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Y-chromosomal STR Variation in Malays of Kelantan and Minang

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ABSTRACT

Malays in Malaysia are a mixture of different races, caused by the history of migration centuries ago and may consist of 14 sub-ethnic groups. We used the Y-chromosomal STR (Y-STRs) to genotype two of the sub-ethnic groups namely, Kelantan and Minang Malays. In this ongoing study we investigated the polymorphisms of six Y-STR loci namely, DYS19, DYS388, DYS390, DYS391, DYS392 and DYS393 in the two populations mentioned. Twenty males (10 Kelantanese and 10 Minang) were analyzed by PCR amplifications followed by 8% non-denatured polyacrylamide gel electrophoresis. Randomly selected samples were sequenced for validation. Results revealed a total of 32 alleles, ranging from three (DYS19) to nine (DYS390). Allele frequency distributions ranged from 0.05 (DYS388, DYS 391, and DYS393) to 0.65 (DYS388). Although the level of polymorphisms of the two sub-groups were similar (average number of alleles, 4; average heterozygosity, 0.6), allele frequency distribution appeared to be imbalanced. Significant differences of allele frequency distributions were observed in loci DYS390, DYS391, and DYS393. None of the individuals shared the same haplotypes. However, errors of scoring and factors like small sample size should be considered. Preliminary results revealed polymorphisms in the six loci among the two Malay sub-ethnic groups. Significant differences of the allele frequency distributions were observed, but a further investigation with a larger sample size is warranted to confirm these findings.

Keywords: Y–STRs, polymorphisms, sub-ethnic groups, PCR amplifications

INTRODUCTION

The Malays in Malaysia are a mixture of different races, caused by history of migration centuries ago. Speculations were made on their pre-historic migration patterns to the Southeast Asia (SEA) region. Dental morphological traits suggested that two migrations into SEA originated from China about 30,000 years before present (Turner, 1987); while the linguistic groups proposed two major wave of migrations (Bellwood, 1985). Today, the Malays are heterogenous. We postulated that they may consist of 14 sub-ethnic groups namely, Melayu Kelantan, Minang, Bataq, Jambi, Kurinchi, Jawa, Riau, Melayu Yunnan, Mendeleng, Banjar, Bugis, Acheh, Champa, and Rawa.

The Y-chromosome, much like mtDNA, is inherited in a sex-specific manner. Its most attractive feature has been an apparent inability to undergo genetic recombination, making the task of assessing the extent of genetic variation in living human relatively straight forward as the accumulation of mutations is fairly by evolutionary forces over the period.

In this ongoing study, we investigated the polymorphisms of two of the unique Malay sub-ethnic groups namely, the Malays of Kelantan (Melayu Kelantan) and the Minangs (Melayu Minang) using six Y-STR markers.

MATERIALS AND METHODS

Sample Collection

Ethical approval was obtained from the Research and Ethics Committee of USM Health Campus. After obtaining the informed consent, 20 healthy and unrelated male subjects were recruited (10 for each sub-ethnic group). The samples of the Malays of Kelantan were collected from various districts of Kelantan including Machang, Kuala Krai, Rantau Panjang, Bachok and Kota Bharu);

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while the samples of Minangs were collected from Seri Menanti and Lenggeng, Negeri Sembilan. Subjects were interviewed to confirm their family history. They must be born at the specified location and their family at least three generations. Those with unknown family history, mixed marriage and consanguineous marriage and consanguity marriage were excluded from this study. Blood samples were collected from these subjects and DNA was extracted using commercial kit (QIAamp ® DNA Blood Mini Kit, Germany).

PCR Amplification

A total of six Y-STR markers were chosen based on the study done by Thomas *et al.* (1999). Details of the selected markers are outlined in Table 1. PCR was performed in a total volume of 10 ml consisting 20 ng DNA, 1 unit of *Taq* polymerase (Promega, Madison, MI, USA), 1x PCR reaction buffer (Promega, USA), 0.5 mM dNTPs (Promega, USA), appropriate concentration of Mg^{2+}, and forward and reverse primers (Table 1). The PCR protocol comprised of 5 min 95°C predenaturation; 38 cycles of 94°C denaturation (1 min); appropriate annealing temperature, and 72°C extension (30 sec; except DYS390, 90 sec). Amplification products were separated by vertical gel electrophoresis through 8% non-denaturing polyacrylamide gel along with 20 bp DNA ladder. Some alleles, which were subsequently used as references, were later confirmed by DNA sequencing.

Statistical Analysis

Allele frequency of the six loci for each ethnic group were estimated and compared. Genetic diversity (h) in each ethnic group was estimated as $1 - \Sigma p_i^2$, where p_i represents the frequency of the i^{th} allele at the locus.

RESULTS

The allele frequency distributions of the six Y-STR loci in the two Malay sub-ethnic groups are summarized in *Fig. 1.* Results revealed a total of 32 alleles, ranging from three (DYS19) to nine (DYS390) of the 20 individuals analyzed. Both groups seemed to have similar diversity both in terms of the number of alleles and their allele frequencies. The allelic variations at one of these loci are shown in *Fig. 2.*

Table 2 represents the diversity statistics for six Y-STR loci in the two Malay sub-ethnic groups studied. Table 3 indicates the haplotype data for both Minang and Kelantan Malays. Interestingly, none of the individuals shared the same six-locus haplotypes. However, sample sizes are too small to permit a complete analysis of the Y-chromosomal structure of these two sub-populations.

TABLE 1
Summary of loci selected, indicating the primer sequences, repeat motif, expected allele sizes, primers and $MgCl_2$ concentrations and annealing temperatures of PCR

Locus	Primer sequence (5' – 3')	Repeat motif	Exp allele sizes (bp)	[primers]+	$MgCl_2$ (mM)	T_a* (°C)
DYS19	F: CTACTGAGTTTCTCTGTTATAGT R: ATGGCATGTAGTGAGGACA	$(TAGA)_n$	186	50 ρmol	2.25	58
DYS388	F: GTGAGTTAGCCGTTTAGCGA R: CAGATCGCAACCACTGCG	$(ATT)_n$	127	50 ρmol	2.25	57
DYS390	F: TATATTTTACACATTTTTGGGCC R: TGACAGTAAAATGAACACATT	$(TCTA/TCTG)_n$	215	40 ρmol	1.5	54
DYS391	F: CTATTCATTCAATCATACACCCATAT R: ACATAGCCAAATATCTCCTGGG	$(TCTA)_n$	171	40 ρmol	1.5	57
DYS392	F: AAAAGCCAAGAAGGAAAACAAA R: CAGTCAAAGTGGAAAGTAGTCTGG	$(TAT)_n$	176	40 ρmol	1.5	57
DYS393	F: GTGGTCTTCTACTTGTGTCAATAC R: AACTCAAGTCCAAAAATGAGG	$(TAGA)_n$	128	50 ρmol	2.25	57

+[primers], concentration of primers; *T_a, annealing temperature

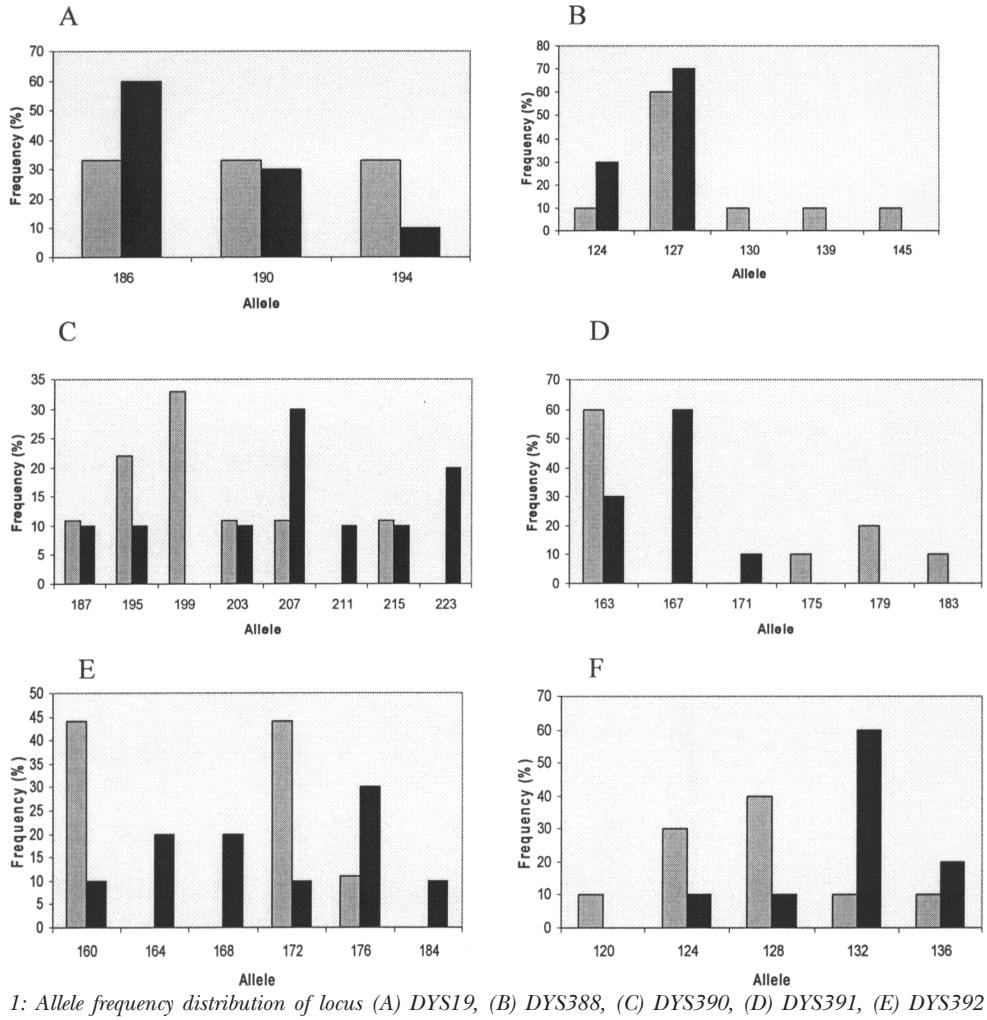

Fig. 1: Allele frequency distribution of locus (A) DYS19, (B) DYS388, (C) DYS390, (D) DYS391, (E) DYS392 and (F) DYS393, among Malays of Kelantan (bright colour) and Minang (dark colour)

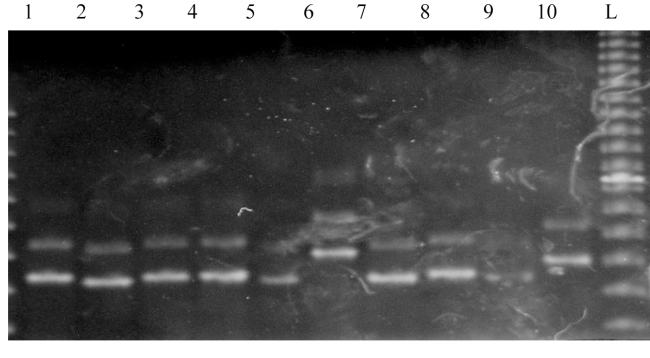

Fig. 2: Resolution of DYS393 on native polyacrylamide gel electrophoresis Lanes 1 to 5, Subjects of Malays of Kelantan; Lanes 6 to 10, subjects of Malays of Minang; Lane L, 25 bp ladder

TABLE 2
Diversity statistics for six Y-chromosome microsatellite loci in two Malay sub-ethnic groups

Population	Average heterozygosity	Total number of allele	Average number of alleles
Melayu Kelantan	0.658	26	4.333
Melayu Minang	0.617	25	4.167

TABLE 3
Y-STR haplotypes in the males subjects of the Malay sub-ethnic group

Sample		Locus*					
		DYS19	DYS388	DYS390	DYS391	DYS392	DYS393
Melayu Kelantan	1	194	127	199	179	160	128
	2	194	124	207	175	160	132
	3		127		175		136
	4	186	127	215	183	156	124
	5	186	127	199	171	160	124
	6	190	145	199	163	172	128
	7	194	127	203	163	172	124
	8	190	130	187	163	172	120
	9	190	127	195	163	184	128
	10	186	139	195	163	176	128
Average locus diversity						0.5926	
Melayu Minang	11	186	127	207	165	164	136
	12	194	127	207	163	184	132
	13	190	127	187	163	164	132
	14	186	127	195	171	172	132
	15	190	127	203	167	176	132
	16	186	127	223	167	176	132
	17	190	124	215	163	168	132
	18	186	124	207	167	160	132
	19	186	127	211	167	176	124
	20	186	124	223	167	168	136
Average locus diversity						0.8000	

* Locus haplotypes were indicated as the size of amplicon, bp

DNA sequencing performed on the selected samples confirmed the number of repeat motifs of the loci (*Fig. 3*).

DISCUSSION

The Minangs originated from West Sumatra, Indonesia. They are believed to be one of the largest martilineal groups in this modern time. Traditionally, the wife remained with her maternal relatives after marriage and inheritance are passed through the women. Islam was brought into the Minangs as a result of increased external trade with India, Acheh, and Melaka; and now they are among the most committed people to practice traditional Islam in the archepelago (Gall, 1998). Their ethnic traditions, was believed to be derived from animistic and Hindu-Buddhist beliefs. During the late 17th and early 18th centuries, migration of the Minangs from West Sumatra to the state of Negeri Sembilan, Peninsular Malaysia took place and their descendants now form the main sub-ethnic group in this state.

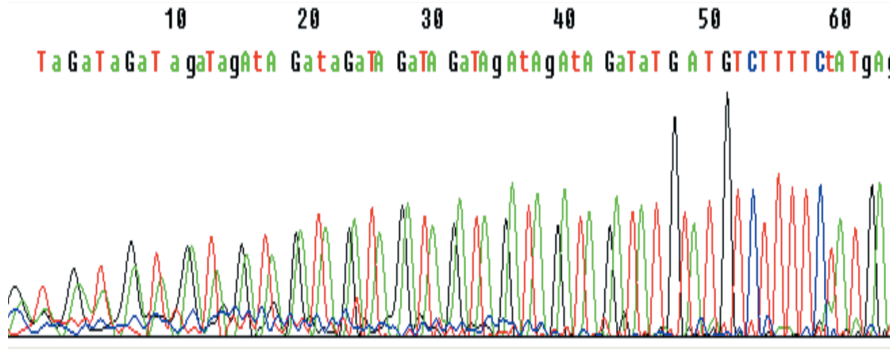

Fig. 3: DNA sequencing performed on a selected sample confirmed presence of the repeat motif $(TAGA)_{11}$ of the loci

Meanwhile, long before the emergence of the Melaka Sultanate, Kelantan was a crucial centre of overland trans-peninsular trade route. Kelantanese also embraced Islam earlier than Melaka and was reputed as the centre of Islamic learning and scholarship. History resources showed that the ancient Hindu-Malay Empire of Langkasuka was centred in Pattani, which encompassed of Modern Malaysia states of Kelantan, Terengganu and Northern Kedah, as well as the provinces of Pattani, Yala, Narathiwat, Songkhla and Satun in Thailand. The Pattani Malays are very much similar in ethnicity, culture and language to the Malays of Kelantan.

Although the level of polymorphisms of the two sub-groups were similar (average number of alleles, 4; average heterozygosity, 0.6), allele frequency distribution appeared to be imbalanced. The differences of the history of settlement and their socio-cultural practices could probably explain this phenomenon.

Significant difference of locus diversity was observed in DYS392, where Kelantan appeared to have lower locus diversity (h = 0.5926) than Minang (h = 0.800). This could be an interesting locus to investigate its ability to differentiate the two sub-ethnic groups. Previous studies found that the locus diversity of this locus ranged from 0.7 – 0.75 (Yong *et al.*, 2006; Chang *et al.*, 2007). Meanwhile, Srikummool *et al.* (2000) reported a relatively lower frequency of allele 132 bp in DYS393 among the Thai population studied. Interestingly, this was found to be similar with the the Malays of Kelantan in the current study, with a contrasting result from the Minangs, which revealed a significantly higher frequency for allele 132 bp, proposing a closer affinity of the Malays of Kelantan to the Thai population. In addition, allele 167 bp of locus DYS391 was also interesting since it was a common allele in Minang but was not found in Malays of Kelantan. Few of these differences may be significant even with the small number of samples and loci currently examined but the consistency of the results, however, is reassuring.

The 8% PAGE offered a rapid, sensitive, and reproducible method for genotyping these six Y- STRs locus. Due to overall large fragment sizes, the point mutations were ignored, as they do not change the migration behaviour in the native PAGE seperation. However, technical errors like miscoring and null alleles should be considered.

In summary, our preliminary results revealed polymorphisms in the six loci among the two Malay sub-ethnic groups. Significant differences of the allele frequency distributions were observed, but a further investigation with larger sample size is warranted to confirm these findings.

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