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Subtyping mtDNA haplogroup H by SNaPshot minisequencing

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Abstract. A population sample from North-Central Italy was analysed to investigate the frequency distribution of subclades of mtDNA haplogroup H. A specific SNaPshot assay was set up to detect diagnostic mutations identifying subhaplogroups from H1 to H7. Haplogroup H subtyping can be useful to discriminate among individuals sharing common mtDNA HVS I/II sequences. © 2006 Published by Elsevier B.V.

Keywords: mtDNA; SNP; Haplogroup; SNaPshot

1. Introduction

Sequencing analysis of hypervariable regions HVSI/II is the most common approach to mitochondrial DNA (mtDNA) typing in the context of anthropological and medical studies [1,2]. In the forensic field, mtDNA analysis is particularly important in human identification caseworks where the amount of genomic DNA recovered from samples as skeletal remains and hair shafts is extremely reduced. However, the discrimination power of mtDNA typing is quite low also as a consequence of the maternal inheritance; in fact, about 7% of the Caucasian population shares the same HVSI/II sequence [3]. In order to increase the discrimination of the common sequences for forensic purposes, it could be useful to characterise other mtDNA polymorphisms, such as single nucleotide polymorphisms (SNPs) of the control region defining the most common European haplogroups. Recently, a SnaPshot minisequencing assay based on ddNTPs single base extension of unlabelled primers immediately adjacent to

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the polymorphic site was set up; this provided the association of each individual to one of the nine major west European haplogroups [4]. In addition, the SnaPshot method was used to type 7 SNPs allowing subtyping of haplogroup H, the most common lineage in the European population (about 50%) [5]. Here we describe the frequency distribution of the same subclades (H1–H7) in our Italian haplogroup H samples and the forensic usefulness of this approach in discriminating common shared mtDNA haplotypes.

2. Materials and methods

DNA was extracted from blood and saliva samples collected from 197 unrelated Northern-Central Italians by phenol–chloroform or Chelex 100 purification. PCR amplification of the hypervariable regions HVS I/II was performed using the following primers: HVS/I: L15997 and H16401; HVS/II: L29 and H408. The amplified fragments were sequenced with the BigDyes Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA) and separated by capillary electrophoresis on ABI PRISM 310 sequencers. RFLP typing was performed following a phylogenetic approach with PCR conditions and primers according to Torroni [6].

A multiplex PCR reaction was set up in order to subtype mtDNA haplogroup H samples. PCR conditions and primer sequences to amplify the DNA fragments containing the H1–H7 polymorphisms and the SNaPshot assay to type H1–H7 diagnostic mutations are according to Quintans et al. [5], with minor modifications.

3. Results and discussion

In this study, we analysed 197 individuals from North-Central Italy by sequencing the hypervariable regions HVS I/II. A phylogenetic analysis was performed on the samples by RFLP typing of diagnostic mutations identifying the major European mtDNA haplogroups. This approach showed that about half of the samples (44.7%) belonged to haplogroup H, in agreement with the distribution found in other European population samples [7]. The SnaPshot minisequencing multiplex reaction set up by Quintans et al. [5] was then used to subcharacterise the Italian haplogroup H samples as shown in Fig. 1.

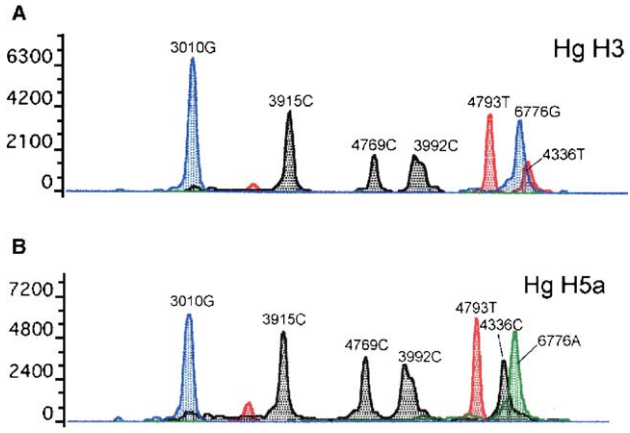

Fig. 1. SNaPshot multiplexes from different samples identifying diagnostic mutations peculiar of subhaplogroups H3 (top) and H5a (bottom).

Table 1
Frequencies of the haplogroup H subclades in the Italian population sample

rCRS	H*	H1	H2	H3	H4	H5a	H6a	H7
3010G	G	**A**	G	G	G	G	G	G
3915G	G	G	G	G	G	G	**A**	G
3992C	C	C	C	C	**T**	C	C	C
4336T	T	T	T	T	T	**C**	T	T
4769A	G	G	**A**	G	G	G	G	G
4793A	A	A	A	A	A	A	A	**G**
6776T	T	T	T	**C**	T	T	T	T
N individuals	41	25	4	4	3	7	3	1
Frequency (%)	20	12.7	2	2	1.5	3.5	1.5	0.5

Diagnostic mutations for each subhaplogroup are boldface in boxes. rCRS: Cambridge Reference Sequence. Frequencies are referred to 197 individuals.

This analysis provided a good definition of haplogroup H subclades in our population sample, as shown in Table 1, with 53.4% of the individuals assigned to a specific H subhaplogroup. The samples not belonging to a specific subclade were included in the paraphyletic group H*.

Our data were then compared with the one calculated for the Spanish (Galician) population sample analysed by Quintans et al. [5] and a statistical significant difference (χ^2, P-value < 0.001) was found in the distribution of the frequencies, probably reflecting a different population history. On the opposite, the 28 (14%) identical rCRS HVSI Italian samples showed the same distribution of H subhaplogroups, if compared with the Spanish ones. These results confirm the utility of this SnaPshot minisequencing assay to increase the discrimination power of HVSI/II sequencing analysis. In fact, the most frequent Italian mtDNA haplotype (CRS, 263G, 315.1C), shared by 8 individuals belonging to the same haplogroup H, was discriminated by the SNPs analysis and subdivided in three H subtypes (H* = 3, H1 = 4, H3 = 1). The SnaPshot approach can be used as a rapid screening method before sequencing, especially if many forensic or reference samples have to be analysed.

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