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THE INTEREST OF MITOCHONDRIAL DNA CODING REGION SNPS

Abstract: As analysis of mtDNA hypervariable regions is a laborious technique, it is desirable to type mtSNPs before performing sequencing. This study presents a 10 SNP multiplex, selected from mitochondrial coding region *loci*. These markers can subdivide any population samples in H* and non-H* haplogroups, assigning non-H* haplogroups, which in same cases is useful for forensic casework. The SNP multiplex detected 13 different non-H* haplogroups encountered in the two most common populations in our casework – European and African ancestry populations. Haplogroups U, J, T and L1/L2 are the most frequent non-H* haplogroups in the Portuguese population samples. A probable new variant was assigned as a U/K haplogroup. This coding SNP assay is a simple method and can increase the discrimination of hypervariable region haploypes.

Keywords: MtSNPs; coding region; SNaPshot analysis; non-H* haplogroups.

Introduction

In recent years, the interest in autosomal, Y-chromosome and mitochondrial Single Nucleotide Polymorphisms (SNPs) has increased in the forensic area. Mitochondrial DNA (mtDNA) is useful for identity testing and, above all, for analysis of degraded material or few DNA containing samples, such as skeletal remains or hair shafts. Analysis of mtDNA HVI and HVII hypervariable regions is sometimes the only available method in forensic casework but provide limited power of discrimination besides being a laborious technique. Coding mtSNP multiplex reaction prior to sequencing analysis can allow for a rapid screening in forensic casework (1). In this study we have selected 10 SNP *loci*, performed in one multiplex assay, for mitochondrial DNA non-H* haplogroup typing of the two most common populations in our casework – European and African ancestry populations.

Material and Methods

DNA was extracted from blood stains by Chelex method in a total of 80 Portuguese population samples studied. HVI and HVII regions were previously sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). A preliminary

classification of sample sequences into haplogroups was done following phylogenetic criteria. Ten non-H* mtSNP *loci*, selected from previous panels from other authors (1-3), were studied with single base extension using SNaPshot® methodology (Applied Biosystems). SNP *loci* selected were the following – G1719A, C3594T, T4216C, G4580A, C7028T, G8251A, A10398G, C10400T, C12705T and A12308G. Amplification products were analyzed in a 3130 Genetic Analyzer with GeneMapper® ID Software v3.2 (Applied Biosystems).

Results

Using this 10 SNP target site multiplex assay, several haplogroups can be detected – U, J, T, I, K, X, W, M, N, V, HV, L3, L1/L2 and H* (Fig.1), some of them shown in Fig.2, which agreed, in the majority of the samples studied, with haplogroups previously obtained from mitochondrial haplotypes. H* haplogroup is caracterized by C7028 and non-H* haplogroups are defined by different SNP polymorphisms as shown in Table1. Haplogroups U, J, T and L1/L2 are the most frequent non-H* haplogroups in the Portuguese population sample, as previously emphasized by HVI and HVII hypervariable region studies. It was not possible to assign a defined haplogroup (U or K) in two samples, as the 10398 SNP *locus* was not detected.

Discussion and Conclusions

This 10 *loci* SNaPshot® multiplex provides a less expensive and simpler method for coding region mtSNP typing compared to control region mtDNA sequencing. With this set of coding mtSNPs it is possible to subdivide any population samples in H* and non-H* haplogroups, assigning non-H* haplogroups, which has been done previously for H* haplogroups (4) and in same cases is useful for forensic casework analysis. With these selected markers, 13 non-H* haplogroups can be detected including the most common European and African population non-H* haplogroups. However, in two samples, it was not possible to assign a defined haplogroup (U or K), as the 10398 SNP *locus* was not detected, probably due to a mutation near this *locus* in our population. The new variant was assigned as a U/K haplogroup. This panel increases phylogenetic haplogroup discrimination which can be very useful for forensic casework, as also emphasized by other authors (5-7).

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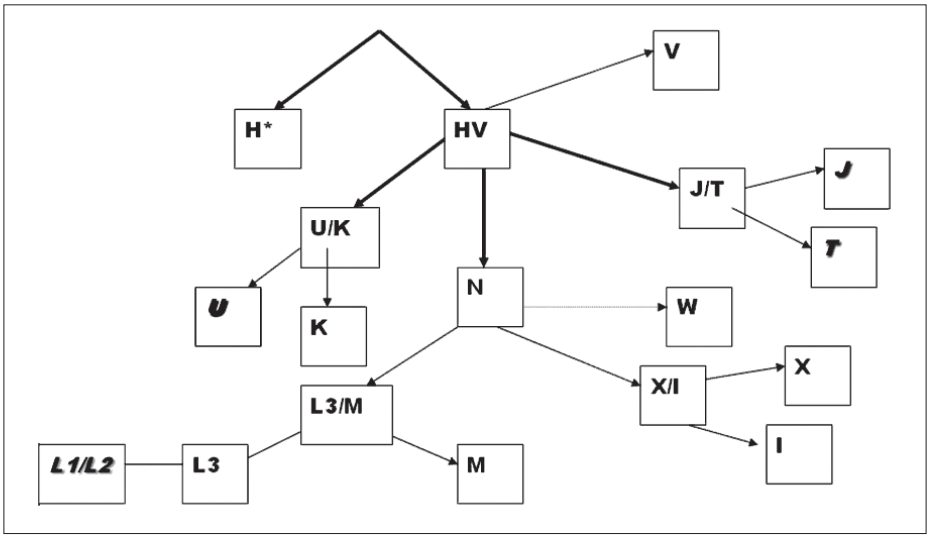

Figure 1 – Mitochondrial phylogenetic tree determined with 10 mtDNA coding region *loci,* emphasizing J, U, T and L1/L2 haplogroups, the most common non-H haplogroups in our population study

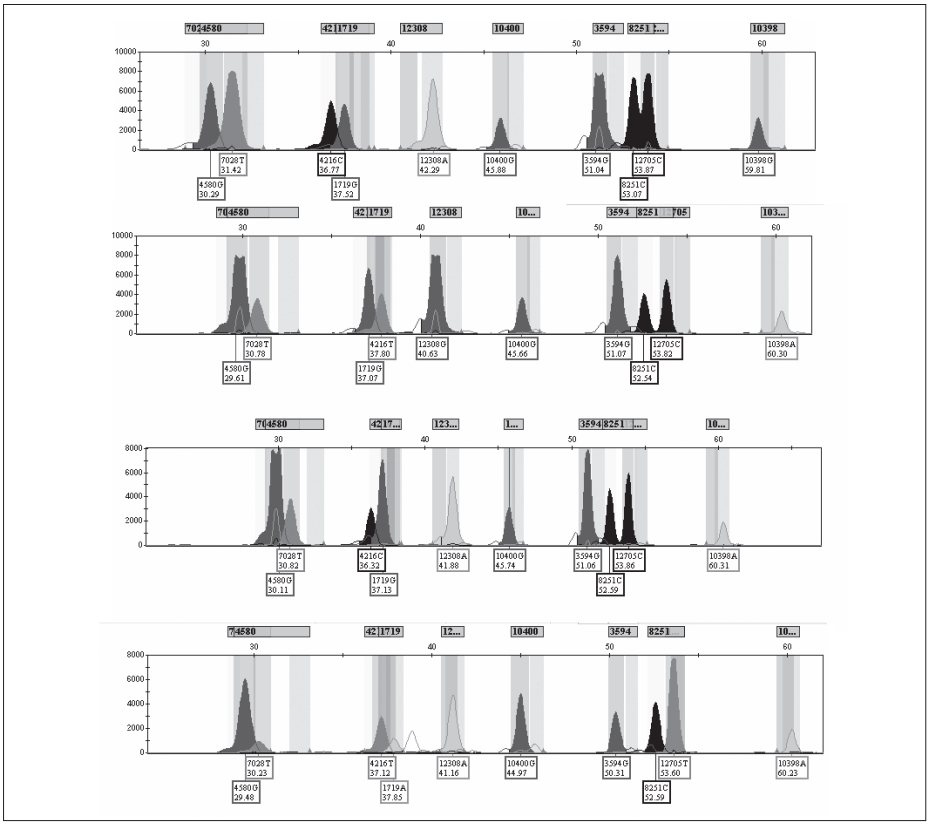

Figure 2 – Several mtDNA haplogroups can be obtained with 10 *loci* SNaPshot multiplex as shown, respectively, for J, U, T and X haplogroups.

G4580A	C7028T	G1719A	T4216C	A12308G	G10400A	G3594A	C8251T	C12705T	A10398G	Haplogroup
G	T	G	T	A	G	A	C	T	G	**L1/L2**
G	T	G	T	A	G	G	C	T	G	**L3***
G	C	G	T	A	G	G	C	C	A	**H***
G	T	G	T	G	G	G	C	C	**?**	**U*/K**
G	T	G	T	G	G	G	C	C	A	**U***
G	T	G	T	G	G	G	C	C	G	**K**
G	T	G	C	A	G	G	C	C	A	**T**
G	T	G	C	A	G	G	C	C	G	**J**
G	T	A	T	A	G	G	C	T	A	**X**
G	T	A	T	A	G	G	T	T	G	**I**
A	T	G	T	A	G	G	C	C	A	**V**
G	T	G	T	A	G	G	C	T	A	**N***
G	T	G	T	A	G	G	T	T	A	**W**
G	T	G	T	A	A	G	C	T	G	**M***
G	T	G	T	A	G	G	C	C	A	**HV***

Table 1 – SNP state for 15 different haplogroups – H* haplogroup is caracterized by C7028 and U*/K haplogroup by a possible mutation near the 10398 SNP *locus.*