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TECHNICAL NOTE

CRIMINALISTICS

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Study of the Cytochrome *b* Gene Sequence in Populations of Taiwan

ABSTRACT: The cytochrome *b* gene (MTCYB) has been widely used in taxonomic research. In this study, the sequence polymorphism of the MTCYB gene was determined in 417 subjects of eight populations living in Taiwan (Taiwanese Han, indigenous Taiwanese, Tao, mainland Chinese, Filipino, Thai, Vietnamese, and Caucasian). Sequence variation from the revised Cambridge Reference Sequence and genetic distance between these populations were analyzed. There were 108 variable positions with a total of 99 haplotypes. Population-specific positions of MTCYB gene were noted in Tao and Caucasian populations. There were statistically significant differences of genetic distance between Taiwanese Han and Caucasian, between Taiwanese Han and Tao, and between Taiwanese Han and Filipino. A phylogenetic tree presents the genetic distances between these populations. In conclusion, there are sufficient sequence polymorphisms of the MTCYB gene in individuals of different populations, which may be used in the analyses of human ethnic groups in forensic casework.

KEYWORDS: forensic science, forensic anthropology, mitochondrial DNA, MTCYB gene, sequence polymorphism, genetic distance, phylogenetic tree

Polymerase chain reaction (PCR)-based analysis of human mitochondrial DNA (mtDNA) has become a useful tool in forensic genetics because of its high copy number, high levels of diversity, haploid maternal inheritance, and lack of recombination (1,2). Many databases of hypervariable region 1 (HV1) and hypervariable region 2 (HV2) sequences of the noncoding region of the mtDNA have been established for forensic casework, ethnic study, and geographic research (3–7). In forensic casework, the significance of mtDNA matching requires comparison to a large mtDNA sequence database in order to determine the relative rarity of the particular case, because of the characteristics of single-linked unit mtDNA. Extending the mtDNA typing database can increase the size of the database and elevate the strength of mtDNA evidence (8).

It has been recognized that assessing variations occurring outside of the HV1/HV2 portions of the control region can provide additional forensic discrimination and augment the sometimes relatively limited forensic power of mtDNA testing (9–11). The cytochrome *b* (MTCYB) gene within the mitochondrial genome, spanning about 1140 bp, has undergone several changes during evolution (12). The MTCYB gene has been used in species identification and phylogenetic studies (13–16). As a possible individual identification marker, subgrouping the MTCYB gene according to different sequences has been discussed (17).

The population in Taiwan is heterogeneous and is made up of two major populations: an indigenous population (1.9%) and a Han population (98.0%) from mainland China. The indigenous population is comprised of twelve tribes (Amis, Atayal, Bunun, Kavalan, Paiwan, Rukai, Puyuma, Saisiyat, Sakizaya, Thao, Truku, and Tsou), living mainly in the mountainous region of Taiwan Island, and a specific tribe (Tao) living on Orchid Island (18). The Taiwanese Han population includes the descendants of individuals who migrated from mainland China (mainly from Fukien and Guangdong Provinces) in a number of waves of immigration from 400 years age to the early 1900s, and a large-scale migration from all mainland China areas in 1949. There is a high degree of admixture and interbreeding between these two groups of Han populations (5,19). There are also populations from other countries throughout the world currently living or working in Taiwan.

The aim of this study was to discover the sequence polymorphisms of the MTCYB gene among populations living in Taiwan, analyze the genetic distances between these populations, and evaluate the polymorphic pattern for its forensic application.

Materials and Methods

This retrospective study was approved by the Institute Review Board. A total of 417 DNA samples from unrelated, outwardly healthy individuals working or living in Taiwan were analyzed. These samples were obtained from 71 Taiwanese Han individuals (Tw), 149 indigenous Taiwanese of Taiwan Island (Ti) (34 Amis, 39 Bunun, 35 Atayal, and 41 Paiwan), 40 Orchid Islanders (Tao), 56 mainland Chinese (Cn), 35 Filipinos (Ph), 20 Thais (Th), 22

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TABLE 1—*Sequences of primers used for mitochondrial DNA amplification and sequencing.*

Primer	Sequences
L14724*,†	CGAAGCTTGATATGAAAAACCATCGTTG
H15915*	AACTGCAGTCATCTCCGGTTTACAAGAC
L15182†	TGAGGACAAATATCATTCTGAGGGGCTACAGTTA
H15149†	TAACTGTAGCCCCTCAGAATGATATTTGTCCTCA

Primers are numbered according to the location of the 3′-ends in the reference sequence. L and H designate the light and heavy strands of the mitochondrial DNA, respectively.
*For PCR amplification
†For DNA sequencing

Vietnamese (Vn), and 24 Caucasians (Cau). Caucasian samples were collected from individuals from the U.S.A. (6), the U.K. (5), Australia (3), Peru (2), Jordan (2), France (1), Germany (1), New Zealand (1), Brazil (1), Egypt (1), and Syria (1).

The blood samples and buccal swab samples were obtained between 1993 and 2007 from volunteer donors with informed consent. Standard methods of phenol–chloroform isoamyl alcohol extraction were used for DNA extraction from peripheral whole blood samples, whereas the QIAamp DNA Mini kit (Qiagene, Valencia, CA) was used for DNA extraction from buccal cells.

The primer pairs for PCR amplification, as described by Kocher et al. and Irwin et al. are listed in Table 1 (15,20). The primers were numbered according to the revised Cambridge reference human mtDNA sequence (rCRS) (21,22). PCR amplification was performed in 50 μL of reaction mixture, which contained 30 ng genomic DNA, reaction buffer (10 mM Tris–HCl, pH 8.3, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% gelatin; Genemark, Taipei, Taiwan), 1 unit of GeneTaq DNA polymerase (Genemark), and 0.15 μM each of primers (L14724, H15915). Amplification was conducted in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA) with the following conditions: initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec, then 72°C for 10 min for further extension. Cycle sequencing of PCR products was also conducted in an ABI 2700 or 9700 thermal cycler with the following conditions: 25 cycles of 95°C for 30 sec, 50°C for 30 sec, and 60°C for 4 min. Sequencing was performed using the primers L14724, L15182, and H15149, and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The DNA sequence was detected with an ABI 3730 DNA sequencer.

Sequences were aligned using the Pile Up program of the GCG computer package (Wisconsin Package, Version 10.3; Accelrys Inc., San Diego, CA). The discrimination powers were calculated. All of the sequences were compared with the rCRS. The genetic distances among different populations were explored by the analysis of molecular variance implemented in Arlequin (http://cmpg.unibe.ch/software/arlequin3) (23). A difference with a *p*-value <0.05 was taken as statistically significant. The phylogenetic tree was constructed by the neighbor-joining methods using the PHYLIP program (24), and visualized by use of Tree View software (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

TABLE 2—*The gene diversity, nucleotide diversity, and discrimination power noted in these populations.*

Population	Gene Diversity	Nucleotide Diversity	Discrimination Power
Ti	0.9254 ± 0.0082	0.0027 ± 0.0016	0.9192
Tao	0.8090 ± 0.0275	0.0025 ± 0.0015	0.7888
Vn	0.8442 ± 0.0582	0.0024 ± 0.0015	0.8058
Th	0.9316 ± 0.0347	0.0027 ± 0.0016	0.8850
Ph	0.9496 ± 0.0174	0.0035 ± 0.0020	0.9224
Cau	0.9565 ± 0.0220	0.0033 ± 0.0019	0.9167
Cn	0.8864 ± 0.0342	0.0028 ± 0.0016	0.8705
Tw	0.9018 ± 0.0240	0.0028 ± 0.0016	0.8891

Ti, Indigenous Taiwanese of Taiwan Island; Tao, Tao of Orchid Island; Vn, Vietnamese; Th, Thai; Ph, Filipino; Cau, Caucasian; Cn, Mainland Chinese; Tw, Taiwanese Han.

Results

The sizes of PCR products were 1190 bp in all samples and were confirmed by DNA sequence analysis. Of these products, the MTCYB gene comprised *c*. 1140 bp from mtDNA position 14747–15886, excluding the primer sequences. The 1140-bp fragment of the MTCYB gene was used as a marker for ethnic group diversity evaluation. Heteroplasmy was not observed in any of these samples.

The diversity of the MTCYB gene for the samples examined was assessed (Table 2). The percentage range of gene diversity in the populations in this study was from 0.8090 (of Tao) to 0.9565 (of Caucasian). Discrimination powers of the MTCYB gene in the eight populations ranging from 0.7888 (Tao) to 0.9224 (Filipino) were observed.

The sequence variation from the rCRS is shown in Table S1. None of these 417 samples was the same as the rCRS at positions 14747–15886. The distribution of varied sequences from the rCRS was from 1 to 9 positions, with an average of 4.26. Deletion or insertion was not found in these samples. The 417 samples presented a total of 108 variable positions with 99 haplotypes. There were 66 silent polymorphisms. All sequenced samples except one revealed an A-to-G transition at position 15326. Other positions with higher frequency that varied from the rCRS among all these populations were 14766T (391/417), 14783C (190/417), 15043A (189/417), and 15301A (206/417). The most common haplotype (haptotype 5 in Table S1) accounts for 65 of the 417 samples. Most of the varied site frequency was lower than 2%. There were 49 positions with only one occurrence (1/417) and 20 positions with two occurrences (2/417) out of 108 positions. We did not find

TABLE 3—*Population-specific polymorphism positions compared to the revised Cambridge Reference Sequence (rCRS).*

			Frequency of Individuals with the Transition							
rCRS Position	Nucleotide Change	Amino Acid Change	Ti (149)	Tao (40)	Vn (22)	Th (20)	Ph (35)	Cau (24)	Cn (56)	Tw (71)
14783	T → C	L13L	0.53	0.45	0.4545	0.45	0.3429		0.5536	0.4366
14798	T → C	F15L						0.2083		
15043	G → A	G99G	0.51	0.45	0.4545	0.45	0.3429	0.0833	0.5536	0.4366
15301	G → A	L185L	0.55	0.675	0.4545	0.45	0.4286		0.5714	0.4366
15777	G → A	S344N		0.25						

Ti, Indigenous Taiwanese of Taiwan Island; Tao, Tao of Orchid Island; Vn, Vietnamese; Th, Thai; Ph, Filipino; Cau, Caucasian; Cn, Mainland Chinese; Tw, Taiwanese Han.

TABLE 4—*Genetic distances between ethnic groups in this study.*

	Ti	Tao	Vn	Th	Ph	Cau	Cn	Tw
Ti	–							
Tao	0.0892*	–						
Vn	0.0186	0.0639	–					
Th	0.0089	0.0695	−0.0177	–				
Ph	0.0648*	0.0863*	0.0735	0.0426	–			
Cau	0.1484*	0.2034*	0.1890*	0.1181*	0.0836*	–		
Cn	0.0464*	0.0575*	−0.0172	−0.0030	0.0582*	0.1816*	–	
Tw	0.0053	0.0614*	−0.0126	−0.0185	0.0405*	0.1337*	−0.0045	–

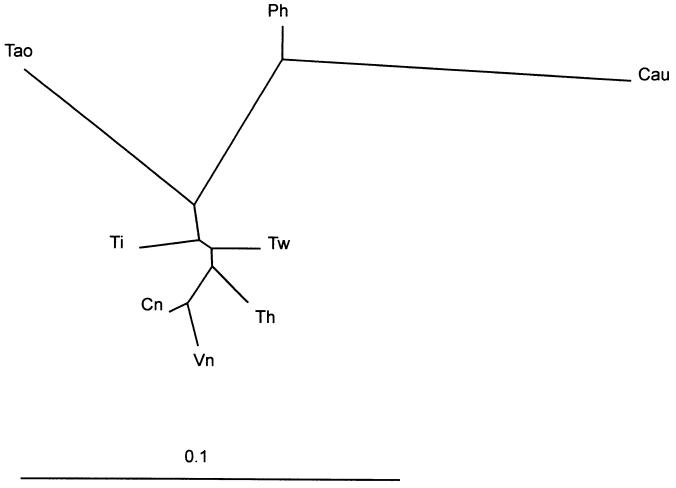
Ti, Indigenous Taiwanese of Taiwan Island; Tao, Tao of Orchid Island; Vn, Vietnamese; Th, Thai; Ph, Filipino; Cau, Caucasian; Cn, Mainland Chinese; Tw, Taiwanese Han.

*p-Value of coancestry coefficient (F_{st}) <0.05.

any hypervariable regions within this gene, because the sequence polymorphisms were evenly distributed from 6 to 14 positions in every 100 bp sequence.

The population-specific polymorphisms are presented in Table 3. The G-to-A transition at nucleotide 15777 was specific to the Tao group, and the T-to-C transition at nucleotide 14798 was specific to the Caucasian population in this study.

The result of analyses of the genetic distances between the populations shows that the Taiwanese Han had a statistically significantly large genetic distance from Caucasians, followed by the Tao and Filipino groups (Table 4). The MTCYB DNA sequences of the Caucasians were significantly different from those of any other group. Figure 1 illustrates the phylogenetic tree of these populations using the genetic distance in Table 4. The neighbor-joining tree groups the Taiwanese Han, indigenous Taiwanese of Taiwan Island, mainland Chinese, Thais, and Vietnamese in the same cluster. The longest branch in the dendrogram is from the Caucasian population. There is another long branch of dendrogram from the Tao population.



Ti: Indigenous Taiwanese in Taiwan Island
Tao: Tao in Orchid Island
Vn: Vietnamese
Th: Thai
Ph: Filipino
Cau: Caucasian
Cn: Mainland Chinese
Tw: Taiwanese Han

FIG. 1—*Phylogenetic tree based on the matrix of the Nei standard genetic distance for the sequences of the MYCYB gene constructed by the neighbor-joining method.*

Discussion

Analysis of mtDNA is a valuable tool in forensic identification and ethnic study. In addition to HV1 and HV2, two mtDNA-coding region segments (10171–10659 and 14055–14590) have been observed to be very informative for East Asian mtDNA haplogroup classification (7,25). However, these two coding regions provide limited information for the further characterization of some common haplogroups in East Asians. Polymorphisms in mtDNA region 14576–16047 serve to further classify the major haplogroups in East Asia (7). In this study, we analyzed the polymorphisms of mtDNA positions 14747–15886, covering the whole MTCYB gene in eight populations. The comparison of the mtDNA sequences of the ethnic populations could reflect their matrilineal ethnohistory.

The sequence polymorphism of MTCYB gene in individuals of different populations was demonstrated and some possible specific positions among the populations were found in this study. The finding of relatively low gene diversity of Tao population and relatively high gene diversity of Caucasian population is consistent with the isolated small population of Tao in a small island and the diversity of the Caucasian population (Table 2). The A-to-G transition at position 15326 was most common and was revealed in almost all subjects, which confirmed the sequence difference reported by Brown et al. and Howell et al. (Table S1). As this substitution changes a nonconserved amino acid only, it was unlikely to be functionally significant (26,27). The second common variation from rCRS was 14766T. The thymine at nucleotide 14766 has been reported in the original CRS (21). The other common variations from rCRS, 14783C, 15043A, and 15301A, were silent polymorphisms. The G-to-A transition at 15777 positions specific to the Tao population induces an amino acid change (Serine to Asparagine) of the cytochrome b protein at a nonconserved region in *Spratelloides delicatulus*, *Neoceratodus forsteri*, and *Elaphodus cephalophus* (http://www.ncbi.nlm.nig.gov/blast/Blast.cgi). Therefore, it may be taken as not significant functionally. The T-to-C transition at nucleotide 14798 specific to the Caucasian population changes a Phenylanine to Leucine in a conserved domain of cytochrome b protein. However, it is a known polymorphism without primary pathogenic significance (21,28). The 15777A and 14789C could serve as single-nucleotide polymorphism markers for Tao and Caucasian populations, respectively. The 14783T-15043G-15301G as described in the rCRS was noted in 91.66% (22/24) of Caucasian, whereas it was detected in only 47.58% (187/393) of Asian individuals in this dataset (Table 3). The relative ratio of the 14783T-15043G-15301G polymorphism of Caucasian was 1.93 in this study. Therefore, the 14783T-15043G-15301G polymorphism may also be a potential ethnic classification marker.

In this study, the genetic distance was analyzed according to MTCYB gene sequences. The values of genetic distances between two populations represent the evolutionary distance or the historical migration route. There was no statistically significant difference between Taiwanese Han and indigenous Taiwanese of Taiwan Island, and between Taiwanese Han and mainland Chinese. However, there was a significant difference between mainland Chinese and indigenous Taiwanese. Taiwanese Han may be a matrilineal combining population of both mainland Chinese and indigenous Taiwanese. It is consistent with the migration history of Taiwanese Han population from the mainland and subsequent interbreeding among indigenous Taiwanese women and Taiwanese Han men,

because the Qing Dynasty government of China prohibited women from migrating from mainland China to Taiwan from 1683 to 1875 (29). Therefore, this phylogenetic tree is different from the previous report of a population study of diversity of HLA among Taiwanese populations (30), because the matrilineality and patrilineality of Taiwanese Han may be different. The MTCYB sequences of the Caucasians were significantly different from the Asian populations, and there was a long branch from the Caucasian population in the phylogenetic tree. These findings present the long genetic distance between Caucasian and Asian populations. The other long branch of dendrogram from the Tao group illustrates the geographical and genetic isolation of this tribe in Orchid Island.

In conclusion, there were sufficient sequence polymorphisms of the MTCYB gene in individuals of different populations. Analysis of the sequence of the MTCYB gene may be valuable for the classification of human ethnic groups and identification of maternal origin in forensic casework.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Distribution of haplotypes of MTCYB among populations in this study compared to the revised Cambridge Reference Sequence. (Nucleotide position in boldface indicates a consequent amino acid change.)

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