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Beyond HV1 and HV2—identification of valuable mitochondrial DNA single nucleotide polymorphisms

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Abstract. The degree of polymorphism characteristic for hypervariable segments of mitochondrial DNA (mtDNA) is sometimes too low to enable differentiation between individuals revealing most frequent HV1 and HV2 haplotypes. An acceptable solution in such a situation seems to be analysis of additional variation present outside the control region. For practical and ethical reasons, analysis of variation present in the coding part of mitochondrial DNA should be limited to selected single nucleotide polymorphisms. Before selective SNP analysis is possible, those particular valuable mutations, which occur appropriately frequently in a population and are free of medical information, must be determined.

Keywords: Mitochondrial DNA; Single nucleotide polymorphism; Population study; Snapshot

1. Introduction

Sequence analysis of the HV1 and HV2 fragments of mitochondrial DNA (mtDNA) is today a routine method applied to forensic identification in cases where evidence specimens are not suitable for STR analysis. The main limitation of the identification process based on mtDNA analysis is the relatively low power of discrimination associated with HV1 and HV2 sequencing. The most frequent haplotype of mtDNA in the Caucasian population (263G, 315.1C) is characteristic for about 4% of individuals [1]. In order to limit this drawback, analysis of variation associated with the remaining sequence of mtDNA has been proposed [2]. There is known association between genetic diseases and particular mutations in mtDNA [3]. This sort of data must not be obtained in a forensic laboratory. Particular positions revealing appropriate variation and no links with genetic diseases (suitable for forensic identification) must be first determined by nucleotide

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sequencing. In the routine identification analyses, the identified SNPs can be selectively analysed with one of the currently available methods, e.g., SNaPshot Multiplex Kit. The aim of the present work was to sequence the portion of the coding region of mtDNA for a population sample from Poland in order to find SNPs that could be further subjected to selective analysis in the identification process.

2. Materials and methods

The study was performed on blood or saliva samples collected from 58 unrelated individuals from Poland. DNA was isolated using the standard organic method and the concentration of DNA extract was measured fluorymetricaly.

The 550-bp fragment (amplicon I) of the coding region, encompassing positions 8368–8917 was amplified in an optimised triplex reaction, using the following primers: (1) GCCCCAACTAAATACTACCG; and (2) GTGGTAAGAAGTGGGCTAGG. Two additional amplicons included in the reaction were:

(II) 403 bp, nucleotide positions 10253–10655, covering SNP positions: A10398G, T10463C, A10550G. Primer sequences: (1) TGATCTAGAAATTGCCCTCC; and (2) GGCAATAGGCACAATATTGGC.
(III) 212 bp, nucleotide positions 16344–16555, covering SNP position: T16519C. Primer sequences: (1) CAGTCAAATCCCTTCTCGTCCC; and (2) ATTTAAGGGGAACGTGTGGGC.

The PCR reaction mixture consisted of 1 U Taq DNA polymerase, 2.0 mM $MgCl_2$, 200 μM dNTP, 0.2 μM of each primer; 0.4 μl BSA, 2.0 μl 10 × concentrated PCR buffer and usually 5 ng of template DNA. PCR conditions were as follows: 95 °C/1 min; (94 °C/10 s, 58 °C/30 s, 72 °C/40 s) × 32; 72 °C/10 min; 4 °C. PCR products were purified and subjected to cycle sequencing of the amplicon I. Genetic diversity for the analysed DNA fragment I was calculated according to the formula: $D = 1 - \Sigma p_i^2$, where p indicates frequency of the observed haplotypes [4].

Amplification products were subjected to a SNaPshot reaction, which was performed according to manufacturers' directions with the following extension primers: SNP-A10398G: GGCCTATGAGTGACTACAAAAAGGATTAGACTGA; SNP-T10463C: AATATTTATGTAAATGAGGGGCATTTGGTA; SNP-T16519C: GACATCTGGTTCCTACTTCAGGG; SNP-A10550G: AGTATATCGCTCACACCTCAT.

3. Results and discussion

The study revealed 12 different haplotypes and 13 polymorphic nucleotide positions among the analysed 58 unrelated individuals. The most frequent variant differed in the single position A8860G, according to the CRS sequence [5]. Table 1 presents information concerning haplotypes, their frequencies and polymorphic sites determined during our study. The genetic diversity equals 0.53. Most of the identified variable positions were discovered in single samples. The only one position—synonymous change G8697A—is characterised by a reasonable degree of polymorphism (86.2% G versus 13.8% A) and can be recommended as a good SNP candidate. Comparative analysis of our results with

Table 1
Haplotypes determined during the study (H1–H12), their frequencies and characteristics of variable positions

	8414	8455	8460	8563	8610	**8697**	8701	8715	8730	8749	8818	8860	8869	Number of individuals
CRS	C	C	A	A	T	**G**	A	T	A	T	C	A	A	
H1		.	.	.	.	**.**	.	.	.	.	.	G	.	39
H2		.	.	.	.	**A**	.	.	.	.	.	G	.	7
H3	T	.	.	.	.	**.**	G	.	.	.	.	G	.	1
H4	.	T	.	.	.	**A**	.	.	.	.	.	G	.	1
H5	.	.	.	T	.	**.**	.	.	.	.	.	G	.	1
H6	.	.	.	.	.	**.**	.	.	.	.	T	G	.	3
H7	.	.	.	.	.	**.**	.	.	.	.	.	G	G	1
H8	.	.	.	.	.	**.**	.	.	G	.	.	G	.	1
H9	.	.	.	.	.	**.**	.	.	.	C	.	G	.	1
H10	.	.	.	.	C	**.**	.	.	.	.	.	G	.	1
H11	.	.	.	.	.	**.**	.	C	.	.	.	G	.	1
H12	.	.	G	.	.	**.**	.	.	.	.	.	G	.	1
	nonsyn	syn	nonsyn	syn/ nonsyn	syn	**syn**	nonsyn	syn	syn	syn	syn	nonsyn	nonsyn	Total = 58

SNP position G8697A marked in bold. CRS—Cambridge Reference Sequence; syn—synonymous change; nonsyn—nonsynonymous change; syn/nonsyn—nucleotide position placed in overlapping fragment of two genes causing amino acid change in one of them.

previously published Chinese data revealed significant differences in variation patterns [6]. This observation supports the thesis that different SNP-type polymorphisms can be strongly associated with a given population. We optimised a SNaPshot reaction enabling selective analysis of the SNPs found during our previous search (A10398G, T10463C, A10550G) and known polymorphic position T16519C. These hot spots, which are amplified in a multiplex reaction and subjected to SNaPshot reaction, give results concordant with sequencing data. The triplex PCR also includes position G8697A, which, together with other mtDNA SNPs described in scientific publications (e.g., Ref. [7]), will be added to the set of SNPs analysed with SNaPshot kit and the method will be validated for casework analysis.

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