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Molecular Analysis of the Two Hypervariable Regions of the Mitochondrial DNA in Taiwanese

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Running title: Analysis of mtDNA HV1 and HV2 in Taiwanese

Abstract

The control region of the human mitochondrial DNA (mtDNA) has two hypervariable regions, designated as HV1 and HV2 respectively. In order to elucidate the genetic heterogeneity of HV1 and HV2 in ethnic Chinese Hans in Taiwan, we have collected blood samples from 310 unrelated individuals for this study. With polymerase chain reaction and direct DNA sequencing, 372 nucleotides from position 16028 to 16400 in HV1 and 344 nucleotides from position 65 to 409 were analyzed. As Anderson sequence as a reference, all of our subjects had 73t, 268g, and 311.1c. In addition to these differences, 163 sites of nucleotide substitution, one of single nucleotide deletion and 2 of nucleotide insertion were found. Eighty three percent of nucleotide substitutions involved transitions. 16189c and insertions at 303 were associated with a high percentage of a length heteroplasmy of a poly-cytosine stretch. The mean nucleotide divergence site of combined HV1 and HV2 from Anderson sequence was 9.8 (range from 4 to 19). The ten most common variations were 16223t (45.5%), 16189c (33.2%), 303.1c (27.1%), 16183c (26.0%), 150t (24.8%), 303.2c (24.2%), 16362c (23.5%), 16129a (22.9%), 249Δa (deletion of one adenine) (21.6%), and 16182c (14.8%). After analysis of nucleotide variations, 304 genotypes were detected among the 310 individuals studied, with only 6 genotypes being shared by two individuals respectively. The mtDNA heterogeneity (0.9998) of the HV1 and HV2 regions in ethnic Chinese Taiwanese was very high. The information obtained from this study should be useful in forensic medicine and anthropology.

Key words: Taiwanese, mitochondrial DNA, hypervariable region, human identification, forensic medicine

1. Introduction

Each cell has thousands of copies of mitochondria DNA (mtDNA) which is a circular molecule of 16569 bases in length. The complete mtDNA sequence has been reported [1] and its control region has two hypervariable regions (HV1 and HV2) which have a high nucleotide substitution rate [2-4].

MtDNA is inherited through maternal lineage and variations in the HV1 and HV2 sequences have been used in anthropology, human identification and forensic medicine [5-20]. Different ethnic groups may have specific mtDNA compositions. In forensic medicine, analysis of mtDNA is especially useful because of the high copy number of the genome in the cells and thus an easier amplification of mtDNA. Therefore in difficult conditions of human identification, such as scanty evidential material and decomposed corpse, when nuclear DNA is minimal in amount or is severely degraded, analysis of mtDNA may offer important clues. Clinical casework has been reported from mtDNA analysis from hair shafts, bones, teeth, and severely decomposed body parts [5,9,10,12,13].

In Asians, HV1 and HV2 sequences have been reported from Japanese and Koreans [21,22]. In this study, we report the heterogeneity of mtDNA HV1 and HV2 regions in Taiwanese (ethnic Chinese Hans).

2. Materials and Methods

A total of 310 unrelated individuals were recruited for this study. These individuals came to the hospital for various reasons, such as health examination, thalassemia screening, cytogenetic diagnosis, or prenatal check-ups. DNA was obtained from peripheral blood using either phenol-chloroform extraction or a purification kit (Puregene, Minneapolis, MN, USA). To amplify HV1 region, we used a forward primer HV1F(5'-ctccaccattagcacccaaagc-3', nucleotide 15977 to 15999)

and a reverse primer HV1R (5'-gatttcacggaggatggtggtc-3', nucleotide 16400 to 16422) to obtain a DNA fragment of 446 basepairs. For HV2, we used a forward primer HV2F (5'-ccactcacgggagctctccatg-3', nucleotide 26 to 47) and a reverse primer HV2R (5'-tgactgttaaaagtgcataccgcc-3', nucleotide 410 to 433) to obtain a DNA fragment of 407 basepairs [1].

Polymease chain reaction (PCR) was performed for 35 cycles in a 50 μL reaction in a programmable thermocycler (Perkin Elmer-Cetus 9600, CA, USA). For each reaction, 20 ng of DNA was mixed with 10 mM Tris-HCl (pH 8.3); 50mM KCl; 1.5 mM $MgCl_2$; 200 μM each of dNTPs; 10 pmoles each of forward and reverse primers; and 1 unit of AmpliTaq DNA polymerase (Perkin Elmer-Cetus, CA, USA). Conditions for cycle reaction were: 94°C for 45 seconds for DNA denaturing, 53°C for 45 seconds for annealing and 72°C for 60 seconds for extension. The last cycle for extension was 72°C for 7 minutes. The DNA fragments were sequenced with HV1F or HV2F respectively. For cycle sequencing, we used fluorescence-labeled BigDye terminator (Perkin Elmer Cetus, CA, USA) method and a semiautomated DNA sequencer (Perkin Elmer-Cetus DNA sequencer model 377). Conditions for cycle sequencing, gel running, and base-calling followed the instructions supplied by the respective manufacturers.

Sequences from nucleotide 16028 to 16400 in HV1 and sequences from nucleotide 65 to 409 in HV2 could be defined. In HV1, a transition of thymine to cytosine at nucleotide 16189 often led to a length heteroplasmy of poly-C from nucleotide 16185 to 16193. In HV2, an insertion of one to five cytosines at nucleotide 303 also led to a length heteroplasmy of poly-C from nucleotide 303 to 309. In both cases of heteroplasmy of poly-C tract, a frame-shift phenomenon was observed downstream, therefore, the 3' end sequences were defined with reverse sequencing

using HV1R and HV2R as primers respectively. With HV1 and HV2 combined, sequences of approximately 716 nucleotides could be determined. Nucleotide differences from the Anderson sequence were checked at least twice by two of the authors and then assigned as the genotype of each sample. Uniqueness of the genotype of each sample was compared among each other using a coding system on Micrsoft Excel software and genetic heterogeneity was calculated accordingly [23]. In addition, we analyzed the sites and types of nucleotide substitution in our samples.

3. Results

As compared to the Anderson sequence, all of the samples had three consensus differences: guanine instead of adenine at nucleotide 73, guanine instead of adenine at nucleotide 263, and an insertion of a cytosine at nucleotide 311 (311.1c), resulting in a stretch of 6 cytosines instead of 5 cytosines. In addition to the above differences, 163 nucleotide substitutions were found in the two hypervariable regions. Transitions involving c ↔t or a ↔ g changes were more common, accounting for 83% of sites of nucleotide substitution. In 9 positions, both transitions and transversions were found (Table 1). Deletion involving one adenine was detected at nucleotide position 249, and insertions of one to five nucleotides were noted at nucleotide positions, 301 and 303, respectively. T to c transition at 16189 almost invariably caused a length heteroplasmy of poly-C tract. Insertions of cytosine(s) at position 303 may also be associated with a length heteroplasmy of poly-C tract. The more the inserted cytosine bases, the higher the possibility of a length heteroplasmy. Twenty (23.8%) of the 84 sample with 303.1c had a length heteroplasmy and 31 (41.3%) of the 75 samples with 303.2c had a length heteroplasmy..

Common differences from the Anderson sequences are listed in Table 2. In HV1, 16223t was the most common (45.5% of the genotypes), while in HV2, insertion of a

cytosine at 303 was the most common (52.5%). All nucleotide substitutions of the common types were transitions. In the 310 samples studied, 304 genotypes were detected with only 6 genotypes being shared by two unrelated individuals (the appendix). Thus, the mtDNA heterogeneity in HV1 and HV2 combined was 0.9998 [23].

4. Discussion

Our results showed a very high heterogeneity of HV1 and HV2 regions of mtDNA of Taiwanese. Almost none of the samples shared a single genotype (the appendix). Taiwanese are the descendants of mainland Chinese who immigrated to Taiwan at different times in the past 200 years. China is a very old country and has 1.3 billion people, accounting for more than 20% of the world total population. A very high genetic heterogeneity can be expected.

Nucleotide transitions were the most common changes, followed by transversions, and insertions and deletions (Table 1). These findings have been reported in other series [21,24]. The sequence variations in the mtDNA control regions have been studied with different methods, such as restriction fragment length polymorphism and sequence-specific oligonucleotide hybridizations [6,16,25]. Using restriction fragment length polymorphism, Pai et al. reported 52 haplotypes of mtDNA control region in 152 Taiwanese [6]. The mtDNA heterogeneity found in this study was higher than that in Pai's report. The difference can be explained by a higher discrimination power of DNA sequencing than restriction fragment length polymorphism analysis.

Melton et al. [16] reported mtDNA variations in 28 Taiwanese aborigines. Fifty sites were investigated using oligonucleotide hybridization. Similar to our study, all samples have 73g and 263g. Table 3 compares the 6 common nucleotide substitutions

between this study and the Melton's report. Two common variants, 16189c and 16223t, have a prevalence rate of approximately 40% in both studies. However, 16362t seems more common in the aborigines, while 16129a, 150t and 249 Δa seem more common in Chinese Hans. The Taiwanese aborigines are composed of at least 9 tribes with a current total population of approximately 300000. In comparison, the ethnic Chinese Taiwanese have a population of over 22 million. Because of a limited number of aborigines studied and different methods used in genotyping, it seems inconclusive whether significant differences really exist in the mtDNA HV1 and HV2 regions between Chinese Hans and Taiwanese aborigines.

In a Korean study involving 306 unrelated people, 265 different mtDNA genotypes were detected with 21 genotypes being shared by 2 to 4 subjects. The five most common sites are 16223t (78%), 16362c (39.9%) 73g (99.1%), 263g (98%), and an insertion at 303 [21]. All of our samples had 73g and 263g, while 16223t was present in 45.5% and insertion of at least one cytosine at 303 was present in 52% of the samples. In a study on 100 Japanese, 68 genotypes were found. 16223t was detected in 75.7% and 16362c was found in 50% of the samples [22]. The mtDNA heterogeneity in this study seemed higher than those on Koreans and Japanese. This might be accounted for by an older and bigger population of the Chinese people.

5. Conclusion

The two hypervariable regions of mt-DNA in ethnic Chinese Hans are highly heterogeneous and genotyping of these two regions should be useful in forensic medicine.

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Table 1.Types of nucleotide substitutions at 163 sites

Nucleotide substitution		Number	%
Transition		135	82.8
	c → t	45	27.6
	t → c	38	23.3
	a → g	38	23.3
	g → a	14	8.6
Transversion		19	11.7
	a → c	7	4.3
	c → a	3	1.8
	a → t	4	2.5
	c → g	3	1.8
	g → c	2	1.2
Transition and transversion	t → a/c, a → g/t, c → t/a, c → t/g, t → a/g, g → a/c	9	5.6

Table 2. Ten most common sites of nucleotide substitution in 310 subjects

Position and substitution	No.	%
16129 g → a	71	22.9
16182 t → c	46	14.8
16183 t → c	78	26.0
16189 t → c	103	33.2
16223 c → t	141	45.5
16362 t → c	73	23.5
150 c → t	77	24.8
249 Δ a	67	21.6
303.1 ins c	84	27.1
303.2 ins c	75	24.2

Δ = deletion, ins = insertion

Table 3. Comparison of 6 positions of mtDNA sequences between Chinese Hans and Taiwanese aborigines

Position	Chinese Hans (n = 310)	Taiwanese aborigines (n =28)*
16129a	22.9%	7%
16189c	33.2%	42.9%
16223t	45.5%	42.9%
16362t	23.5%	46.4%
150t	24.8%	10.7%
249 Δa	21.6%	17.9%

Δ = deletion, * Reference no. 16

Legend to the appendix: The complete HV1 and HV2 sequences of the 310 individuals. The numbers in the upper panels indicate the nucleotide position and those in the left-handed column indicate the tested sample numbers. "A" stands for Anderson sequence. Dots indicate the same sequence as that in the Anderson sequence. "cs" indicates a nucleotide substitution with a cytosine and a length heteroplasmy (thus a nucleotide shift in the downstream sequence). "x" indicates a deletion of the nucleotide.