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MITOCHONDRIAL DNA POLYMORPHISM IN JAPANESE LIVING IN HOKKAIDO

Shinji HARIHARA, Momoki HIRAI, and Keiichi OMOTO

Department of Anthropology, Faculty of Science, The University of Tokyo, Tokyo 113, Japan

Summary Restriction enzyme fragment patterns of human mitochondrial DNA (mtDNA) were analyzed using total DNAs from the blood cells of 122 Japanese (Ainu and Non-Ainu) living in Hokkaido, northern Japan. Polymorphisms were detected and the mtDNAs were classified into eleven types using four enzymes, *Ava*II, *Hinc*II, *Hpa*I and *Pvu*II. The distribution of the mtDNA morphs was not significantly different between the Ainu and the Non-Ainu samples, supporting the views that the Ainu are genetically one of the Mongoloid populations.

INTRODUCTION

Human mtDNA is a 16.5 kilo base (kb) circular molecule which has been completely sequenced and its gene composition analyzed (Anderson *et al.*, 1981; Chomyn *et al.*, 1985). Since the pioneer work by Brown (1980), polymorphism of human mtDNA has attracted a great deal of attention of human population geneticists and anthropologists (Brown, 1980; Denaro *et al.*, 1981; Blanc *et al.*, 1983; Cann and Wilson, 1983; Johnson *et al.*, 1983; Cann *et al.*, 1984; Horai *et al.*, 1984; Wallace *et al.*, 1985; Horai and Matsunaga 1986).

The Ainu of Hokkaido, the northernmost island of Japan, is considered to be derived from the original population of northern Japan. Although earlier anthropological observations based on morphological features gave birth to the belief that the Ainu belonged to the Caucasoid race, recent genetic studies indicated their close relations to the Mongoloid race (Misawa and Hayashida, 1968; Mittal *et al.*, 1972; Omoto, 1972; Omoto, 1975). However, because of the considerable admixture in the present-day Ainu (Omoto, 1973), the findings based on the variation of nuclear genes are subject to the question as to racial origins of the Ainu.

In the present study, polymorphism of mtDNA of the Ainu and the Non-Ainu Japanese living in Hokkaido was analyzed. Since males of immigrant populations

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are likely to be the predominant source of gene introduction into the aboriginal populations, it was hoped that the information of mtDNA, because of its maternal inheritance (Hutchison *et al.*, 1974; Giles *et al.*, 1980; Case and Wallace, 1981), may give a clue to answer the question, whether the genetic similarity between the Ainu and the Mongoloid populations including the Non-Ainu Japanese is ascribed to gene flow.

MATERIALS AND METHODS

Samples. Five ml of blood samples were obtained from 122 adult Japanese (48 Ainu and 74 Non-Ainu) living in Hidaka district, Hokkaido.

DNA extraction. Total human DNA was extracted from the buffy coat by the method of Kan *et al.* (1977). After an overnight digestion of blood cells with proteinase K in 50 mM Tris-HCl at pH 7.5/100 mM NaCl/1 mM EDTA/0.5% SDS, DNA was extracted with saturated phenol and then dialyzed against 10 mM Tris-HCl at pH 8.0/5 mM EDTA. After the dialysis, DNA was precipitated with ethanol, dried and stocked in 20 mM Tris-HCl at pH 8.0/0.1 mM EDTA.

Restriction enzyme fragment analysis. Two to five μg of total DNA was used for the digestion of each restriction endonuclease. Five restriction enzymes used in the present study (*Ava*II, *Bam*HI, *Hinc*II and *Hpa*I) were purchased from Takara Shuzo Co., Ltd., Kyoto, and the digestion buffers were those recommended by the manufacturer. After the overnight digestion at 37°C, dye markers of bromophenol blue and xylene cyanol and glycerine (to a final concentration 10% v/v) were added to digested DNA. The digested DNA fragments were separated in 0.7%–1.8% agarose gel. The buffer for electrophoresis used was E-buffer (40 mM Tris-acetate at pH 7.9/20 mM sodium acetate/1 mM EDTA) (Mickel *et al.*, 1977; Hadler *et al.*, 1983). The DNA fragments were then transferred to nitrocellulose filter (Southern, 1975) and hybridized with ^{32}P-labeled human mtDNA. The mtDNAs used as probe were purified from human placentae or human cultured cells (Raji) and were nick translated (Rigby *et al.*, 1977). After hybridization in X6 SSC/X1 Denhardt's solution/0.5% SDS (Denhardt, 1966; Botchan *et al.*, 1976), digested mtDNA patterns were analyzed using autoradiography.

RESULTS AND DISCUSSION

Cleavage patterns

*Ava*II: Six different cleavage patterns were observed with this enzyme in the Non-Ainu sample (Fig. 1), five of which (morph 1, 2, 3, 10 and 12) have been reported by Johnson *et al.* (1983) and Horai and Matsunaga (1986). Morph 1 is the most common type in most racial groups, while morph 2 and 3 also have been detected previously in Caucasians, African groups and the Japanese (Johnson *et al.*, 1983; Horai and Matsunaga, 1986). Morph 2 and 3 correspond to morph 3 and 4, re-

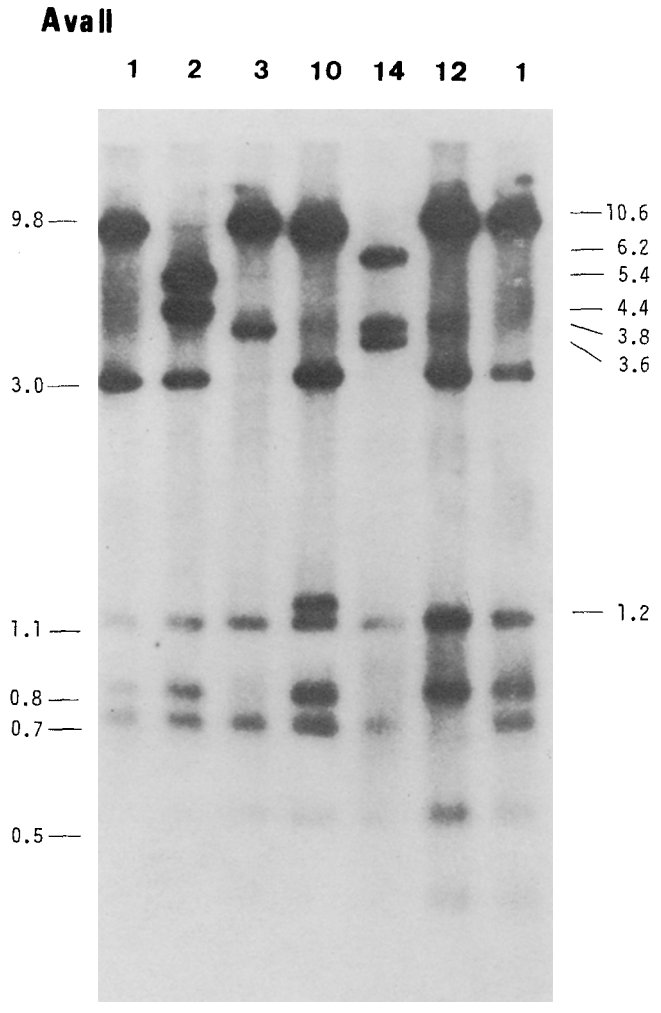

Fig. 1. Autoradiogram of *Ava*II digestion patterns. Fragments were separated in 1.5% agarose gel. Morphs are denoted by numbers at top of lanes and fragment sizes are designated in kilo bases.

spectively, reported by Horai and Matsunaga (1986). Morph 10 has been reported only in Caucasians (Johnson *et al.*, 1983), whereas morph 12 has been reported only in Japanese by Horai and Matsunaga (1986).

One other digestion pattern, which has not been reported previously, is named as morph 14. In morph 14, 9.8 kb fragment is cleavaged into 6.2 kb and 3.6 kb fragments. In addition to this change, 3.0 kb and 0.8 kb fragments are fused to form 3.8 kb fragment. Using a double digestion with *Ava*II and *Eco*RI, the new site was found to be in the coding region of cytochrome *c* oxidase subunit I (COI). The

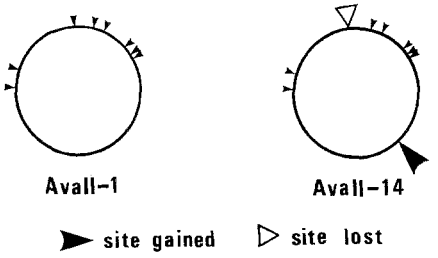

Fig. 2. Site changes in *Ava*II morph 14. The locations of site loss and site gain are designated on the circular mtDNA map. The top of the circle corresponds to the site 0 bp of the sequence of human mtDNA.

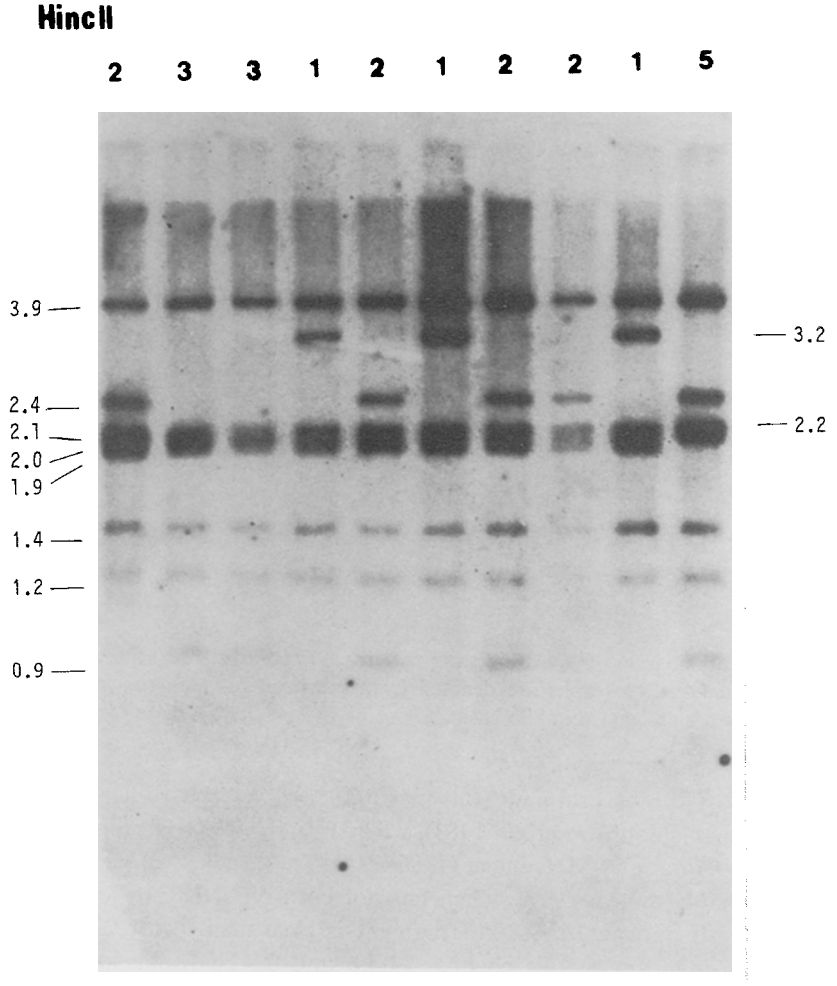

Fig. 3. Autoradiogram of *Hinc*II digestion patterns. Fragments were separated in 1.8% agarose gel. Morphs are denoted by numbers at top of lanes and fragment sizes are designated in kilo bases.

base substitution may be G to A, a transition, or G to T, a transversion, at the site 6,384 base pair (bp) of the human mtDNA sequence (Anderson *et al.*, 1981). This position corresponds to the first base of the codon for alanine. If the base change is G to A, the amino acid encoded by this codon would change to threonine. If the base change is G to T, alanine would be replaced by serine. A site loss occurs at the site 16,390 bp in the non-coding region, and it seems to be the same change as found in morph 3. The changes giving birth to morph 14 are diagrammatically shown in Fig. 2.

*Hinc*II: Four different patterns were found in the Non-Ainu Japanese (Fig. 3). They were morph 1, 2, 3 and 5 reported by Blanc *et al.* (1983). In the Ainu, only three patterns, morph 1, 2 and 3, were detected. The four morphs detected in the Japanese population in the present study, have been reported in Orientals (Blanc *et al.*, 1983; Horai *et al.*, 1984). Among them, morph 1 and morph 3 are common in Orientals but thus far have not been detected in Caucasians.

*Hpa*I: Three distinct morphs, morph 1, 2 and 4, which have already been reported (Brown, 1980; Denaro *et al.*, 1981), were detected when mtDNAs of the Ainu and the Non-Ainu Japanese were digested with *Hpa*I (Fig. 4). Morph 1 has been detected in Africans (Bantu) and Orientals (Denaro *et al.*, 1981). Morph 4 has

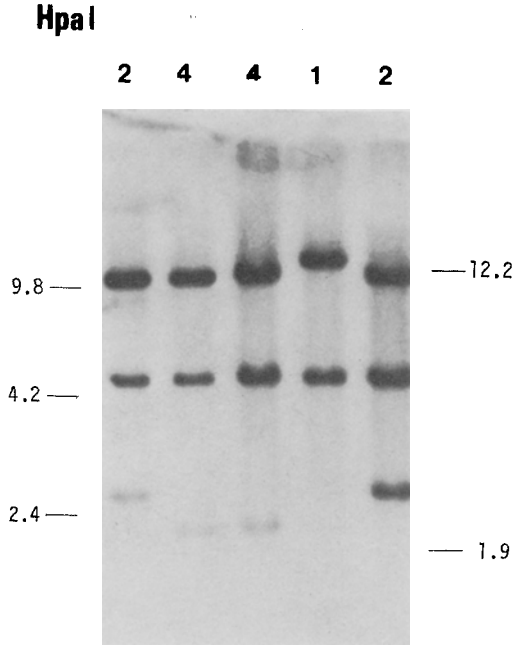

Fig. 4. Autoradiogram of *Hpa*I digestion patterns. Fragments were separated in 1.0% agarose gel. Morphs are denoted by numbers at top of lanes and fragment sizes are designated in kilo bases.

been detected only in Orientals (Denaro *et al.*, 1981).

The variations in mtDNAs detected by *Hpa*I were explained by *Hinc*II digestion patterns in the present study. *Hinc*II, which recognizes the sequence GT($\frac{T}{C}$)($\frac{A}{G}$)AC, also recognizes *Hpa*I sites (GTTAAC). *Hpa*I morph 1 and 4 completely correspond to *Hinc*II morph 1 and 3 respectively. Base changes, which cause *Hpa*I site loss but not *Hinc*II site loss at the same site, or which generate new *Hpa*I site at the *Hinc*II site, could not be detected.

*Pvu*II: *Pvu*II digestion patterns of human mtDNA usually show only one band, which is 16.5 kb fragment of total mtDNA. Horai *et al.* (1984) have reported one *Pvu*II variant morph, morph 2, in the Japanese. Another variant type was found in the present study and named as morph 3. In morph 3, two fragments (10.1 kb and 6.4 kb) were detected (Fig. 5). The double digestion with *Pvu*II and *Bam*HI and that with *Pvu*II and *Eco*RI showed that the extra site is in the coding region of URF-5, which is one of the components of respiratory-chain NADH dehydrogenase (Chomyn *et al.*, 1985). The base change in this case may be A to G, a transition, at the site 12,753 bp, which is the third base of the glutamine codon, and the base substitution may be a silent mutation. The location of the extra site in *Pvu*II morph 3 is shown in Fig. 6.

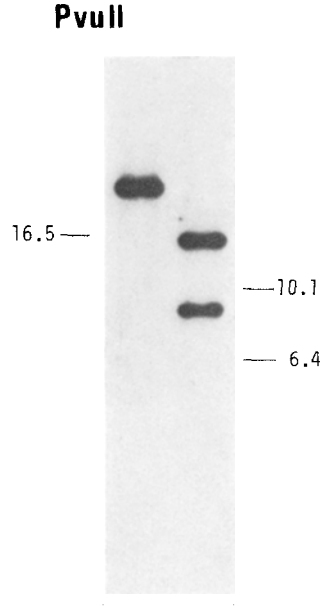

Fig. 5. Autoradiogram of *Pvu*II digestion patterns. Fragments were separated in 0.7% agarose gel. Morphs are denoted by numbers and fragment sizes are designated in kilo bases.

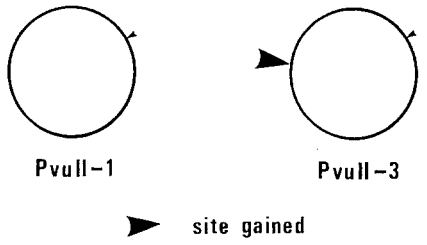

Fig. 6. Site gain in *Pvu*II morph 3. The location is designated. The top of the circle corresponds to the site 0 bp of the human mtDNA sequence.

*Bam*HI: No variant could be detected with *Bam*HI and all the individuals showed morph 1 reported by Brown (1980) and Johnson *et al.* (1983). Other morphs, which have been observed only in Caucasians (Brown, 1980; Johnson *et al.*, 1983), were found in neither the Ainu nor the Non-Ainu Japanese.

Distribution of mtDNA morphs

The Non-Ainu Japanese were highly polymorphic with *Ava*II and *Hinc*II digestion patterns, whereas the Ainu were less polymorphic with these enzymes (Table 1). However, the difference in the distribution of morphs between the two groups were not statistically significant for each enzyme (Table 2).

Horai and Matsunaga (1986) reported that five *Ava*II morphs were detected in the Japanese from central Japan. Four of them, morph 1, 2, 3 and 12, were found also in the present study. Among them, morph 12, which has been detected only in the Japanese may have potential value as a specific marker for Japanese. For the distribution of the *Ava*II morphs, the frequency of morph 1 in the Non-Ainu in the present study is similar to that reported by Horai and Matsunaga (1986). Though the frequency of other morphs are different, there is no significant difference between the distribution of *Ava*II morphs in the present study and that reported by Horai and Matsunaga ($\chi^2=8.8$, df=6, $0.1<p<0.2$).

The distribution of *Hinc*II morphs in the Non-Ainu Japanese is also similar to the results of Horai *et al.* (1984) on the Japanese from central Japan, although morph 9, 10 and 11 reported by them could not be detected in the present study. The frequency of morph 1 is slightly higher (4.9%) than that in the study of Horai *et al.* (3.3%). However, the distribution of *Hinc*II morphs in the present study is not significantly different from that reported by Horai *et al.* ($\chi^2=4.2$, df=6, $0.5<p<0.7$).

mtDNA classification by the combination of enzymes

The mtDNAs of 122 Japanese examined in this study could be classified into eleven types by combining the results of three enzymes: *Ava*II, *Hinc*II and *Pvu*II (Table 3). Though the frequency of the most common type (type 1: *Ava*II morph 1-*Hinc*II morph 2-*Pvu*II morph 1) is higher in the Ainu than in the Non-Ainu

Table 1. Number of individuals and frequencies of all morphs listed by four restriction enzymes.

		Ainu Japanese		Non-Ainu Japanese		Total	
Sample size		48		74		122	
Morph		No.	Freq.	No.	Freq.	No.	Freq.
*Ava*II	1	48	100.0	66	89.2	114	93.4
	2	0	0.0	1	1.4	1	0.8
	3	0	0.0	2	2.7	2	1.6
	10	0	0.0	3	4.1	3	2.5
	12	0	0.0	1	1.4	1	0.8
	14	0	0.0	1	1.4	1	0.8
*Hinc*II	2	46	95.8	64	86.5	110	90.2
	1	1	2.1	5	6.8	6	4.9
	3	1	2.1	3	4.1	4	3.3
	5	0	0.0	2	2.7	2	1.6
*Hpa*I	2	46	95.8	66	89.2	112	91.8
	1	1	2.1	5	6.8	6	4.9
	4	1	2.1	3	4.1	4	3.3
*Pvu*II	1	46	100.0	73	98.6	121	99.2
	3	0	0.0	1	1.4	1	0.8

Table 2. χ^2 value to test difference between distribution of the Ainu and that of the Non-Ainu.

Digestion	χ^2	df	p
*Ava*II	5.7	5	$0.3<p<0.5$
*Hinc*II	3.3	3	$0.3<p<0.5$
*Hpa*I	1.8	2	$0.3<p<0.5$
*Pvu*II	0.7	1	$0.3<p<0.5$

sample, and there are fewer variant types in the Ainu sample, the distribution of the two groups are not significantly different ($\chi^2=9.3$, df=10, $0.3<p<0.5$).

Frequencies of other types except for type 1 are low both in the Ainu and in the Non-Ainu Japanese. Since *Pvu*II morph 3 was found only in one individual, the variability of distribution is mainly attributed to the digestion patterns of the following two enzymes: *Ava*II and *Hinc*II.

Relation between the Ainu and the Non-Ainu Japanese

In the Ainu, 46 individuals (95.8%) had mtDNA type 1, while, two individuals

Table 3. Number of individuals and frequencies of mtDNA types classified by all morphs of three enzymes. Enzyme morphs are listed in parentheses in the order: *Ava*II, *Hinc*II, *Pvu*II.

	Ainu Japanese		Non-Ainu Japanese		Total	
Sample size	48		74		122	
mtDNA type	No.	Freq.	No.	Freq.	No.	Freq.
1 (1-2-1)	46	95.8	57	77.7	103	84.4
2 (1-1-1)	1	2.1	4	5.4	5	4.1
3 (10-2-1)	0	0.0	3	4.1	3	2.5
4 (1-3-1)	1	2.1	2	2.7	3	2.5
5 (1-5-1)	0	0.0	2	2.7	2	1.6
6 (2-2-1)	0	0.0	1	1.4	1	0.8
7 (3-2-1)	0	0.0	1	1.4	1	0.8
8 (3-3-1)	0	0.0	1	1.4	1	0.8
9 (12-1-1)	0	0.0	1	1.4	1	0.8
10 (14-2-1)	0	0.0	1	1.4	1	0.8
11 (1-2-3)	0	0.0	1	1.4	1	0.8

(4.2%) showed other types (type 2 and type 4) differing from type 1 as to *Hinc*II morph. In the Non-Ainu, the mtDNAs of 74 individuals were classified into eleven types and the frequency of type 1 was 78.4%.

Though the mtDNAs of the Ainu were less polymorphic than those of the Non-Ainu, the present result does not show great divergence between the two groups. The low level of variation of the Ainu sample may be ascribed to the small sample size and the random genetic drift. The three mtDNA types found in the Ainu were also found in the Non-Ainu and no significant difference was detected between the two groups. These results are compatible with those of the recent genetic studies using blood protein and antigen markers suggesting that the Ainu have closer genetic relation to Mongoloids than to Caucasoids or other racial groups (Misawa and Hayashida, 1968; Mittal *et al.*, 1972; Omoto, 1972).

The present-day Ainu population show considerable degree of admixture with Non-Ainu Japanese immigrants and it is known that the intermarriages predominantly took place between Ainu women and Non-Ainu men (Omoto, 1973). Therefore, mtDNA with its maternal inheritance may have advantages over nuclear genes as the genetic marker for studying the origins of the Ainu. Although more material is clearly needed for a final conclusion, the result of this study may be taken as a clue to substantiate the views that the genetic affinities of the Ainu to Mongoloids are the true ones and are not due to gene flow in the recent times (Omoto, 1975).

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