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Analysis of the cytochrome b gene in Taiwanese populations

Li-Chin Tsai,[1]Ph.D.; James Chun-I Lee,[2,3]Ph.D.; Apollo Chun-Yen Lin,[1,3]M.S.; Cheng-Hsien Su,[1,4]B.S.; Szu-Jung Chen,[5]B.S.; Hsing-Mei Hsieh,[1,*]Ph.D.

[1] Department of Forensic Science, Central Police University, 56 Shu Jen Road, Taoyuan 33334, Taiwan ROC.
[2] Department of Forensic Medicine, College of Medicine, National Taiwan University, 1 Jen-Ai Road Section 1, Taipei 10051, Taiwan ROC.
[3] Institute of Forensic Medicine, Ministry of Justice, No. 166-1 Keelung Road Section 2, Taipei 10675, Taiwan ROC.
[4] Forensic Division of Taoyuan County Bureau, 3 Shiann Fuu Road, Taoyuan City, Taoyuan 33053, Taiwan ROC.
[5] Department of Bioengineering, Tatung University, 40 Chungshan North Road 3rd Section, Taipei 10452, Taiwan ROC.

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Abstract

In this study, 250 samples, 25 samples from each of the nine tribes (Amis, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tsou and Yami) and Han population of Taiwanese, were collected and the polymorphisms of partial sequence of cytochrome b gene were analyzed for forensic purposes. Totally there were 9 sequence types and 9 variation sites in 402 bp. All the types but Type6 completely matched the sequence of human cytochrome b gene registered in EMBL databank. There was 2 base difference with the sequence registered in databank for Type6. Type1 and Type2 existed in all the tribes and Han population and composed 85.2 % samples. Type4, Type6 and Type7 existed only in Tsou tribe, and Type5 only in Amis tribe. Although the polymorphisms of cytochrome b gene were observed in Taiwanese populations, however, more samples must be analyzed to investigate the unique distribution for forensic applications.

Keywords: cytochrome b, Taiwanese population, sequence variation, forensic application

Introduction

Mitochondrial cytochrome b gene is widely used in species identification, evolution analysis and phylogenetic studies [1-10]. Furthermore, the cytochrome b gene was evaluated to be used as a candidate for individual identification on forensic application when conventional STR typing is unavailable based on the nature of its changes during evolution. Lee SD et al. reported that a total of 30 polymorphic sites were found evenly distributed along cytochrome b gene in 98 unrelated Koreans [11]. Mishmar D et al. also reported that natural selection shaped regional mtDNA variation in humans and attributed to genetic drift [12]. Mitochondrial DNA sequence polymorphisms of five ethnic populations from northern China were reported by Kong QP et al., the polymorphisms of cytochrome b sequence were found to be very informative for defining the haplogroup status of East Asian mtDNAs [13].

There are two major populations living in Taiwan, one is Han population and the other is indigenous population. Han population included plain people in Taiwan and the immigrating Chinese population. The indigenous population included thirteen tribes, twelve (Amis, Atayal, Bunun, Kavalan, Paiwan, Puyuma,

* Corresponding author, e-mail: mei@mail.cpu.edu.tw

Rukai, Saisiat, Sakizaya, Taroko, Thao and Tsou) of them living in the mountains or the eastern plain of Taiwan and the only other tribe (Yami tribe) living in Orchid island. The four tribes of Kavalan, Sakizaya, Taroko and Thao are named during recent years. In this study, samples from the Taiwanese populations were collected and the polymorphisms of partial sequence (402 bp) of cytochrome b gene were analyzed to show the distribution variations between these tribes or populations. Unique sequence observed only in a given tribe or population will be valuable for investigation of evidence origin on forensic applications.

Materials and methods

Sample sources

The blood samples from 250 unrelated healthy males were randomly collected with informed consent. Twenty-five samples were collected from each of the 9 aboriginal tribes (Amis, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tsou and Yami) and Han population respectively. The parents of each collected sample were from the corresponding tribe respectively. DNA was extracted with salt-chloroform method [14] and quantified with the QuantiBlot kit (Roche Molecular Systems, Alameda, Calif.).

PCR amplifications and DNA sequencing

The primers adopted in this study were designed according to the report of Irwin et al. [1]. The sequences of primers L14724 and H15149 are 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' and 5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3' respectively. PCR amplifications were performed in a reaction mixture of 50 µl, which contained about 10 ng of isolated genomic DNA, 0.15 µM each of primers, reaction buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl2, 50 mM KCl, 0.01%(w/v) gelatin), 200 µM dNTP, and 1 unit of VioTaq DNA polymerase (Viogene, Taipei, Taiwan). The PCR amplifications were performed for 35 cycles according to the program of 94 ℃ for 1 minute, 50 ℃ for 1 minute and 72 ℃ for 2 minutes, and extension at 72 ℃ for 10 minutes in a thermal cycler (GeneAmp PCR System 2400, Applied Biosystems, Foster, CA, USA).

Sequencing of the PCR products was performed using the L14724 primer or H15149 primer and the BigDye™ Terminator Kit (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems). The cycle sequencing products were separated with the polyacrylamide gel POP-7 and detected by an ABI 3730 Genetic Analyzer (Applied Biosystems).

Sequence analysis

Sequences were compared with those registered in EMBL databank by the Fasta program through the website (http://www.ebi.ac.uk/fasta33/nucleotide.html). Different type sequences were aligned and the consensus sequence was deduced by the PileUp and Pretty programs of GCG software (GCG Version 11.1, Accelrys Inc., San Diego, CA).

Results and discussion

DNA from all samples were amplified successfully and sequenced. The 250 sequences were classified as 9 sequence types, named from Type1 to Type9. These 9 type sequences were compared with those registered in EMBL databank, and the accession no. and similarity of the most similar sequence for each sequence type were showed in Table 1. All the types but Type6 completely matched the sequence of human cytochrome b gene registered in databank. Sequence of Type6 was novel sequences for human cytochrome b gene. There was 2 base difference in 402 bp with the sequence registered in databank (accession no. AY255154). Different type sequences were aligned and the consensus sequence was deduced by the PileUp and Pretty programs of GCG software. Totally there were 9 variation sites in 402 bp. It showed that the polymorphisms of cytochrome b sequence were informative and was concordant with the report by Kong QP et al. [13]. The sequence variations were showed in Table 2 and compared with the sequence reported by Anderson S et al. [15]. Type1 sequence was the same with Anderson sequence, and there were from 1 to 6 nucleotide variations for the other sequence types. There were 1, 2, 3, 4 and 6 variations observed for Type4/Type5/Type8, Type2, Type3/Type9, Type7 and Type6 respectively. For nucleotide 14783, only three sequence types (Type1, 4 and 8) were with the same base (T) as the Anderson sequence. The variations between these sequence types were all nucleotide substitutions and no

insertions or deletions. The results were concordant with the report of Lee SD et al. [11].

Type distribution of the nine tribes (Amis, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tsou and

Table 1. Accession no. and similarity of the most similar sequence in EMBL databank for each sequence type.

Sequence type	Accession no.	Similarity
Type1	AP008385	100.0 %
Type2	AY255150	100.0 %
Type3	AY922308	100.0 %
Type4	DQ112744	100.0 %
Type5	DQ462234	100.0 %
Type6	AY255154	99.5 %
Type7	AY255178	100.0 %
Type8	DQ372869	100.0 %
Type9	AY289097	100.0 %

Table 2. Sequence variations of the nine sequence types.

Sequence Type	Nucleotide								
	14766	14783	14869	15040	15043	15046	15071	15106	15109
Anderson	T	T	G	C	G	A	T	G	T
Type1	·	·	·	·	·	·	·	·	·
Type2	·	C	·	·	A	·	·	·	·
Type3	C	C	·	·	A	·	·	·	·
Type4	·	·	·	·	·	·	·	A	·
Type5	·	C	·	·	·	·	·	·	·
Type6	·	C	A	T	A	·	C	·	C
Type7	·	C	·	T	A	·	C	·	·
Type8	·	·	·	·	·	G	·	·	·
Type9	·	C	·	T	A	·	·	·	·

* Nucleotide numbering is according to the human mtDNA sequence [15]. The symbol of '·' represents the same base as the Anderson sequence.

Yami) and Han population was summarized in Table 3. Number of sequence types ranged from 2 to 6 for each tribe or population. There were 6 sequence types in Tsou tribe. Type1 and Type2 existed in all the tribes and Han population. Most of the samples (213 in 250) were of these two types, and the rate was 85.2 %. Rates for the other types were 8.0 % (Type3), 0.4 % (Type4, Type6 and Type7), 0.8 % (Type5), 3.2 % (Type8) and 1.6 % (Type9) respectively. There was only one sample for each type of Type4, Type6 and Type7. All these three types existed only in Tsou tribe, and Type5 only in Amis tribe, however, the sample number was only 1 (for Type4, Type6 and Type7) and 2 (for Type5). Six types, from type4 to type9, were only observed in the indigenous population. From analysis of the distribution frequency of 9 variation sites in each tribe or population, the results showed that nucleotides 14869A, 15071C, 15106A and 15109C were observed only in Tsou tribe (Table 4). However, more samples must be collected and analyzed to investigate the unique sequences observed only in a given tribe or population and to determine the evidence origin for criminal investigation.

In this study, partial cytochrome b gene of 250 samples from the indigenous and Han populations

Table 3. Sample no. of the nine tribes and Han population for different sequence type.

Tribe or population	Sequence Type								
	Type1	**Type2**	**Type3**	**Type4**	**Type5**	**Type6**	**Type7**	**Type8**	**Type9**
Amis	10	13			2				
Bununs	17	2	6						
Puyuma	10	7	7					1	
Tsou	13	7	2	1		1	1		
Rukai	11	14							
Paiwan	15	5						5	
Saisiat	10	8	3					1	3
Atayal	9	14						1	1
Yami	14	11							
Han	11	12	2						
Total	120	93	20	1	2	1	1	8	4
Rate (%)	48.0	37.2	8.0	0.4	0.8	0.4	0.4	3.2	1.6

Table 4. Distribution frequency of 9 variation sites in each tribe or population.

	Nucleotide								
	14766	**14783**	**14869**	**15040**	**15043**	**15046**	**15071**	**15106**	**15109**
Non Anderson sequence	C	C	A	T	A	G	C	A	C
Amis	0	60	0	0	52	0	0	0	0
Bununs	24	32	0	0	32	0	0	0	0
Puyuma	28	56	0	0	56	4	0	0	0
Tsou	8	44	4	8	44	0	8	4	4
Rukai	0	56	0	0	56	0	0	0	0
Paiwan	0	20	0	0	20	20	0	0	0
Saisiat	12	56	0	12	56	4	0	0	0
Atayal	0	60	0	4	60	4	0	0	0
Yami	0	44	0	0	44	0	0	0	0
Han	8	56	0	0	56	0	0	0	0

* Nucleotide numbering is according to the human mtDNA sequence [15]. Non-Anderson sequence represents the sequence different from the Anderson sequence. Distribution frequency is recorded as the rate (%) of each respective base.

of Taiwanese was analyzed and totally there were 9 sequence types observed. Type4, Type6 and Type7 existed only in Tsou tribe, and Type5 only in Amis tribe. Although the polymorphisms of cytochrome b gene were observed in Taiwanese populations, however, more samples must be analyzed to investigate the unique distribution for forensic applications.

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