SEQUENCE POLYMORPHISM OF MITOCHONDRIAL DNA HYPERVARIABLE REGIONS I AND II IN MALAY POPULATION OF MALAYSIA

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Abstract: The analysis of the control region of human mitochondrial genome (mtDNA) especially hypervariable regions I (HVI) and II (HVII) segments have been proven to be useful for human identification. For forensic application of mtDNA profiling in Malaysia, a comprehensive database on both HVI and HVII regions are essential. In order to identify polymorphic positions and to determine their frequency in the Malay population, mtDNA HVI and HVII regions of 103 maternally unrelated individuals were amplified ,sequenced and compared with Cambridge reference sequence (CRS). Sequence comparison led to the identification of a total of 446 and 604 location polymorphisms in mtDNA HVI and HVII regions respectively. This polymorphisms defined by 88 haplotypes (81 unique) in the HVI and 78 haplotypes (64 unique) in the HVII regions. In combined HVI and HVII defined 101 haplotypes (99 unique) was defined. In the HVII region All the individuals in HVII showed nucleotide transition event from A→G at nucleotide position 073 and 263 and an insertion of cytosine (315.1C) at nucleotide position 315. The genetic diversity and probability of random match in combined HVI and HVII of 103 Malay individuals was found to be 0.9996 and 0.0101 respectively.

Keywords: mtDNA; Polymorphism; HVI; HVII; Haplotype.

INTRODUCTION

Currently, most of the forensic science laboratories used STR DNA profiling of chromosomal DNA as a standard DNA profiling method. However, when dealing with biological samples of human remains and problematic samples (e.g. old stains, decomposed tissue, hair shaft, teeth or bone) STRs DNA profiling has limitations due to sample conditions caused by environmental effects [1]. In this situation, mtDNA sequence analysis is found to be valid alternative and reliable tool for genetic characterization [2, 3, 4]. mtDNA HVI and HVII are found highly variable in the human population [5]. Since the sequence of these hypervariable regions provides a high degree of information for discriminating between unrelated individuals, the forensic identity testing has focused on sequence variation within these two hypervariable regions [6,7,8]. In addition, any living relative may provide a reference sample when an individual is not available for direct comparison with a biological sample.

Malaysia consists of a multiracial population with the Malays, Chinese and Indians forming the main ethnic groups. Published database for both HVI and HVII segments of mtDNA on major ethnic Malay population of Malaysia is almost negligible. For forensic application of mtDNA profiling in Malaysia, a comprehensive database on both HVI and HVII regions are essential. Therefore, in this study 103 maternally unrelated Malay individuals were used for the purposes of mtDNA polymorphism study.

MATERIALS AND METHODS

DNA extraction

Buccal swabs were collected from 103 maternally unrelated Malays residing in Peninsular Malaysia. Genomic DNA was extracted from buccal swabs using organic extraction method.

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Amplification and sequencing of mtDNA

PCR amplification was performed using established primers for HVI (L15997-H16391) and HVII (L048-H408) [9]. DNA template (50 ng) were amplified in a 20 µl reaction volume containing 160 µM dNTPs, 2.5 mM $MgCl_2$, 1XPCR buffer, 20 pmol primers [5] and 1.5 unit of *Taq* polymerase. The PCR condition for both HVI and HVII were 95°C for 3 minutes, followed by 30 cycles at 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec, followed by 72°C for 5 minutess. Amplified PCR products were purified using QIAquick gel purification kit (QIAGEN, Germany) and was sequenced using ABI 3700 DNA sequencer (PE Applied Biosystems, USA). The sequencing was performed in both directions using the same forward and reverse primers.

Analysis of data

The nucleotide positions 15997-16391 (HVI) and 048-408 (HVII) were analysed [10]. The haplotype diversity (h) was calculated according to Tajima [11] and random match probability (p) was calculated according to Stoneking et al [5].

RESULTS

The sequence data of all 103 unrelated ethnic Malay individuals were compared with CRS [10]. The analysis of mtDNA HVI of 103 Malay individuals shown 446 location polymorphisms and 88 different haplotypes (81 unique) was observed. In mtDNA of 103 individuals a total of 604 location polymorphisms and 78 different haplotypes (64 unique) was identified. In combined data of HVI and HVII, a total of 101 different haplotypes were recorded with 99 individuals show unique haplotype sequence. The haplotype results and other parameters for mtDNA HVI and HVII of the 103 Malays are presented in Table 1 and Table 2 respectively. The genetic diversity for 103 Malays for HVI and HVII was 0.9958 and 0.9905 respectively. The probability of random match of HVI and HVII among 103 Malays was 0.0139 and 0.0191 respectively. The genetic diversity and probability of random match of in combined HVI and HVII was 0.996 and 0.0101 respectively.

DISCUSSION

Both mtDNA HVI and HVII regions exhibit transitions as the most frequently occurring events within sequence deviations with 85.87% and 56.46% respectively. However, the majority of transition in mtDNA HVI region is pyrimidine transition 35.77% (C→T) whereas in the mtDNA HVII region, the majority is purine transition 64.51% (A→G), This purine A→G transition in HVII also found occurring more frequently in other populations with in Korean 70.12% [13], German 62.68% [14], Southern Spanish 58.4% [15], Caucasian Danes 58.04% [16] and Brazilian 97.5% [17]. The nucleotide position 16223 exhibits the most frequent C→T transition with a distribution of 37.22% within Malay population and the same polymorphism also reported in Argentine, Amerindian, Asian and African populations [12]. In mtDNA HVI, A→C transversion at nucleotide position 16183 coupled with T→C transition at nucleotide position 16189 exhibited in 18 Malay individuals. Five individuals exhibited a double transition at nucleotide position 16182 and 16183 coupled with T→C transition at nucleotide position 16189. This type of C-stretches has also been reported in Italian Caucasians and Solvenians [18]. The insertions in the HVII region was observed at distribution of 35.59% in Malay individuals whereas in mtDNA HVI region the insertions was observed at lower frequency which is 0.9%. A similar pattern was seen in other populations such as in the Korean 22.62% [13], German 32.58% [14], Polish 37.26% [19], Southern Spanish 38.53% [15], Caucasian Danes 34.30% [16] and Brazilian 21.18% [17] . In mtDNA HVII region all the Malay individuals showed nucleotide transition event from A→G at nucleotide position 073 and 263 and an insertion of cytosine (315.1C) at nucleotide position 315. The other insertions were also identified at nucleotide positions at 309 (+C, +2C) in 98 individuals (78 individuals with 309.1C and 20 individuals with 309.1C, 309.2C).The presence of insertion C residues between nucleotide positions 303-309 and 311-315 in mtDNA HVII region also reported in many populations [12,13,14,15,16,17,19,20].

The most frequent mtDNA HVI haplotype (3.88%) is defined by polymorphism at nucleotide position 16223 C→T transiton in Malay population, which also found in South Indians (2.1 %) [9], Argentine population (8.9 %) [12], Taiwanese Han population [21], Japanese population (6 %) [22], Russian (1.59 %) [23], Indigenous Indian tribes (5 %) [24], Korea ethnic Chinese (1.81 %) [25]. The most frequent mtDNA HVII haplotype (6.79 %) was defined by polymorphisms at nucleotide positions 073 and 263 A→G transitions, 249 A-del and the insertion of a C-residues at 309.1C and 315.1C in Malay population, where the similar polymorphisms also observed in Korea ethnic Chinese (1.81 %) [25], North east Chinese (5.88 %) [26] and North East Germany population (0.33 %) [27].

CONCLUSION

The total number of polymorphic sites in mtDNA HVI (91) and HVII (52) of Malay individuals clearly shows that the control region of mtDNA region is highly polymorphic. The compilation of mtDNA HVI and HVII in this study provides a comprehensive mtDNA database for Malay population of Malaysia that will be useful for forensic purposes.

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Table 1: List of mtDNA HVI and HVII haplotypes in the Malay population (n=103).

Sample	mtDNA Haplotype	
No.	HVI (15997-16391)	HVII (048-408)
M1	16108, 16129, 16162, 16172, 16240,	16304073, 195, 249d, 263, 309.1C, 315.1C
M2	16147, 16182.1C, 16189, 16217, 16235, 16293	073, 249d, 263, 309.1C, 315.1C
M3	16190, 16223, 16327, 16330	073, 113, 263, 309.1C, 309.2C, 315.1C
M4	16140, 16183C, 16189, 16266A, 16304	073, 125, 128, 263, 309.1C, 315.C
M5	16172, 16304	073, 249d, 263, 309.1C, 315.1C
M6	16182C, 16183 C, 16189, 16223	073, 088, 263, 309.1C, 315.1C
M7	16086, 16148, 16223, 16259, 16278, 16319	073, 150, 152, 200, 263, 315.1C
M8	16091, 16223, 16298, 16327	073, 249d, 263, 309.1C, 315.1C
M9	16218, 16362	073, 146, 150, 199, 263, 309.1C, 315.1C
M10	16182C, 16183C, 16189, 16219C	073, 146, 263, 309.1C, 309.2C, 315.1C
M11	16223, 16295, 16362	073, 146, 199, 263, 309.1C, 315.1C
M12	16093, 16209, 16223, 16263, 16278, 16319	073, 146, 150, 151, 263 309.1C, 309.2C, 315.1C
M13	16140, 16189, 16355	073, 146, 150, 151, 263, 309.1C, 309.2C
M14	16223	073, 150, 195A, 263
M15	16126, 16184A, 16223, 16278	073, 263, 315.1C
M16	16172, 16183C, 16189, 16223	073, 185, 189, 263, 309.1C, 315.1C
M17	16176, 16278, 16354	073, 199, 263, 315.1C
M18	16027G, 16074.1C, 16140, 16183, 16189, 16266A	073, 210, 263, 309.1C, 315.1C
M19	16037, 16192, 16288, 16304, 16309, 16390	073, 143, 183, 263, 309.1C, 309.2C, 315.1C
M20	16037, 16129, 16148, 16183C, 16189, 16319	073, 093, 113, 263, 315.1C
M21	16140, 16189	073, 210, 263, 309.1C315.1C
M22	16108, 16129, 16162, 16172, 16304	073, 150, 249d, 263, 309.1C, 315.1C
M23	16126, 16181, 16209, 16362	073, 152, 242, 263, 309.1C, 315.1C
M24	16223	073, 146, 150, 199, 263, 309.1C, 315.1C
M25	16126, 16231, 16311 , 16362	073, 204, 207, 263, 309.1C, 309.2C, 315.1C
M26	16223, 16295, 16362	073, 146, 199, 263, 315.1C
M27	16129, 16140, 16271, 16364, 16367G	073, 143, 146, 151, 263,315.1C
M28	16177, 16223, 16263, 16266, 16274, 16311, 16343 G	073, 146, 152, 263 309.1C, 315.1C
M29	16129, 16172, 16304	073, 249d, 263, 309.1C, 309.2C, 315.1C, 332, 362 366, 389, 394A
M30	16108, 16129, 16162, 16172, 16278, 16304	073, 249d, 263, 309.1C, 315.1C, 366
M31	16192, 16223, 16304, 16309, 16390	073, 263, 309.1C, 315.1C
M32	16093, 16223, 16243, 16270, 16319, 16352	073, 121T, 195, 204, 227, 263, 309.1C, 315.1C
M33	16188, 16209, 16223, 16325	073, 263, 309.1C, 315.1C
M34	16162, 16172, 16304, 16366G	073, 249, 263, 309.1C, 309.2C, 315.1C
M35	16136, 16261, 16292,16294	073, 086.1G, 207, 263 309.1C, 315.1C
M36	16223, 16295, 16362	073, 085d, 094.1G, 199, 263, 309.1C
M37	16145, 16181, 16192, 16223, 16291, 16304	073, 210, 263, 309.1C, 315.1C
M38	16172, 16223, 16261	073, 097, 200, 263,309.1C, 315.1C
M39	16027G, 16260, 16298,16355, 16362, 16367, 16383	073, 207, 249d, 263, 309.1C, 315.1C
M40	16108 A, 16129, 16162, 16172	073, 249d, 263, 309.1C, 315.1C
M41	16037d, 16140,16182C, 16183C, 16189, 16217,16278, 16335, 16343C	073, 152, 263, 309.1C, 315.1C, 366
M42	16037, 16140, 16183C, 16189, 16213, 16217, 16242, 16274, 16335	073, 085d, 146, 150, 195, 263, 309.1C, 315.1C
M43	16037, 16140, 16183C, 16189, 16261, 16267A, 16274	073, 085d, 152, 210, 263, 309.1C, 315.1C
M44	16223, 16261, 16362, 16390	073, 152, 263, 309.1C, 315.1C
M45	16223, 16257A, 16261,16292, 16294	073, 085, 150, 263, 309.1C, 315.1C
M46	16126, 16184A, 16223, 16278	073, 263, 315.1C
M47	16091, 16223, 16298, 16327	073, 94.1G, 151.1 G, 249d, 263, 315.1C
M48	16108, 16129, 16162, 16172, 16278, 16304	073, 085d, 249 d, 263, 309.1C, 315.1C
M49	16111, 16223, 16278, 16291	073, 204, 207, 263, 309.1C, 309.2C, 315.1C, 321, 332
M50	16108, 16129, 16162, 16172, 16304	073, 249d, 263, 315.1C
M51	16126, 16231, 16311	073, 085d, 094.1G, 204, 207, 263, 309.1C, 309.2C, 315.1C
M52	16140, 16183C, 16189, 16362	073, 085d, 092.1T, 094.1G, 097, 210, 263, 309.1C, 309.2C, 315.1C
M53	16223	073, 094.1G, 124, 150, 195A, 263, 315.1C

M54	16223	073, 146, 150, 199, 263, 309.1C, 315.1C
M55	16140, 16182C, 16183C, 16217, 16274, 16335	073, 085d, 146, 195, 263, 309.1C, 309.2C
M56	16182C, 16183C, 16189, 16266A	073, 146, 263, 309.1C, 315.1C, 389
M57	16129, 16188, 16209, 16223, 16325	073, 085d, 263, 309.1C, 315.1C
M58	1603d, 16136, 16223, 16257A, 16261 16294	073, 094.1G, 207, 263, 309.1C, 315.1C
M59	16027G, 16129, 16192, 16223, 16258C, 16289,16297	073, 150, 263, 309.1C, 315.1C, 332
M60	16037,16140, 16183C, 16190	073, 151.1G, 210, 263, 309.1C, 315.1C
M61	16295, 16362, 16380	07, 146, 199, 263, 309.1C, 309.2C, 315.1C
M62	16192, 16304,16309, 16390	073, 094.1G, 124, 263, 309.1C, 315.1C
M63	16140, 16183C, 16189,16274, 16372G	073, 085d, 210, 263, 315.1C
M64	16223, 16311, 16362	073, 146, 152, 187, 263, 315.1C
M65	16129, 16172, 16304	073, 094.1G, 124, 249d, 263, 309.1C, 309.2C, 315.1C
M66	16027G, 16108, 16129,16172, 16304, 16364G, 16367	073, 149, 263, 309.1C, 315.1C
M67	16037, 16129, 16190, 16223, 16234, 16290	073, 094.1G, 146, 195, 263, 315.1C
M68	16196T,16223, 16233C, 16273T, 16291	073, 263, 309.1C, 315.1C
M69	16223, 16261, 16362, 16367,16382G, 16390	073, 146, 150, 195, 263, 315.1C
M70	16027G, 16074.1C, 16129, 16172, 16304, 16367,16382	073, 150, 249d, 263, 315.1C
M71	16183C, 16189, 16223, 16372	073, 124, 263, 309.1C, 315.1C
M72	16177, 16263, 16266, 16274, 16311, 16343	073, 146, 152, 263, 309.1C, 315.1C
M73	16295, 16362	073, 146, 199, 263, 309.1C
M74	16223, 16261,16362, 16390	073, 146, 187, 263, 315.1C
M75	16209, 16223, 16233C16274	073, 143, 146, 263, 309.1C, 315.1C
M76	16183, 16223	073, 113, 263, 309.1C, 309.2C, 315.1C, 375
M77	16129, 16189, 16223,1633	073, 121T, 263, 315.1C
M78	16196T, 16223, 16291, 16362	073, 263, 309.1, 315.1C
M79	16108, 16129, 16162, 16172, 16304	073, 094.1G, 249d, 263, 315.1C
M80	16223, 16325, 16362	073, 199, 263, 309.1C, 309.2C, 315.1C
M81	16051, 16156, 16219C, 16223,16233C, 16258, 16362, 16384, 16390	073, 195, 263, 309.1C, 315.1C
M82	16079, 16171, 16209, 16243, 16278	073, 195, 263, 309.1C, 315.1C
M83	16108, 16129, 16162, 16172, 16304	073, 249d, 263, 309.1C, 315.1C
M84	16129, 16272, 16311	073, 199, 249d, 263, 309.1C, 309.2C, 315.1C, 316
M85	16223, 16311	073, 146, 263, 309.1C, 315.1C
M86	16223, 16261, 16294	073,152, 263, 315.1C
M87	16129, 16172, 16304	073, 249d, 263, 309.1C, 315.1C
M88	16093, 16129, 16223 16261, 16356	073, 150, 152, 263, 309.1C, 315.1C
M89	16147, 16182.1C, 16189, 16217, 16235	073, 146, 263, 309.1C, 309.2C, 315.1C
M90	16176, 16223,16278,16354	073, 199, 263, 315.1C
M91	16129, 16172, 16256, 16305, 16309	73, 152, 263, 309.1C, 315.1C
M92	16288, 16304, 16309, 16390	073, 085d, 143, 183, 263, 309.1C, 309.2C, 315.1C
M93	16129, 16223, 16259, 16278, 16291, 16362	073, 195, 263, 286, 292, 293, 294, 309.1C, 315.1C
M94	16037d, 16140, 16183C, 16189, 16266A, 16298	073, 092T, 097,210, 263, 309.1C, 315.1C, 366
M95	16037, 16140, 16183C, 16189	073, 210, 263, 309.1C, 315.1C
M96	16134, 16223, 16362	073, 263, 309.1C, 315.1C, 366, 383
M97	16093, 16223,16295,16362	073, 150, 199, 263, 309.1C, 309.1C, 315.1C, 366, 380, 389
M98	16223, 16234 16300, 16311	073, 146, 263, 315.1C
M99	16126, 16129, 16192, 16223, 16297	073, 146, 199, 263, 309.1C, 315.1C
M100	16147, 16189, 16217, 16235	073, 263, 309.1C, 315.1C
M101	16086, 16156, 16189, 16223,16234	073, 210, 263, 315.1C
M102	16209, 16298, 16355, 16362	073, 152, 207, 249d, 263, 309.1C, 315.1C
M103	16223, 16257A, 16261, 16292, 16294	073, 150, 207, 263, 309.1C, 309.2C, 315.1C

Note:

1.M15 and M46 samples show same type of combined HVI and HVII haplotypes.

2.M24 and M54 samples show same type of combined HVI and HVII haplotypes.

3.All other 99 samples show unique type of combined HVI and HVII haplotypes.

Table 2: Distribution of location polymorphisms of mtDNA HVI and HVII in Malay population (n = 103).

Mutation Type	HVI		HVII	
	Number of positions	Total number of mutations	Number of positions	Total number of mutations
Transitions				
C-T	26	137	7	29
T-C	22	153	7	63
A-G	16	46	6	220
G-A	7	47	9	29
Total	**71**	**383**	**29**	**341**
Transversions				
C-A	5	11	2	2
A-C	5	29	0	0
C-G	3	5	0	0
G-C	0	0	1	1
T-G	2	7	2	2
G-T	2	3	3	5
T-A	0	0	1	2
Total	**17**	**55**	**9**	**12**
Insertions				
+C	2	4	3	201
+G	0	0	3	13
+T	0	0	1	1
Total	**2**	**4**	**7**	**215**
Deletions				
-A	1	4	2	21
-G	0	0	2	12
-T	0	0	3	3
Total	**1**	**4**	**7**	**36**
Total polymorphic positions	**91**	**446**	**52**	**604**

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