Int J Legal Med (2010) 124:165–170
DOI 10.1007/s00414-009-0355-6

SHORT COMMUNICATION

MtDNA control region sequence polymorphisms and phylogenetic analysis of Malay population living in or around Kuala Lumpur in Malaysia

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Received: 23 February 2009 / Accepted: 12 May 2009 / Published online: 16 June 2009

Abstract Control region polymorphisms in the mitochondrial DNA of 124 unrelated individuals from the Malay population living in or around Kuala Lumpur in Malaysia were investigated and phylogenetic haplogroup lineages were determined. The intergenic COII/tRNALys 9-bp deletion, 3010 and 5178 mutations, and several coding region polymorphisms were examined to discriminate some phylogenetic haplogroups. Sequence comparison of the control regions led to the identification of 117 mitochondrial haplotypes, in which 103 types were observed in only one individual and the other nine types were shared by more than two individuals. Gene diversity was estimated to be 0.997. Phylogenetic haplogroup determination revealed that the gene pool of the modern Malay population in Malaysia consisted mainly of southeast Asian, east Asian, unidentified and unique, and aboriginal southeast-specific haplogroups. These results suggest a multi-original nature for the modern Malay population. The present database may help not only in personal identification but also in determining geographic origin in forensic casework in Malaysian, Southeast Asian and East Asian populations.

Keywords Mitochondrial DNA · Polymorphism · Control region · Haplogroup · Malay population

Introduction

Knowledge of the frequencies with which certain mitochondrial DNA sequences occur in a given population is of crucial importance for the application of mitochondrial markers to forensic studies because mitochondrial DNA (mtDNA) sequence types are strongly correlated with geographic origin [1]. Recent research on mtDNA sequence data in the forensic field has begun to encompass the complete control region sequence, with the aim of obtaining more information on polymorphisms and more reliable indicators of phylogenetic haplogroup than those based on the HV1 and HV2 regions alone. Although knowledge of mtDNA polymorphisms of the control region in the East Asian population is increasing, only two reports have investigated large numbers of the modern Malay population [2, 3]. One of these reports did not present a database on control region polymorphisms [2] and the other reported only HV1 (16024–16364) and HV2 (73–340) region information and did not show haplogroups [3]. Therefore, the details of the phylogenetic characteristics and control region variations of the modern Malay population remain to be clarified. In this study, we investigated the complete control region and some specific coding region poly-

Electronic supplementary material The online version of this article (doi:10.1007/s00414-009-0355-6) contains supplementary material, which is available to authorized users.

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morphisms to obtain more reliable indicators of mtDNA haplogroups and compared them with other datasets on the Southeast Asian, East Asian, and neighboring populations.

Materials and methods

Samples

Genomic DNA was extracted from tooth samples from 124 healthy, unrelated, Malay individuals. Informed consent was obtained from the donors. This study was approved by the ethics committee of the Tokyo Dental College and met with the conditions for cooperative study at the University of Malaya. All samples were from Malay individuals living in or around Kuala Lumpur. A family history was also taken to ensure that their parents were of Malay origin. Tribal population samples were not included in the study. Isolation of genomic DNA from tooth samples was performed as described previously [4], except that tooth samples were decalcified with ethylenediaminetetraacetic acid for 2 days.

Polymerase chain reaction amplification and sequencing of control region

We determined the nucleotide sequences of the 1.15-kb control region from 16024 to 576. The PCR primer sequences used in this study were the same as those used previously [5]. The complete control region was amplified using the primer pair L15978 and H601. When amplification efficiency was low, two overlapping fragments (L15978–H67 and L8–H601) were amplified.

PCR amplification, purification of amplicons, sequencing PCR, and removal of excessive dye were performed as described previously [5]. Sequence analysis was performed with the ABI 3130 DNA Sequencer (Applied Biosystems, Tokyo, Japan). Primers used for DNA sequencing were essentially the original PCR primer pairs, H68 and L8.

Typing of other polymorphisms

The intergenic COII/tRNALys 9-bp deletion and the 3010 and 5178 mutations were determined as described elsewhere [6, 7]. Mutations of 1189, 5186, 6620, 7598, 7759, 8270, 8584, and 10834 were determined by sequencing. To sequence the corresponding region, we selected primers from our primer sets depending on the region targeted for analyzing each haplogroup. Information on primers is available upon request.

Data analysis

Gene diversity was calculated as follows: $h = n\left(1 - \Sigma x_i^2\right)/(n-1)$ (where n is sample size and x_i is the frequency of the i-th mtDNA type) [8]. The probability of two randomly selected individuals from a population having identical mtDNA types was calculated as $P = \Sigma x_i^2$.

Results and discussion

Population data

We determined the nucleotide sequences of the control region in 124 matrilineal unrelated individuals (Table S1). Among 1,122 nucleotides in the control region (16024–576), 159 (14.1%) sites were variable, excluding the number of C-stretches in HV1 and HV2. A total of 111 different haplotypes were observed, among which two types were shared by three individuals, nine types were observed in two individuals, and 100 types were unique. We did not include variation in the C-stretch in calculating number of haplotypes because this variation frequently shows length heteroplasmy in most haplogroups [9] and haplotypes with a different length of C-stretch are sometimes obtained from biological materials from the same individuals. However, we did include differences in the number of CA repeats from nt 514–523, as this is comparatively restricted to certain haplogroups. Gene diversity was calculated as 0.9900. The probability of two randomly selected individuals from a population having identical mtDNA types was 1.01%.

Phylogenetic studies

Phylogenetic haplogroup lineages were estimated using control region sequence variation together with polymorphisms of the intergenic COII/tRNALys 9-bp deletion, 3010 and 5178 mutations to discriminate D and D4 lineages, and several other haplogroup-specific mutations in the coding region (Table S1). We referred to the classification trees proposed by Palanichamy et al. [10], Howell et al. [11], Sun et al. [12], Kong et al. [13], Hill et al. [14, 15], Friedlaender et al. [16], and Soares et al. [17]. Mutation 489 is informative for defining macrohaplogroups M and N. The present Malay population showed 52% for the M macrohaplogroup and 48% for the N macrohaplogroup. We distinguished 42 haplogroups, among which 20 samples in the M* haplogroup and two samples in the D* haplogroup could not be specified further. Characteristic sites defining haplogroup lineage and diagnostic mutations defining final haplogroup lineage are shown in Table S1.

The M7b1 haplotype has been found throughout East and Southeast Asia in the Korean (0.3%) [18-21], Chinese (1.8%) [22-29], Japanese (0.15%) [5, 9, 30-35], Thai (1.9%) [36], Singapore Malay (2.4%) [3], Southern Chinese

Daic and Austro-Asiatic (7.7%) [37], Vietnamese (6.95%) [38], Aboriginal Taiwanese (3.3% and 0.74%) [39, 40], and Island Southeast Asian populations (1.4%) [14, 15, 41], but not in the Aboriginal Malay population [14, 41]. Of the present M7b1 individuals, two had the intergenic COII/tRNALys 9-bp deletion. When PCR products of these samples were sequenced, both samples had an 8270 mutation adjacent to the 8271–8279 deletion. This pattern is not identical to the ordinary 9-bp deletion found in haplogroup B, but is found in the 9-bp deletion type in other haplogroups [32, 42]. The present M7b1 possessing this 9-bp deletion may constitute a different lineage in this haplogroup.

Of the nine individuals in haplogroup M7c, eight shared the 16223–16295–16362 HV1 motif designated as M7c1c by Kivisild et al. [23]. This motif is frequent in the Island Southeast Asian (8.3%) [15], Aboriginal Taiwanese (4.4% and 7.87%) [39, 40], Aboriginal Malay (5.8%) [14, 41], Singapore Malay (10%) [3], and Vietnamese (2.7%) [38] populations; is rare in the Chinese (0.5%) [22-29], Thai (0.5%) [36], and Southern Chinese Austro-Asiatic and Daic (0.1%) [37] populations; and has never been found in the Japanese [5, 9, 30-35] or Korean [18-21] populations.

The branches of haplogroup E were determined using control and coding region mutations. Estimation of E1a, E1b, and E2 lineages is possible from information on HV1 and HV2. However, discrimination between E1a and E2 is not possible from HV1 alone in some cases [17]. E1a, E1b, and E2 haplogroups have not been found in the Japanese [5, 9, 31-35], Korean [18-21], Chinese [22-29], Thai [36], Southern Chinese Daic and Austro-Asiatic [37], Vietnamese [38], or Aboriginal Malay populations [14, 41]. The E lineage is mainly found in the Aboriginal Taiwanese (11.1% and 11.38%) [40, 41], Island Southeast Asian (11%) [15], and Melanesian populations (4.9%), in which the E1a and E1b lineages showed different distributions [16, 17]. The E1a and E1b lineages were also found in Singapore Malays (2.0% and 3.5%, respectively) [3]. One individual (Mal-70) had a COII/tRNALys 9bp deletion at the same location (8271–8279) as that of haplogroup B.

The haplogroup Q1 is a major haplogroup in Melanesia (13.9%) [16] and Polynesia (7.9%) [43], is found at a low frequency in Island Southeast Asia (2.9%) [15], but is rarely found in modern or Aboriginal Malay populations [3, 15]; neither has it been observed in East Asian populations such as the Chinese [22-29], Korean [18-21], Japanese [5, 9, 30-35], Thai [36], Vietnamese [38], Southern Chinese Daic and Austro-Asiatic [37], or Aboriginal Taiwanese populations [39, 40].

The M12 lineage has been found in the Thai (3%) [37], Southern Chinese Daic (1.55%) [38], Singapore Malay (1.5%) [3], Vietnamese (0.53%) [39], Chinese (0.36%) [23-30], and Island Southeast Asian populations (0.2%) [14], but has never been found in the Aboriginal Malay [14, 15], Southern Chinese Austro-Asiatic [38], Korean, or Japanese populations [5, 9, 19-22, 31-36].

M21a has been found only in the Aboriginal Malay (21.5%) [14, 41], Thai (7.73%) [36], and Singapore Malay populations (1.49%) [3]. M21b has been found in Aboriginal Malay (3.97%) [14, 41] and in Southeast Asian populations on rare occasions [15]. M22 has been found mainly in Aboriginal Malay (2.3%) [14, 41], and rarely in Singapore Malay (0.5%) [3] and Thai (0.5%) populations [36]. These haplogroups may essentially be Malay markers.

Several kinds of Indian haplogroup, M3 [12], K1, U2b, R30, and R8 [10], were found. It was possible to determine the M3 lineage by control region mutations only. The K and U2 haplogroups could be estimated by control region mutations. However, to determine their sub-branches, coding region mutations were examined. The R30 lineage was determined by coding region mutations. Because the control region sequence of this sample was almost completely different from that of the reported Indian haplotype, except mutation 373 [10], this Malay lineage may constitute a new branch in the R30 haplogroup. No specific mutation motif in the control region has been proposed for the R8 lineage [10]. Therefore, it is necessary to examine coding region mutations to determine this haplogroup. These five lineages are not usually found in East Asian populations. Although the available databases including HV1 and HV2 polymorphisms are not ideal to estimate these lineages, the K and U2 haplogroups have been found in the Silk Road region in north China [29], and the U2b haplotype has been found in the Singapore Malay population [3].

The G2a1 lineage is found widely in East Asia, including in Chinese [23-30], Korean [18-21], Japanese [5, 9, 30-35], Central Asian [44], and Southern Chinese Daic populations [37], but not in Southeast Asian populations, such as the Thai [36], Vietnamese [38], Southern Chinese Austro-Asiatic [37], Island Southeast Asian [15], Aboriginal Malay [14, 41], or Singapore Malay populations [3].

Regarding the D haplogroup, the same haplotype as that of the D4a3 lineage has been found in East Asia, including in the Korean (0.9%) [18-21], Thai (0.5%) [36], Southern Chinese Daic (0.4%) and Austro-Asiantic (0.8%) [37], Japanese (0.3%) [5, 9, 30-35], and Chinese populations (0.2%) [22-29], but not in the Vietnamese [38], Island Southeast Asian [15], Aboriginal Malay [14, 41], or Singapore Malay populations [3]. The same haplotype as that of the D4* lineage (Mal-40) has not been found in East [5, 9, 18-35] or Southeast Asia [16, 36-40], including in the Aboriginal Malay [14, 41] or Singapore Malay populations [3]. A similar haplotype to that of the D5'6 lineage (Mal-65) has been found in a Chinese individual of the D6 haplogroup [45].

The 20 samples of macrohaplogroup M type appear unrelated to any established lineage in East Asia [5, 13, 45], Southeast Asia [14, 15, 17, 41], Melanesia [16, 46], or

India [12]. These samples could be classified into at least 17 different haplogroups, of which three kinds of haplotype (Mal-13 and 75, Mal-41 and 110, and Mal-16 and 53) were shared by two individuals each out of the 20 samples. These unclassified M* types suggest the presence of various very old lineages in the modern Malay population.

N9a1 is sporadically found in East Asia [5, 9, 18-35, 37], but has almost never been found in the Thai [36], Vietnamese [38], Island Southeast Asian [15], Aboriginal Taiwanese [39], Singapore Malay [3], or Aboriginal Malay [14, 41] populations. In contrast, the N9a6 designated by Hill et al. [14] has been found only in the Vietnamese (2.7%) [38], Island Southeast Asian (1.1%) [15], Thai (0.5%) [36], and Malay populations [3, 14, 41]. In the Malay population, N9a6a, which further shares the 16294 mutation, is commonly observed and found in 6.5% of the Aboriginal Malay and 3.5% of the Singapore Malay populations [3, 14]. The Y2 haplogroup is also observed in the Island Southeast Asian (2.9%) [15], Singapore Malay (1%) [3], and Aboriginal Taiwanese populations (1.1%) [39, 40]. The Y2 lineage is proposed to come from some Neolithic movement from the Philippines [15].

The present N21 haplotype is rare and has been found in only one sample of Sumatrans [15]. The N22 haplogroup is also very rare and has been found only in Sumba island Indonesian (8.0%) [15], Aboriginal Malay (1.5%) [14, 41], and Vietnamese populations (0.5%) [38].

B and F are major haplogroups in the present population. Haplogroup B is widely distributed throughout Continental East Asia, Island Southeast Asia, Melanesia, Micronesia, and Polynesia. Among six kinds of B haplogroups, B4c1b3 was the most common. Although this lineage is widely distributed but not frequent throughout East Asia, including in the Japanese [5, 9, 30-35], Korean (0.1%) [18-21], Taiwanese Han (0.7%) [22], Daic (1.0%) and Southern Chinese Austro-Asiatic (0.8%) [37], Continental Southern Chinese (1.7%) [23, 27], Vietnamese (1.6%) [38], and Singapore Malay populations (0.5%) [3], it is comparatively frequent in the Aboriginal Taiwanese (5.5%) [39] and Island Southeast Asian populations (2.8%) [15]. The B4c2 lineage was also comparatively restricted to the Island Southeast Asian (2.2%) [15], Singapore Malay (2.5%) [3], Thai (2.4%) [36], and Southern Chinese Daic (0.8%) and Austro-Asiatic (2.4%) populations [37], but was rare or not found at all in the East Asian [5, 9, 18-35], Vietnamese [38], Aboriginal Taiwanese [39], and Aboriginal Malay populations [14, 41]. The other B lineages are common haplogroups and are distributed widely in East and Southeast Asian populations [3, 5, 9, 15, 18-39], and some of them (B4a) even further into Oceanian populations [16, 40].

Among the four kinds of the present F haplogroup, F1a was very frequent in all East, Continental Southeast, and Island Southeast Asian populations [3, 5, 9, 15, 18-40], but F1a1a was relatively restricted to Continental Southeast and Island Southeast Asia populations [3, 22, 23, 26, 27, 36-38], including Aboriginal Malays [14, 41]. F1a2 seems to be a rare lineage in East and Southeast Asian populations [3, 5, 21, 24, 27, 29, 32, 34, 36-39], and F1a3 is also not frequent in East and Southeast Asian populations [5, 9, 18-39], but is more frequent in Island Southeast Asian (2.3%) and Singapore Malay populations (2.0%) [3, 15].

In the two types of the Southeast Asian R haplogroup, the R22 lineage was mainly found in the Island Southeast Asian population (2.3%) [15], but has rarely been found in other Southeast and East Asian populations [3, 5, 9, 18-35, 37, 39, 40]. It is reported that R9b diversified in Indochina and then spread southward into the Malay Peninsula [14]. R9b is most frequent in the Aboriginal Malay population (9.6%) [14, 41], and is distributed mainly among continental Southeast Asian populations, such as the Southern Chinese Daic (4.4%) and Austro-Asiatic (3.2%) [37], Vietnamese (2.1%) [38], and Thai populations (1.4%) [36]. It is further found in some parts of Island Southeast Asia (0.9%) [15] and in Singapore Malays (1.0%) [3], but it is almost never found in East Asia [5, 9, 18-27, 29-35, 39].

Finally, the African haplogroup L2a was found. Because a very similar control region haplotype is found in African American individuals [47], it is not clear whether this haplotype represents an early expansion from Africa.

In the present study, we analyzed control region polymorphisms and haplogroup lineages in modern Malay individuals living in or around Kuala Lumpur, Malaysia. The results demonstrated that modern Malay individuals of this population consisted of characteristic Aboriginal Malay haplogroups (M21a, M21b, M22, and N21), Southeast Asian haplogroups (M7c with 16295, E1a, E1b, E2, M12, N9a6a, Y2, N22, B4c1b3, B4c2, F1a1a, R22, and R9b), East Asian haplogroups (M7b, M7b1, C, G2a1, D4a3, N9a1, B4a, B4b1a, B4b1, B5a, B5b, F1a, and F1a3), Indian haplogroups (M3, K, U2b, R30, and R8), a Melanesian haplogroup (Q1), an African haplogroup (L2a), and unclassified haplogroups (M*, D*, and D5'6). The high frequency of the M* haplogroup lineages, which probably diverged in a deep node of the M* lineage, in particular, suggests a highly diverse and unique nature for the modern Malay population. The results demonstrate the complexity of the modern Malay population, and the present database will be useful not only for personal identification in forensic casework in Malaysia, but also as an aid for estimation of geographic origin in forensic casework in East and Southeast Asian populations.

Acknowledgements We would like to thank Associate Professor Jeremy Williams, Laboratory of International Dental Information, Tokyo Dental College, for editing this manuscript. This research was partially supported by the Ministry of Education, Science, Sports and Culture; Grant-in-Aid for Scientific Research (B) (14370701); and Grant-in-Aid for Young Scientists Research (B) (29791646).

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