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Announcement of population data

Sequence polymorphism of the mitochondrial DNA hypervariable regions I and II in 205 Singapore Malays

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Abstract

Mitochondrial DNA sequences of the hypervariable regions HV1 and HV2 were analyzed in 205 unrelated ethnic Malays residing in Singapore as an initial effort to generate a database for forensic identification purposes. Sequence polymorphism was detected using PCR and direct sequencing analysis. A total of 152 haplotypes was found containing 152 polymorphisms. Out of the 152 haplotypes, 115 were observed only once and 37 types were seen in multiple individuals. The most common haplotype (16223T, 16295T, 16362C, 73G, 146C, 199C, 263G, and 315.1C) was shared by 7 (3.41%) individuals, two haplotypes were shared by 4 individuals, seven haplotypes were shared by 3 individuals, and 27 haplotypes by 2 individuals. Haplotype diversity and random match probability were estimated to be 0.9961% and 0.87%, respectively.

Keywords: mtDNA; Haplotypes; Haplogroups; Heteroplasmy; Sequence polymorphism; Singapore Malay population

Population: Blood samples from 205 unrelated ethnic Malays residing in Singapore were collected on FTA™ paper (Whatman, Middlesex, UK) [1].

Purification: Purification of the FTA punches was performed in accordance with the manufacturer's protocol.

PCR amplification: V1 (position 16024 –16365) and HV2 (position 73–340) regions were amplified in a single reaction using primers F15971 (TTA ACT CCA CCA TTA GCA CC) and R484 (TGA GAT TAG TAG TAT GGG AG). The PCR was performed in a total volume of 50 μl consisting of 1× PCR buffer (containing 1.5 mM $MgCl_2$, 10 mM Tris–HCl and 50 mM KCl), 200 μM of each dNTP, 400 nM of each primer and 8 μg of BSA with 5 U AmpliTaq Gold® (Applied Biosystems Inc., Foster City, USA). Amplification was performed on PTC-200™ DNA Engine® Peltier Thermal Cycler (MJ Research, Inc., Massachusetts, USA) using the following conditions: 96 °C for 10 min, followed by 32 cycles of 94 °C for 20 s, 56 °C for 10 s, 72 °C for 30 s and hold at 4 °C. Postamplification products were purified using ExoSAP-IT® (USB Corp., OH, USA) in a 5:1 ratio [2]. Samples were randomly chosen from each batch of PCR amplification and subjected to electrophoresis on a 2% agarose gel to obtain an estimated yield of the PCR products.

Sequencing: Sequencing reactions were performed in a total reaction volume of 20 μl consisting of ~20 ng of template amplicon, 10 pmol of primers, ABI BigDye® Terminator Cycle Sequencing kit on a PTC-200™ under the following conditions: 96 °C for 1 min followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min and hold at 4 °C. The primers used for HV1 were F15971 (TTA ACT CCA CCA TTA GCA CC) and R16410 (GAG GAT GGT GGT CAA GGG AC), while primers used for HV2 were F15 (CAC CCT ATT AAC CAC TCA CG), R408 (CTG TTA AAA GTG CAT ACC GCC) and

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Table 1
Mitochondrial DNA HV1 and HV2 control region sequence polymorphism in 205 unrelated Malays residing in Singapore

Haplotype	Variation in HV1[a]	Variation in HV2[b]	Frequency
HA1	37G, 183C, 189C, 223T, 261T, 362C	152C, 315.1C	2
HA2	37G, 223T, 278T, 311C, 320T	146C, 150T, 152C, 269T, 315.1C	1
HA3	42A, 70G, 183C, 189C, 209C, 223T, 233G	143A, 152C, 315.1C	1
HA4	47A, 51G, 168T, 184A, 189C, 201T, 304C	93G, 146C, 315.1C	1
HA5	47A, 93C, 129A, 223T, 261T, 356C	150T, 152C, 315.1C	1
HA6	51G, 93C, 182C, 183C, 189C, 218T, 292T, 362C	207A, 228A, 234G, 249D, 315.1C	1
HA7	51G, 168T, 311C	146C, 315.1C	1
HA8	51G, 179T, 234T	146C, 152C, 315.1C, 334C	1
HA9	51G, 182C, 183C, 189C, 362C	315.1C	1
HA10	51G, 183C, 189C, 194C, 195C, 266T	315.1C	2
HA11	51G, 184T, 223T, 325C, 362C	195, 315.1C	1
HA12	51G, 185T, 223T, 362C	195C, 315.1C	1
HA13	70G, 93C, 140C, 182C, 183C, 189C, 266A	210G, 315.1C	1
HA14	86C, 129A, 183C, 189C, 223T, 297C	150T, 152C, 199C, 315.1C	1
HA15	86C, 129A, 192T, 223T, 297C	150T, 199C, 315.1C	3
HA16	86C, 129A, 209C, 223T, 272G	151T, 152C, 225A, 249D, 315.1C, 316A	1
HA17	86C, 129A, 209C, 223T, 272G	152C, 225A, 249D, 291T, 315.1C, 316A	1
HA18	86C, 147T, 183C, 184A, 189C, 217C, 235G	146C, 315.1C	1
HA19	86C, 172C, 189C, 223T, 234T, 290T	125C, 127C, 128T, 146C, 195C, 315.1C	1
HA20	92C, 140C, 172C, 189C, 223T, 278T	249G, 315.1C, 319C	1
HA21	92C, 140C, 182C, 183C, 189C, 261T, 266A	152C, 210G, 315.1C	1
HA22	92C, 148T, 182C, 183C, 189C, 223T, 362C	150T, 152C, 185A, 315.1C	1
HA23	92C, 164G, 182C, 183C, 189C, 223T, 266T, 362C	150T, 315.1C	1
HA24	93C, 126C, 207G, 292T, 309G, 318T	151T, 152C, 315.1C	1
HA25	93C, 129A, 140C, 182C, 183C, 189C, 261T, 266A	152C, 210G, 315.1C	1
HA26	93C, 129A, 223T, 256T, 271C	204C, 315.1C	1
HA27	93C, 129A, 223T, 256T, 271C	315.1C	1
HA28	93C, 140C, 183C, 189C, 266A	146C, 210G, 315.1C	1
HA29	93C, 172C, 223T, 298C, 327T	249D, 315.1C	1
HA30	93C, 183D, 186T, 189C, 223T, 271C, 311C	151T, 315.1C	1
HA31	93C, 184A, 223T, 278T	151T, 315.1C	2
HA32	93C, 185T, 223T, 260T, 294T, 298C	152C, 189G, 207A, 249D, 315.1C	2
HA33	93C, 192T, 223T, 266T, 271C, 316G, 362C	184A, 249D, 315.1C	1
HA34	93C, 209C, 223T, 224C, 263C, 278T, 319A	146C, 150T, 151T, 152C, 315.1C	1
HA35	93C, 209C, 223T, 224C, 263C, 278T, 319A	146C, 150T, 151T, 234G, 315.1C	2
HA36	93C, 223T, 295T, 362C	146C, 199C, 315.1C	3
HA37	93C, 260T, 298C, 311C, 355T, 362C	249D, 315.1C	1
HA38	93C, 260T, 298C, 355T, 362C	204C, 207A, 249D, 315.1C	1
HA39	93C, 295T, 362C	146C, 199C, 315.1C	1
HA40	108T, 129A, 162G, 172C, 189C, 304C	249D, 293C, 315.1C	1
HA41	108T, 129A, 162G, 172C, 189C, 304C	249D, 315.1C	4
HA42	108T, 129A, 162G, 172C, 288C, 304C	150T, 249D, 315.1C	1
HA43	108T, 129A, 162G, 172C, 304C	150T, 249D, 315.1C	2
HA44	108T, 129A, 162G, 172C, 304C	249D, 315.1C	2
HA45	108T, 129A, 172C, 223T, 234T, 290T, 311C	153G, 185A, 189G, 315.1C	1
HA46	108T, 129A, 172C, 223T, 234T, 290T	153G, 185A, 189G, 315.1C	1
HA47	111T, 140C, 182C, 183C, 189C, 234T, 243C, 291T	131C, 204C, 315.1C	1
HA48	111T, 168T, 172C,183C, 189C, 223T, 362C	152C, 315.1C	1
HA49	124C, 179A, 223T, 261T, 262T	315.1C	1
HA50	124C, 223T, 248T, 362C	315.1C	1
HA51	126C, 140C, 182C, 183C, 189C, 261T, 266A	152C, 210G, 315.1C	1
HA52	126C, 214A, 223T, 271C, 278T, 298C	152C, 195C, 204C, 315.1C	2
HA53	126C, 223T, 290T	315.1C	1
HA54	126C, 231C, 311C	143A, 228A, 315.1C	1
HA55	126C, 231C, 311C	315.1C	1
HA56	126C, 292T, 294T, 296T	152C, 315.1C	1
HA57	129A, 140C, 182C, 183C, 189C, 261T, 266A	131C, 152C, 210G, 308D, 309D, 315.1C	1
HA58	129A, 140C, 182C, 183C, 189C, 266A	152C, 210G, 315.1C	1
HA59	129A, 140C, 223T, 271C	146C, 151T, 315.1C	1
HA60	129A, 140C, 271C	143A, 146C, 151T, 315.1C	2
HA61	129A, 145A, 249C, 288C, 301T, 304C, 311C	152C, 315.1C, 329A	2
HA62	129A, 148T, 172C, 223T, 256T, 305G, 309G	152C, 315.1C	1
HA63	129A, 172C, 293G, 304C, 311C	249D, 315.1C	1