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Forensic Parameter and Paternity Testing of Tpa-25 Element in Kelantan-Malay and Jawa-Malay

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ABSTRACT

Malays consist of multi sub-ethnic group believed to have different ancestral origins based on their migrations centuries ago. The DNA profiling for every individual in Malaysia is not recorded, making Malaysia lacking in genetic data of its own citizens. This research aimed to study the geographic-ancestry origin of two Malay sub-ethnic population; Kelantan-Malay and Jawa-Malay by looking into the variation of TPA-25 insertion in each population. It specifically studied on several areas of Peninsular Malaysia in the region of Kelantan, Selangor and Johor as the representative of main areas with high percentage of Kelantan-Malay and Jawa-Malay populations. All the data were obtained from an application of TPA-PCR method, forensic parameter (F-statistic) and survey questionnaire that polled genetically on their ancestry origin in each sub-ethnic population. The research showed that population with high percentage of heterozygous allele (Tt) of TPA-25 insertion was likely to have high possibility of genetic drift occurrence. Jawa-Malay showed the highest percentage of heterozygous allele (Tt) with approximately 48% of the population. The FIS value of Kelantan-Malay and Jawa-Malay populations were recorded positive with the values of 0.678 and 0.366 respectively.

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Moreover, the FIT value recorded was 0.535 which suggested that these two populations were deficits of heterozygotes.

Keywords: F-statistic, genetic drift, heterozygosity, TPA-25 element

INTRODUCTION

Malays (Melayu) belongs to an ethnic group who speak Malayo - Polynesian language which is a member of the Austronesian family (Bellwood, 2007; Omar, 2004). They dominantly inhabit the Malay Peninsula, the east coast of Sumatra and the coast of Borneo (Bellwood, 2007). In Peninsular Malaysia, the Malays consist of multi sub-ethnic group believed to have different ancestral origins based on their migrations centuries ago (Wheatley, 1961). The Malay Peninsula was once a very strategic port and trading centre, connecting Indochina and the Indonesian archipelago (Jacq-Hergoualc'h, 2018). However, migrating populations from surrounding areas have further confounded the investigation of the origin of Malays. Recently, there has been deep interest in using Alu elements for application to forensic casework in studies of human population genetic structure and inference of individual geographic origin (Asari et al., 2012; Bamshad et al., 2003; Ray et al., 2005). Alu elements are transposable elements which reach over one million copies in human genome (Ade et al., 2013; Cordaux et al., 2007). These elements are approximately 300 base pairs sequence long which have expanded in human genome for more than 60 million years (Batzer & Deininger, 2002). Alu elements are characterized by their ability to "copy and paste" via a mechanism to produce new copies by using RNA transcript to be reverse trancripted as cDNA and the duplicate is inserted at a new genomic location (Batzer & Deininger, 2002).

These Alu elements are expanded in human genome at a substantial rate (Cordaux et al., 2006) resulting in multiple copies in human genome, making them as the most successful mobile elements (Batzer & Deininger, 2002; Lander et al., 2001) and represented as an important source of human genomic variation (Batzer & Deininger, 2002). TPA-25 sequence is an Alu element within tissue plasminogen activator gene, a dimorphic gene not represented in all individuals (Batzer et al., 1991). This marker has been used to study various population in central Argentina Patagonia, North Africa (Tunisian) and South India (Ennafaa et al., 2011; Lotfi et al., 2011; Moncer et al., 2011; Parolin et al., 2017; Veerraju et al., 2001). TPA-25 suitable for both ancestry and admixture analysis because of the present of importance source of information regarding on genetic diversity, both from the current diversity, and throughout the process of human evolution (Cherni et al., 2011; Rishishwar et al., 2015).

These criteria suit the sequence as a better gene marker for screening the genetic structure of human population. This study aimed to determine the variation of TPA-25

insertion in each population of two sub-ethnic groups, Kelantan-Malay and Jawa-Malay, the two groups that were categorized as the one of the biggest ethnic groups in Peninsular Malaysia by using TPA-PCR-based method, to calculate the allele and genotype frequencies of each human DNA sample by using Hardy-Weinberg equation and to determine the geographic-ancestry origin of each population by an application of forensic parameters and parental lineage investigation.

MATERIALS AND METHODS

Sample Collection

Total human genome DNA samples in the form of buccal cell were collected on the FTA Mini Cards (WHATMAN, Germany). Fifty-one samples were from unrelated Kelantan-Malay population and another thirty-three samples from unrelated Jawa-Malay population. Figure 1 shows the geographical map of populations used in this study. The samples were limited within Peninsular Malaysia and collected from different regions. Consideration of looking into their skin colour and appearance was excluded. The ethical approval on sample collection was obtained from the Research and Ethics Committee of Universiti Teknologi MARA (UiTM) [Ref no: 600-RMI (5/1/6/01)].

Figure 1. Geographic location of the Malay sub-ethnic groups in Peninsular Malaysia used in this study.

PCR Amplification

Oligonucleotide primers for TPA-25 element PCR were TPA-forward 5'GTAAGAGTTCGTAACAGGACAGCT3' and TPA-reverse 3' CCCCACCCTAGAGAACTTCTCTTT5' with size product of ~400 bp (TPA-25 insertion) and ~100 bp (TPA-25 deletion). PCR conditions were optimized for each assay regarding annealing temperature and oligonucleotide primers.

Amplification of each Alu Polymorphism (TPA-25 sequence) was performed in 22 µl reaction using 2 mm FTA card disc, 10x Optimized DyNAzymes buffer, 20 mM dNTPs (Solis BioDyne), 10 mM oligonucleotide primer (AITBIOTECH PTE LTD, Singapore), DyNazyme II DNA polymerase and double distilled water. Each reaction contained 49 µl of PCR master mix and 1 FTA disc of DNA template. The samples were then subjected to an initial denaturation of 13 minutes at 95 °C followed by 35 amplification cycles of denaturation at 94 °C for 15 seconds, annealing temperature for 30 seconds and followed by extension at 72 °C for 30 seconds. After the final extension at 72 °C for 10 minutes, the samples were kept at 4 °C.

Statistical Analysis

Allele frequency of each samples was calculated by using Hardy-Weinberg equation (Eq.1). The data was represented as the heterozygosity of each Malays sub-ethnic population; Kelantan-Malay and Jawa-Malay.

$$p + q = 1$$
$$(p+q)^2 = p^2 + 2pq + q^2 \qquad [1]$$

The Fixation index, FST value was measured through F-statistics (Roewer et al., 2000). Three levels fixation indices that reflect the biological of organization, individual, subpopulation and the total population (i) inbreeding coefficient (FIS), (ii) fixation index (FST) and (iii) overall inbreeding coefficient (FIT) (Saltkin, 1995).

The reduction in heterozygosity of an individual due to non-random mating within its subpopulation was estimated by following formula of FIS :

FIS = (HS – HI) / HS [2]

It shows the degree to which heterozygosity is reduced below the expectation. The value of FIS ranges between -1 and +1. Negative FIS values indicate heterozygote excess (outbreeding) and positive values indicate heterozygote deficiency (inbreeding) compared with Hardy-Weinberg Equilibrium expectations.

The reduction in heterozygosity of an individual relative to the total population was estimated by following formula of FIT :

FIT = (HT – HI) / HT [3]

The degree of the genetic differentiation between sub-populations was estimated by following formula of FST :

FST = (HT - HS) / HT [4]

FST value is always positive, ranges between 0 (no subdivision, random mating occurring, no genetic divergence within the population) and 1 (complete isolation with extreme subdivision).

RESULTS AND DISCUSSION

The allele frequencies of TPA-25 sequence in each sample for both Malays sub-ethnic populations were determined based on the existence of TPA-25 insertion (~400bp) and TPA-25 deletion (~100bp) which is shown in Figure 2 and Figure 3. Meanwhile, the size estimation of the insertion and deletion are shown in Table 1 and Table 2. After collecting the genotyping data, Table 3 and Table 4 were created to show the distribution of three different types of group which are (i)group of homozygous insertion (+ve), (ii) homozygous deletion (-ve), and (iii) group of heterozygous alleles (+ve/-ve).

Figure 4 shows that from the total of 33 individuals sampled from Jawa-Malay population, 27.27% of the population possessed TPA-25 insertion, 12.12% possessed TPA-25 deletion and the remaining of the population (48.48%) possessed heterozygous allele of TPA-25 element. Meanwhile, from 51 individuals of Kelantan-Malay population, about 17.64% of the population possessed TPA-25 insertion, 37.25% possessed TPA-25 deletion and the remaining of the population (29.41%) possessed heterozygous allele of TPA-25 element.

It shows the heterozygous group in Jawa-Malay population is higher than heterozygous group in Kelantan-Malay. This indicates the high possibility of genetic drift in the Jawa-Malay population as may be due to the result of continuous network of migrations, invasions and admixture of people from different origins (Cherni et al., 2011; Tishkoff & Kidd, 2004). These practices and the ancient movements were active in hundreds of years prior while many different ethnics from neighbouring nations came and abode in Tanah Melayu (Hugo, 1993; Shamsul, 2001).

Eventually, the activities then contributed to the existence of different Malays sub-ethnic population resulting from the intermarriage between the Proto-Malay and other ethnic groups. Different ethnic population also could become unified under singular cultural, religion practices, and language family by simply accepting, adoption and adaptation process (Keita, 2010). Meanwhile, the low heterogeneous gene in a population reflecting their reproductive isolation which might be due to religious and cultural differences seemingly to have provide an obstacle to their intermixing (Cherni et al., 2011; Ennafaa et al., 2006).

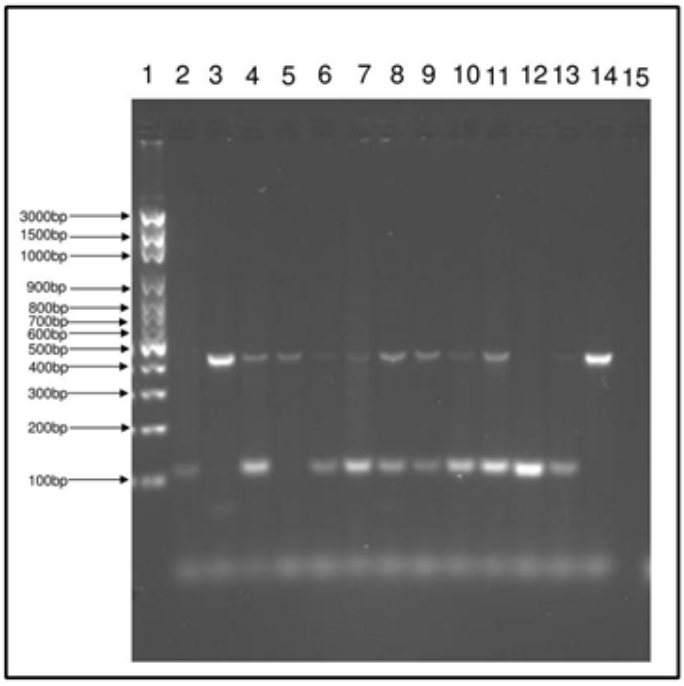

Figure 2. A total of 13 samples of PCR products for TPA-25 sequence of Jawa-Malay population on 2% (w/v) Agarose gel.

Table 1

Size estimation of TPA-25 element of 13 samples of Jawa-Malay population

Lane No.	Sample	TPA-25 Insertion	TPA-25 Deletion
1	100 bp DNA ladder		
2	PCR product of sample 43J	No amplification	114bp
3	PCR product of sample 44J	478bp	69bp
4	PCR product of sample 45J	436bp	114bp
5	PCR product of sample 49J	478bp	No amplification
6	PCR product of sample 50J	478bp	114bp
7	PCR product of sample 52J	478bp	114bp
8	PCR product of sample 53J	478bp	114bp
9	PCR product of sample 54J	478bp	114bp
10	PCR product of sample 55J	524bp	204bp
11	PCR product of sample 56J	478bp	186bp
12	PCR product of sample 57J	No amplification	169bp
13	PCR product of sample 58J	No amplification	186bp
14	PCR product of sample 59J	478bp	No amplification
15	Negative control	No amplification	No amplification

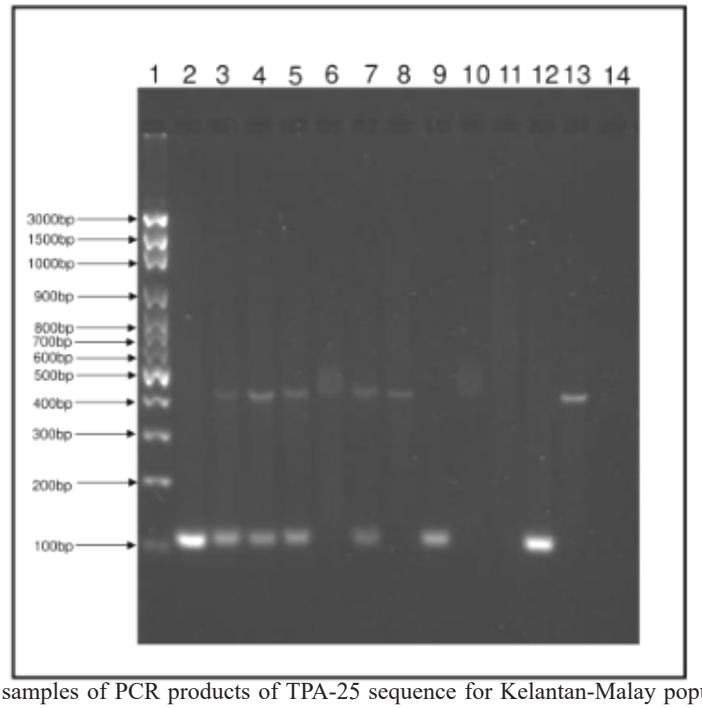

Figure 3. A total 12 samples of PCR products of TPA-25 sequence for Kelantan-Malay population on 2% (w/v) Agarose gel

Table 2

Size estimation of TPA-25 element of 14 samples of Kelantan-Malay population

Lane No.	Sample	TPA-25 Insertion	TPA-25 Deletion
1	100 bp DNA ladder		
2	PCR product of sample 4K	No amplification	109bp
3	PCR product of sample 5K	449bp	117bp
4	PCR product of sample 6K	420bp	117bp
5	PCR product of sample 7K	449bp	117bp
6	PCR product of sample 8K	No amplification	117bp
7	PCR product of sample 9K	449bp	No amplification
8	PCR product of sample 10K	449bp	117bp
9	PCR product of sample 11K	No amplification	No amplification
10	PCR product of sample 12K	No amplification	109bp
11	PCR product of sample 13K	No amplification	No amplification
12	PCR product of sample 14K	No amplification	No amplification
13	PCR product of sample 15K	420bp	109bp
14	Negative Control	No amplification	No amplification

Table 3

Allele and heterozygosity of TPA-25 element of Jawa-Malay population

No	Sample	Heterozygosity of TPA-25
1	43J	-ve
2	44J	+ve
3	45J	-ve/+ve
4	49J	+ve
5	50J	-ve/+ve
6	52J	-ve/+ve
7	53J	-ve/+ve
8	54J	-ve/+ve
9	55J	-ve/+ve
10	56J	-ve/+ve
11	57J	-ve
12	58J	-ve/+ve
13	59J	+ve
14	20J	-ve/+ve
15	21J	+ve
16	24J	-ve
17	25J	+ve
18	29J	-ve/+ve
19	31J	-ve/+ve
20	30J	+ve
21	32J	-ve/+ve
22	33J	+ve
23	34J	No allele
24	16J	-ve
25	17J	-ve/+ve
26	11J	No allele
27	10J	-ve/+ve
28	7J	No allele
29	6J	-ve/+ve
30	5J	-ve/+ve
31	4J	+ve
32	41J	No allele
33	42J	+ve

+ve = homozygous insertion; -ve = homozygous deletion; -ve/+ve = heterozygous allele.

Table 4

Allele and heterozygosity of TPA-25 element of Kelantan -Malay population

No	Sample	Heterozygosity of TPA-25	No	Sample	Heterozygosity of TPA-25
1	4K	-ve	28	32K	+ve
2	5K	-ve/+ve	29	35K	+ve
3	6K	-ve/+ve	30	37K	-ve
4	7K	-ve/+ve	31	39K	-ve
5	8K	No allele	32	42K	+ve
6	9K	-ve/+ve	33	44K	+ve
7	10K	+ve	34	46K	-ve
8	11K	-ve	35	50K	-ve
9	12K	No allele	36	55K	-ve/+ve
10	13K	No allele	37	81K	-ve/+ve
11	14K	-ve	38	89K	-ve/+ve
12	15K	+ve	39	91K	No allele
13	16K	-ve	40	95K	-ve
14	17K	-ve	41	97K	-ve/+ve
15	18K	-ve/+ve	42	99K	-ve/+ve
16	19K	-ve/+ve	43	101K	-ve
17	20K	-ve	44	86K	-ve
18	21K	No allele	45	57K	-ve/+ve
19	23K	-ve	46	58K	+ve
20	24K	+ve	47	63K	+ve
21	25K	-ve/+ve	48	68K	No allele
22	26K	-ve	49	70K	-ve/+ve
23	27K	-ve/+ve	50	71K	-ve
24	28K	-ve	51	72K	-ve/+ve
25	29K	-ve			
26	30K	-ve/+ve			
27	31K	No allele			

+ve = homozygous insertion; -ve = homozygous deletion; -ve/+ve = heterozygous allele.

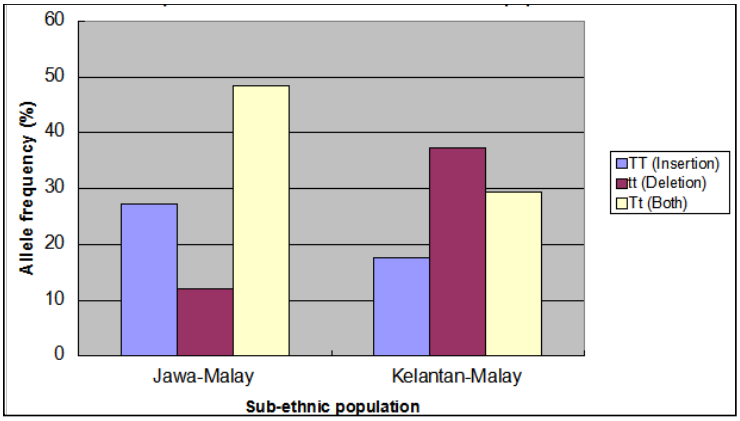

Figure 4. Percentage of allele frequency of TPA-25 (heterozygosity)

The heterozygosity and F-statistic for TPA-25 element among the two Malays sub-ethnic populations in peninsular Malaysia were calculated in F-statistic indices which had been formalized by Wright (1978) using the three different hierarchical measures of heterozygosity, H (HI, HS and HT) as shown in Table 5.

The inbreeding coefficient (FIS) assesses the global variation in individuals, relative to the variation in their subpopulations. From the results shown in Table 6, the FIS values for two sub-populations were positive which indicate that the subpopulation is deficit of heterozygotes. However, the FIS value for Kelantan-Malay population was higher than Jawa-Malay population. This data indicates that occurrence of inbreeding is higher in Kelantan-Malay population compared to Jawa-Malay population.

The overall inbreeding coefficient (FIT) assesses the variation in individuals relative to the variation in the total set of subpopulations. FIT value for the populations was 0.5359 which was within range 0 to 1, meaning the TPA-25 element had significant variation among individuals within the total population of Jawa-Malay and Kelantan-Malay studied. Fixation index, FST is the statistic used to assess the variation in the subpopulations relative to that in the total population.

Table 5

Heterozygosity value of TPA-25 element for two Malays sub-ethnics

	Alleles		Subpopulation		Total	
	p	H	P	H	P	H
Jawa-Malay	0.4848	0.7650	0.1905	0.4848		
Kelantan-Malay	0.2941	0.9135	0.1785	0.2941	0.1845	0.8393
Average Heterozygosity	H_S =	0.8393	H_I =	0.3895	H_T =	0.8393

Table 6

F-statistic of TPA-25 element for two Malays sub-ethnics

Subpopulation	Jawa-Malay	Kelantan-Malay
F_{IS}	0.3663	0.6781
All population		
F_{IT}	0.5359	
F_{ST}	0.0	

The value of FST for the total population was 0. As referred to qualitative guidelines for FST interpretation by Wright (1978), the FST value indicates that there is no subdivision, random mating occurring and no genetic divergence within the populations. However, the expected result for FST value was opposite from the data recorded as these two Malays sub-ethnic populations should show some genetic variation because the possibility of genetic drift happened within the population was high if referred to the FIT and FIS values. This misinterpreted data may be caused by the small scale of sample that was used in this study preventing the use of the parameter to determine the genetic differentiation of overall populations.

In the comparison with other world population, using TPA-25 element as the ancestry marker, this Alu insertion is proven as a useful tool that can reveal the patterns of human genetic diversity over the last 100,000 years (Rishishwar et al., 2015). Table 7 shows the TPA-25 frequencies in various world populations with range of frequencies within 0.3 to 0.6.

Table 7

Allele frequencies of TPA-25 in the world populations

Population	n	f	References
Europe			
Albania	60	0.557	Comas et al. (2000)
Albania Aromuns	49	0.500	Comas et al. (2000)
Andalusia	67	0.590	Comas et al. (2000)
Basque	96	0.568	Comas et al. (2000)
Catalonia	60	0.608	Comas et al. (2000)
Genova	30	0.450	Santovito et al. (2007)
Germany	70	0.514	Romualdi et al. (2002)
Valencia	101	0.556	García-Obregón et al. (2006)
North Africa			
Algeria	47	0.532	Comas et al. (2000)
NSC Tunisia	96	0.572	Bahri et al. (2008)
N. Morocco	111	0.617	Comas et al. (2000)
Sahara	58	0.397	Comas et al. (2000)
Sejnane	47	0.521	Frigi et al. (2011)
SE. Morocco	49	0.510	Comas et al. (2000)
Tunisia	48	0.604	Comas et al. (2000)
Takrouna	33	0.469	Frigi et al. (2011)
W. Morrocco	140	0.575	Comas et al. (2000)

Table 7 *(Continued)*

Population	n	f	References
South India			
Koya Dora	59	0.458	Veerraju et al. (2001)
Konda Reddi	57	0.481	Veerraju et al. (2001)
Central Patagonia			
Comodoro Rivadavia	50	0.500	Parolin et al. (2017)
Esquel	50	0.388	Parolin et al. (2017)
Puertp Madryn	50	0.620	Parolin et al. (2017)

n: number of samples; f: allele frequency

CONCLUSION

Kelantan-Malay population has genetically conserved their own special traits with low occurrence of intermarriage with other Malays sub-ethnic group and high occurrence of inbreeding within the population. Meanwhile, low inbreeding in Jawa-Malay population indicates is big genetic diversity exists for this population in peninsular Malaysia compared to Kelantan-Malay population.

Moreover, migration flows and interactions between sub-ethnic groups are suggested to be the main cause for genetic diversity occuring in both populations. This research suggests that culture and social life of these populations affect the genetic flows as nowadays, majority of them kept standard Malays lifestyle and cultures instead following their ancestry practices.

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