Detection of Sequence Variants in Hypervariable Segments of Mitochondrial DNA in the Asian Population

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Introduction

The analysis of highly polymorphic regions of mitochondrial DNA is one of the most commonly used methods for personal identification. The recent advances of fluorescent detection in automated DNA sequencing (Smith et al. 1986) has made it possible a rapid analysis of sequence variants without using isotopic labeling.

We chose to analyze the control region in mitochondrial DNA because it displays many polymorphisms (Aquadro et al. 1983, Sullivan et al. 1992)

Materials and method

We collected saliva as samples from total 171 individuals of Asian population (Japan, China, Mongolia, Myanmar, Bangladesh, and Europe).

DNA extraction - one drop of saliva (3μl)was collected in 500μl microtube by the straw from the oral cavity. Next, 250 μl of 5 % chelex was added into the tube, incubated at 56℃ for 30 minutes and at 94℃ for 10 minutes after vortex mixing. DNA were extracted in 45 minutes by chelex based method.

After microcentrifugation for 5 minute at 5000g, 5μl of supernatant was added to PCR mixture.

The following primers and thermocycle were used for Segment I and II amplifications (Stoneking et al. 1991)

Seg I	L15996: 5'-CCACCATTAGCACCCAAAGC	20mer
	H16401: 5'-TGATTTCACGGAGGATGGTG	20mer
Seg II	L29: 5'-TCTATCACCCTATTAACCAC	20mer
	H408: 5'-GTTAAAAGTGCATACCGCCA	20mer
Thermocycle	94℃-45s/ 56℃-1m/ 74℃-1m (27+20 cycles)	

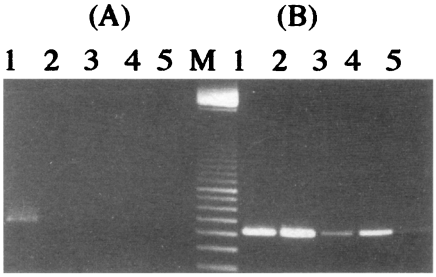
The two hypervariable segments of mitochondrial control region were amplified separately by the method of semi-nested PCR (Honda et al. 1994; 1995). In the first PCR, the 982 bp segment was amplified with the primers L15996 and H408. In the second PCR, 406bp segment was amplified with primers of L15996 and H16401 (Segment I), and 380bp segment was amplified with primers of L29 and H408 (Segment II). The sequence variants of the two

hypervariable segments were examined by the one-lane-direct-sequencing labeled by dye-deoxy-terminator using DNA- sequencer (ABI; model 373A).

Results

The seminested PCR is extremely sensitive and specific so that as low as 0.01pg. concentration of the template DNA can be amplified without unspecific band (Fig.1).

In our result, more than 12% of sequence variants were detected in each segment (Fig.2). Some of them were suspected to be Asian specific (e.g.16085: C to G change, 150: C to T change) because they were rarely found in Caucasians. About 4 to 18 of each sequence variants per individual were found in comparison with full sequence data by Anderson et al. (1981). In both segments, Cytosine to Guanine replacement was most frequently found, and the next was Cytosine to Thymine.



lane 1 :PCR product from 100.00pg template DNA
lane 2 :PCR product from 10.00pg template DNA
lane 3 :PCR product from 1.00pg template DNA
lane 4 :PCR product from 0.10pg template DNA
lane 5 :PCR product from 0.01pg template DNA

Fig 1 - Comparison of sensitivity between Simple PCR and Nested PCR
(A) Agarose gel electrophoresis of Mitochondrial Control Region Seg.I amplification products after 30 cycles of standard PCR with decreasing concentration of template DNA. M : 100 bp ladder
(B) Agarose gel electrophoresis of Nested PCR (using 1μl aliquot from first amplification mixture and 20 cycles further amplification). Concentrations of template DNA correspond to that of (A).

Position No.	16085 (C)	16091(A)	73(A)	150(C)	152(T)
Japanese	G: 50%	T: 28%	G:95%	T:27%	C:27%
Chinese	G:95%	T: 5%	G:75%	T:13%	C:25%
Mongolian	G:95%	T: 90%	G:80%	rare	C:60%
Myanmar	G:70%	T: 40%	G:67%	T:17%	C:35%
Bangladeshi	G:97%	rare	G:95%	T:18%	C:33%
European	rare	rare	G:45%	rare	C:20%

Table 1 - Percentage of nucleotide replacements observed in various population

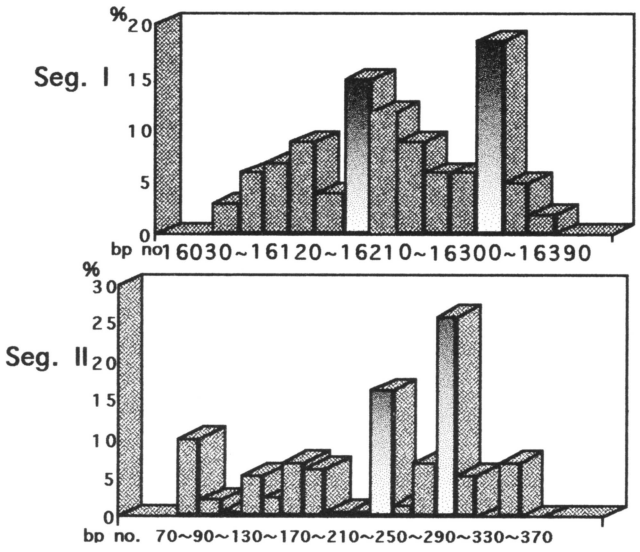


Fig.2 Distribution of sequence variants in segment I and II

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