indicated that the PCR is free from contamination. DYS 287 controls used for PCR amplification are female's sample and it showed no amplification of DYS 287 Y

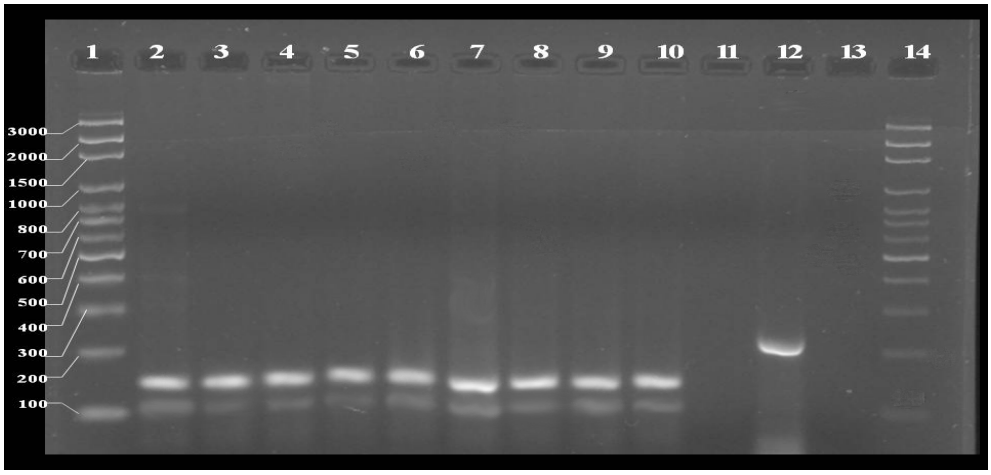

Fig. 3 Photograph of a 2% agarose gel containing the PCR products of Minangkabau Malays YAP-, negative control, positive control and female sample.

Lane from extremely left:
Lane 1: 100 bp DNA ladder
Lane 2: Minangkabau Malays 4
Lane 3: Minangkabau Malays 7
Lane 4: Minangkabau Malays 19
Lane 5: Minangkabau Malays 24
Lane 6: Minangkabau Malays 25
Lane 7: Minangkabau Malays 27
Lane 8: Minangkabau Malays 31
Lane 9: Minangkabau Malays 30
Lane 10: Minangkabau Malays 33
Lane 11: PCR negative control
Lane 12: PCR positive control
Lane 13: DYS 287 control
Lane 14: 100 bp DNA ladder

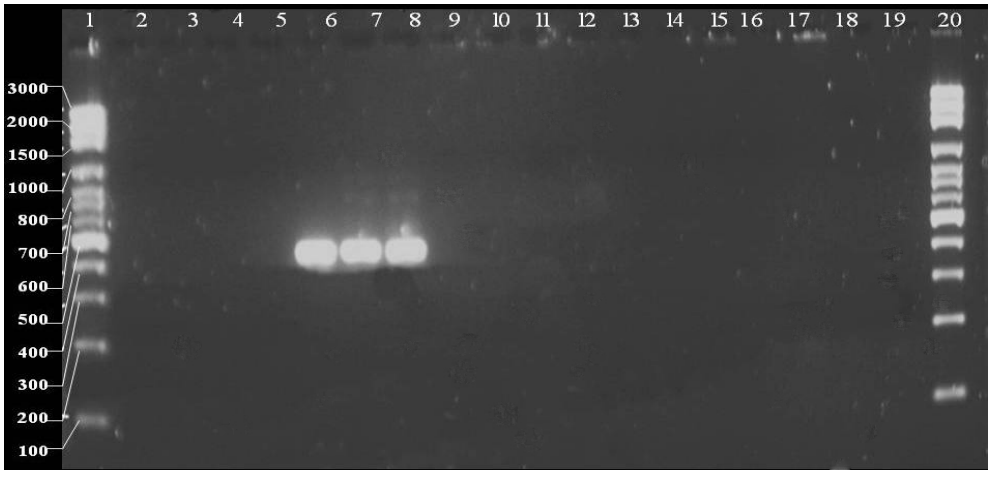

Fig. 4 Photograph of a 2% agarose gel containing the PCR products YAP+ of Minangkabau Malays.

Lane from extremely left:
Lane 1: 100 bp DNA ladder
Lane 2-5: No PCR product amplified
Lane 6: Minangkabau Malays 14
Lane 7: Minangkabau Malays 15
Lane 8: Minangkabau Malays 16
Lane 9-19: No PCR product amplified
Lane 20: 100 bp DNA ladder

Table 2 Allele frequency distribution DYS 287 minagkabau Malays.

Sub Ethnic Group	State	District	Total Samples	Yap+	Yap+ frequencies (%)
Minangkabau	Negeri Sembilan	Jelebu	27	0	0.0
		Seremban	14	3	7.3

Alu element. It stated that the Y chromosome does not exist in female genome because it is a single haploid entity that passed from father to son only [11]. The allele frequencies distribution of the DYS 287 is reported in Table 2.

This study has shown that the Minangkabau Malay population lacks of YAP element and only 7.3% of subjects are having YAP+. Out of 41 subjects, 92.7% are having YAP-. This means their father did not inherit DYS 287 Y *Alu* polymorphisms from their common ancestor because *Alu* insertion polymorphism is identical by descent [12].

4. Discussion

A broad study of YAP insertion and deletion polymorphism in African, Europe, Asian and Oceania populations was carried out by [5]. The frequency YAP insertion polymorphism (YAP+) was significantly appeared at high frequency in African, Japan and Tibetans populations followed by the Western Eurasians populations and at low frequency in some Asian, and Oceania populations [5, 13, 14].

Recent study on YAP element among Ahmadiyya Muslim from Qadian, district of Punjab, Pakistan appeared no presence of YAP+ [15] and also research done by [16] where the study focused on polymorphism of Y chromosome at YAP locus among 25 ethnics groups in Yunnan China showed Primi, Tibetan, Naxi and Naxi (Mosuo) has the highest YAP+ frequency.

In Southeast Asians, studies conducted by [13], could not find the frequency of YAP+ in any populations (108 Filipinos, 42 Indonesians, and 74 Vietnamese) except in one Thai male sample. Previous study done by [17] also did not find among any of the Southeast Asians populations (3 Cambodian, 7 Laotian, 12 Philippine, 1 Thai and 3 Vietnamese).

Until now, there is yet to have any research carried out in Southeast Asians population involving ethnic group except Malaysia. In the case of DYS 287 among Malaysian population, research has been done by [9] in Kadazan-Dusun ethnic of Sabah population and recently, a local study conducted by [10] in Bidayuh ethnic of Sarawak population. Results obtained show a small percentage presence of YAP+ only 14.4% in Bidayuh and 2% in Kadazan-Dusun.

The results obtained in this study on Minangkabau Malay subjects is supported by previous studies [5] and [15] which conclude that most among Asian populations lack the YAP+ with the exceptions of the Japanese and Tibetan populations. At this moment, it is not clearly defined why the YAP element has this characteristics distribution high around Africa, Central Asia and East Asia.

5. Conclusions

Further studies examining more Malay sub-ethnic groups will provide a better understanding of DYS 287 Y *Alu* polymorphisms pattern in Peninsular Malaysia.

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