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Short communication

Characteristics of Hypervariable Regions of Mitochondrial DNA in Korean Population

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The nucleotide sequence of two hypervariable regions of the D-loop and the frequency of the 9-bp repeat in the region V of mitochondrial DNA (mtDNA) were investigated in the Korean population. Alignment of these sequences with the published reference revealed a unique pattern of base substitution and deletion compared with those of other races. The deletion and addition frequency of the 9-bp repeat in the region V was also distinct.

Keywords: Hypervariable region, Korean population, Mitochondrial DNA, 9-bp repeat.

Introduction

Analysis of mtDNA has many advantages in the fields of evolution, forensic sciences and many other areas (Cann *et al.*, 1983; Greenberg *et al.*, 1983; Shields *et al.*, 1992; Piercy *et al.*, 1993; Yoshii *et al.*, 1995; Wilson *et al.*, 1995; Soodyall *et al.*, 1996). In particular, the noncoding regions of mtDNA have shown more rapid changes of nucleotide sequences than a single copy of nuclear genome (Greenberg *et al.*, 1983). There are two major noncoding segments in mtDNA. One is the D-loop region and the other is the region V between the COII and $tRNA^{Lys}$ genes (Anderson *et al.*, 1981; Wrischnik *et al.*, 1987). The D-loop region shows many nucleotide changes caused by substitution, deletion, and addition (Greenberg *et al.*, 1983). The region V also has Asian-specific length polymorphism (Wrischnik *et al.*, 1987). This study was carried out to determine the variation of polymorphism present in both noncoding regions and to find a unique pattern in the Korean population.

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Materials and Methods

Extraction of mtDNA Blood samples were obtained from 100 unrelated Korean donors from the Red Cross National Blood Center. DNA extraction was carried out as previously published (Park *et al.*, 1997).

Sequence analysis of the HV1 and HV2 segments in the D-loop region DNA samples were amplified and sequenced as previously published (Wilson *et al.*, 1995; Park *et al.*, 1997) with the following primers: H408 (5′-CTGTTAAAAGTGCATACCGCCA-3′) and L16159 (5′-TACTTGACCACCTGTAGTAC-3′). The total reaction volume was 100 μl under the following condition; 30 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min. The sequencing reaction was performed using a Circumvent™ thermal cycle sequencing kit purchased from New England Biolabs (Beverly, USA) according to the manufacturer's instructions. Separation of these samples was carried out in a 6% polyacrylamide gel in TBE-buffer containing 8.3 M urea, and the gel was dried on Whatman filter paper and exposed to X-ray film.

Deletion analysis of 9-bp repeats in the region V The primers used were the same as Wrischnik *et al.* (1987). Cycle conditions were as follows; 30 cycles at 94°C for 40 s, 57°C for 40 s, and 72°C for 40 s. The protocols and methods of amplification were the same as previously described (Park *et al.*, 1997). The PCR products were separated in a 10% polyacrylamide gel and the results were visualized with ethidium bromide.

Results and Discussion

Sequence analysis of the HV1 and HV2 segments in the D-loop region DNA sequence alignment was made to the original Anderson sequence with its numbering system (Anderson *et al.*, 1981). There were several differences from the Anderson sequence as shown in Table 1, in which the unique positions are shaded. The substitution rates were 84% at nucleotide (nt) 16223, 22% at nt 16319, and 48% at nt 16362, respectively. These rates were higher

Table 1. Nucleotide sequences of the D-loop region of mtDNA of the Korean population.

Anderson sequence[a]

	HV1																												
	16217	16223	16231	16234	16243	16247	16248	16249	16257	16261	16266	16278	16287	16288	16290	16291	16293	16294	16298	16304	16311	16316	16319	16324	16325	16327	16355	16362	16399
Ref.[a]	T	C	T	C	T	A	C	T	C	C	C	C	C	T	C	C	A	C	T	T	T	A	G	T	T	C	C	T	A
S1	.	T	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S2	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S3	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S4	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S5	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S6	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S7	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S8	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S9	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S10	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S11	.	T	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S12	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S13	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.
S14	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S15	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.
S16	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.
S17	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S18	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
S19	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
S20	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.
S21	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.
S22	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	A	.	.	.	.	.	.
S23	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
S24	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.
S25	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.
S26	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.
S27	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.
S28	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S29	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
S30	.	T	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S31	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S32	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
S33	.	T	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
S34	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.
S35	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
S36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
S37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.
S38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.
S39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S40	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.
S41	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.
S42	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.
S43	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.
S44	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	C	.
S45	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.
S46	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	T	.	C	.
S47	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
S48	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S49	.	.	C	.	C	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.	.	G
S50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.

	HV1																			
	146	150	151	152	153	194	195	199	200	204	207	222	228	235	249	257	263	280	C1[b]	C2[c]
Ref.[a]	T	C	C	T	A	C	T	T	A	T	G	C	G	A	A	A	A	C	7	5
S1	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S2	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	G	.	9	6
S3	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	G	.	9	6
S4	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	G	.	8	6
S5	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S6	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S7	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	G	.	8	6
S8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S9	.	.	.	C	.	.	.	.	.	.	.	T	A	.	.	.	G	.	7	6
S10	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S11	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	7	6
S12	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S13	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S14	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S15	.	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	G	.	7	6
S16	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S17	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S19	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S20	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S21	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	8	6
S22	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.	.	G	.	8	6
S23	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	8	6
S24	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	8	6
S25	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	G	.	8	6
S26	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.	8	6
S27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S28	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S29	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S30	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	9	6
S31	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	9	6
S32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S33	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S34	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	7	6
S36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	G	.	7	6
S37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	G	.	7	6
S38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	G	.	8	6
S39	.	.	.	.	.	.	C	.	.	.	.	.	.	.	D	.	G	.	7	6
S40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	G	.	7	6
S41	.	.	.	.	.	.	C	.	.	.	.	.	.	.	D	.	G	.	8	6
S42	C	.	.	.	.	.	.	.	.	.	.	.	T	.	D	.	G	.	8	6
S43	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	7	6
S44	C	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	G	.	8	6
S45	.	.	.	C	.	.	.	.	.	.	.	.	.	.	D	.	G	.	9	6
S46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	G	.	7	6
S47	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	G	G	.	7	6
S48	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	9	6
S49	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	8	6
S50	.	.	.	C	.	.	.	.	.	.	.	.	.	.	D	.	G	.	8	6

[a]Reference sequence and its numbering were published by Anderson *et al.* (1981).
Dots represent the same sequence as the reference and deletions are shown by D.
C1: poly (C) region in nt 303–309.
C2: poly (C) region in nt 311–315.
The numbers in columns of C1 and C2 mean real numbers of C in those positions.

Table 2. Frequency of the 9-bp repeats of the mtDNA noncoding region in nt 8266–8294*

Population+	Sample size	Deletion (%)	Addition (%)
Caucasian[a]	46	0 (0%)	0 (0%)
Japanese[b]	63	12 (19%)	0 (0%)
Korean[b]	64	5 (7.8%)	0 (0%)
negrito[b]	37	34 (91.9%)	0 (0%)
vedda[b]	20	0 (0%)	0 (0%)
Native Beringians[c]	836	0 (0%)	0 (0%)
African[d]	919	81 (8.8%)	0 (0%)
Korean[e]	100	12 (13%)	1 (1%)

* There are normally two 9-bp repeats in the region. Therefore, deletion and addition mean one 9-bp repeat and three 9-bp repeats, respectively.

+ a, adopted from the report (Cann *et al.*, 1983); b, (Harihara *et al.*, 1992); c, (Shields *et al.*, 1992); d, (Soodyall *et al.*, 1996); e, our study of the Korean population.

than those of Caucasians (8%, 0%, and 5%, respectively) (Piercy *et al.*, 1993), and similar to those of the Japanese and Koreans (Yoshii *et al.*, 1995; Lee *et al.*, 1997). We have also found a nucleotide deletion at nt 249 which was not detected in Caucasians (Piercy *et al.*, 1993). These characteristics present in the Korean population can be useful as a genetic marker.

Deletion analysis of 9-bp repeats in the region V Analysis of 9-bp repeats in the region V is shown in Table 2. This deletion was not found in Caucasians but specifically found in Asian populations, and the frequencies of the 9-bp repeats of each race appeared to be very distinct (Cann *et al.*, 1983; Horai *et al.*, 1986; Wrischnik *et al.*, 1987; Harihara *et al.*, 1992; Shields *et al.*, 1992; Soodyall *et al.*, 1996). The frequency of 9-bp repeats in the Korean population in the present study was slightly different from previous data reported (Harihara *et al.*, 1992). It was rather similar to the frequency of the Japanese and somewhat different from the case of Koreans (Horai *et al.*, 1986; Harihara *et al.*, 1992). This result may be due to the smaller sample size used. In addition, we have found one case of a 9-bp addition in the Korean population. The addition was first reported by Cann and Wilson (1983). We verified the exact composition of 9-bp repeats by DNA sequence analysis and we have also confirmed the unit sequence of the three tandem repeats of 9-bp, CCCCCTCTA, in the Korean population.

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