International Congress Series 1261 (2004) 410–412

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Sequence analysis of mitochondrial DNA HVIII region in a Japanese population

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Abstract. Sequence polymorphism of the hypervariable region HVIII of mitochondrial DNA (mtDNA) was analyzed in a sample of 150 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan). A total of 21 different haplotypes resulting from 20 variable sites was observed. The most frequently observed nucleotide substitution was at position 489 (T to C transition).

Keywords: mtDNA; HVIII; Japanese population data

1. Introduction

The sequence analysis of the hypervariable region of mitochondrial DNA (mtDNA) is a very useful tool for forensic investigations. Recently, Lutz et al. [1] reported the existence of the third hypervariable region called HVIII (positions 438–574). The main aim of this study was to evaluate the variability of the HVIII region in the Japanese population.

2. Materials and methods

Blood samples were obtained from 150 unrelated Japanese individuals living in Gifu Prefecture (central region of Japan). DNA was extracted using the phenol–chloroform method. PCR amplification conditions were based on the method of Bini et al. [2]. The PCR products were directly sequenced using the Thermo Sequenase core sequencing kit (Amersham). The PCR primers labeled with Texas Red were used as the sequence primers. Sequence analysis was performed on an SQ5500-S DNA Sequencer (Hitachi Electronics Engineering). The resulting sequence data were compared with the reference sequence described by Anderson et al. [3].

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0531-5131/
doi:10.1016/S0531-5131(03)01786-2

3. Results and discussion

The sequence polymorphisms of the mtDNA HVIII region observed in this study are summarized in Table 1. A total of 21 different haplotypes resulting from 20 variable sites was found in a sample of 150 unrelated Japanese individuals living in Gifu Prefecture. The most common haplotype was found in 38.0% ($n=57$) of the individuals. Transitions were more prevalent than transversions (Table 2). Screening CA repeat at positions 514–523 (−CA, +CA, +2CA), we found 53 sequences with $(CA)_{5-1}$, 92 with $(CA)_5$, 3 with $(CA)_{5+1}$ and 2 with $(CA)_{5+2}$ repeats (Tables 1 and 2). At C stretch at positions 568–573 (+C, +2C, +3C), we found 1 sequence with 5, 145 sequences with 6, 1 sequence with 7, 1 sequence with 8 and 2 sequences with 9 C residues (Tables 1 and 2). The most frequently observed nucleotide substitution was at position 489 (T to C transition), and secondly at positions 522 (C deletion) and 523 (A deletion). The genetic diversity and the genetic identity for this population sample were calculated to be 0.782 and 0.223, respectively, based on the calculation method described by Tajima [4].

Table 1
Sequence polymorphisms in the mtDNA HVIII region of 150 unrelated Japanese

456	469	482	483	489	493	499	501	522	523	523a	523b	523c	523d	542	573	573a	573b	573c	593	*n*
C	C	T	C	T	A	G	C	C	A	–	–	–	–	C	C	–	–	–	T	
•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	57
•	•	•	•	•	•	•	•	del	del	•	•	•	•	•	•	•	•	•	•	26
•	•	•	•	C	•	•	•	del	del	•	•	•	•	•	•	•	•	•	•	24
•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	22
•	•	•	•	C	•	•	•	•	•	C	A	•	•	•	•	•	•	•	•	3
•	•	•	•	C	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	3
•	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1
•	•	C	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1
•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	C	•	•	1
•	•	•	•	•	•	•	•	•	•	C	A	C	A	•	•	•	•	•	•	1
•	•	•	•	C	•	•	•	del	del	•	•	•	•	•	•	C	C	C	•	1
•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	1
•	•	•	•	C	•	•	T	•	•	•	•	•	•	•	•	•	•	•	•	1
•	G	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1
•	•	•	•	C	•	•	•	•	•	C	A	C	A	•	•	•	•	•	•	1
•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	C	C	C	•	1
•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	del	•	•	•	•	1
•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	C	1
T	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1
•	•	•	•	•	•	•	•	del	del	•	•	•	•	T	•	•	•	•	•	1
•	•	•	•	•	•	•	•	del	del	•	•	•	•	•	•	C	•	•	•	1
Total																				150

The nucleotide in the reference sequence [3] is shown below the order for polymorphic sites. Letters following a number (a–d) indicate additions of nucleotides not found in the reference sequence.
(•) Identical nucleotide with the reference, (–) nucleotide absence in the reference, del: deletion from the reference, *n*: observed number.

Table 2
Nucleotide substitutions, insertions and deletions in the mtDNA HVII region of 150 unrelated Japanese

Mutation type	HVIII	
	Number of positions	Total number of mutations
Transition		
C → T	4	4
T → C	3	99
A → G	1	3
G → A	1	1
Total	9	107
Transversion		
C → G	1	1
Total	1	1
Insertion		
+CA	1	3
+2CA	1	2
+C	1	1
+2C	1	1
+3C	1	2
Total	5	9
Deletion		
−C	1	1
−CA	1	53
Total	2	54

This database of mtDNA HVIII region for Japanese population would be useful for forensic examinations and human genetic studies.

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