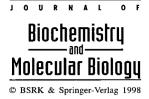
Short communication



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 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'-CTGTTAAAAGTGCATA-CCGCCA-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

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

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^aReference 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.

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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.

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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⁺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.