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Analysis of the HVI, HVII and HVIII regions of mtDNA in 400 unrelated Japanese

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Abstract. Sequence polymorphism of the hypervariable regions HVI, HVII and HVIII of mitochondrial DNA (mtDNA) was analyzed in a sample of 400 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan) by PCR amplification and direct sequencing. A total of 308 different haplotypes resulting from 187 polymorphic positions was found in our Japanese population sample. The most common haplotype was shared by 10 individuals. The genetic diversity and the genetic identity were calculated to be 0.9975 and 0.0050, respectively. © 2005 Published by Elsevier B.V.

Keywords: Mitochondrial DNA; Hypervariable region; Japanese population data

1. Introduction

The sequence analysis of the hypervariable region of mitochondrial DNA (mtDNA) is a very useful tool for forensic investigations. Sequence data for mtDNA have been obtained from many different populations (e.g. [1–4]). The main aim of this study was to evaluate the variability of the three hypervariable regions HVI, HVII and HVIII in the Japanese population.

2. Materials and methods

Blood samples were obtained from 400 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan). DNA was extracted using the phenol-chloroform method. The primers F16047/R16464 for HVI [5], F29/R408 for HVII [6], F182/R619 for

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HVIII [7], and F15971/R599 for HVI, HVII and HVIII [4] were used for amplification of mtDNA. PCR was carried out using the amplification conditions described in each reference, with minor modifications. The PCR products were directly sequenced using the Thermo Sequenase core sequencing kit (Amersham) or the Thermo Sequenase Primer Cycle Sequencing Kit (Amersham). In addition to the PCR primers, F16047/R16464, F29/R408 and F182/R619, primers F15989 [8] and R274 [4] were used for sequencing. Each sequencing primer was labelled with Texas Red. Sequence analysis was performed on a SQ5500-S DNA Sequencer (Hitachi Electronics Engineering). The resulting sequence data were compared with the reference sequence described by Anderson et al. [9].

3. Results and discussion

A total of 111 polymorphic positions were found in HVI, 53 in HVII and 23 in HVIII which resulted in 308 different haplotypes for 400 unrelated Japanese individuals living in Gifu Prefecture. The most common haplotype (16129A, 16223T, 16362C, 73G, 152C, 263G, 309.1C, 315.1C, 489C) was shared by 10 individuals. The genetic diversity and the

Table 1
Sequence polymorphism in the mtDNA hypervariable regions HVI, HVII and HVIII from 400 unrelated Japanese individuals

Mutation type	HVI	HVII	HVIII
Transitions			
C–T	588	121	14
T–C	716	213	274
A–G	79	862	3
G–A	143	47	12
Total	1526	1243	303
Transversions			
C–G	4	0	2
C–A	47	0	0
T–A	0	1	0
A–C	130	0	0
A–T	4	0	0
Total	185	1	2
Insertions			
+C	4	749	3
+2C	0	0	1
+3C	0	0	3
+A	0	12	0
+CA	0	0	4
+2CA	0	0	5
Total	4	761	16
Deletions			
−C	2	2	1
−A	0	39	0
−CA	0	0	132
Total	2	41	133

genetic identity for this Japanese population sample were calculated to be 0.9975 and 0.0050, respectively, based on the calculation method described by Tajima [10]. Transitions were more prevalent than transversions (Table 1). The most frequent nucleotide substitutions were at positions 73 (A to G transition) and 315.1 (C insertion), and secondly at position 263 (A to G transition). The length heteroplasmy in the homopolymeric C-stretch regions located at positions 16184–16193 in HVI, at positions 303–315 in HVII and at positions 568–573 in HVIII was observed in 26.1%, 8.6% and 4.1% of individuals, respectively. Insertions of C residues were located at positions 16184–16188 (+C), at positions 16190–16193 (+C), at positions 16259–16262 (+C), at positions 303–309 (+C, +2C), at positions 311–315 (+C) and at positions 568–573 (+C, +2C, +3C). Deletions of a single C residue were also located at positions 16190–16193 and at positions 568–573. Screening CA repeat at positions 514–523 (−CA, +CA, +2CA), we found 132 sequences with $(CA)_{5-1}$, 254 with $(CA)_5$, nine with $(CA)_{5+1}$ and five with $(CA)_{5+2}$ repeats.

This database of mtDNA HVI, HVII and HVIII regions for Japanese population would be useful for forensic examinations and human genetic studies.

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