MITHOCONDRIAL HVR1 POLYMORPHISM IN ITALY

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INTRODUCTION

Polymorphism within the mainframe of mitochondrial DNA sequence (mtDNA) is an interesting source of human molecular diversity. Most of the mtDNA sequence variation occurs at two regions (namely, HVRI and HVRII) encompassing a relatively short area (800 base pairs) of the entire mtDNA genome. Both have been used in studies addressing the structure and relationships of human populations (Vigilant 1989).
Recently, evidence has been given that there is considerable advantage in using mtDNA for forensics (Holland 1993, Piercy 1993), two hyperpolymorphic domains have been used to solve special problems of identification which would have not been possible to deal with nuclear loci. To properly exploit the potential informativeness of mtDNA, an adequate database of the appropriate population should be available as reference for statistical analysis. We recently undertook a study aimed at determining the sequence polymorphism shown by our reference population (Central and Southern Italy) at HVRI domain of d-loop region. In this report, we give information on 74 individual sequences.

MATERIALS AND METHODS

MtDNA was extracted from whole blood samples from 74 unrelated individuals. To amplify HVRI fragment, two separate sets of primers were used (L15990 and H16239 for set 1 and L16159 and H16410 for set 2; primers sequence is summarized in table n.1), each generating a 280 base pair length fragment. The two segments overlapped on a 50 base pair long region in the middle. All fluorescent primers were synthesized on a 391 PCR-mate (ABI) using a slightly modified cycle to label the 5'-end of one primer of any given pair by a fluorescein-amidite (FluorPrime TM, Pharmacia).
The PCR products were controlled on agarose, then they were purified and concentrated by a Microcon 30 spin-dialysis column to remove unincorporated primers. An aliquot of 50 nanograms template was administered to the TAQ cycle sequencing reaction and forward\reverse sequences were produced (sequencing primers as follow: L15997, H16236, L16163 and H16395; primers sequence is summarized in table n.1). Sequencing cycles (15 on average for good quality DNA) were specifically designed for each fluorescent-primers (1 μM each). The reaction products were denaturated for 10 minutes at 95°C, then loaded onto the ALF DNA sequencer (Pharmacia); electrophoresis was performed on a denaturing polyacrilamide gel (7M urea, 6%T) for 4-5 hours depending on the fragment length to sequence. The sequencing data were analyzed by internal ALF software facilities and sequences were alligned on the original Cambridge consensus sequence (Anderson 1981).

RESULTS AND DISCUSSION

All collected data and a shortenend version of the resulting database are shown in Fig. 1 and Table 2. As internal controls, mother-child samples were processed whenever possible. No mutation was observed in these cases.
A total of 55 different haplotypes were identified, containing 55 overall mutation sites. The majority of sequences were represented only once in the database. The most common genotype (identical to the Cambridge consensus sequence) was observed with a frequency of 12%. Except for eleven transversion, all polymorphisms were transitional events (with pyrimidine mostly involved). No insertion/deletion was documented. Most mutations (76 %) were C➔T or T➔C.
A comparison with previously published Caucasian data is in progress and will be detailed elsewhere.

REFERENCES

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Fig.1. Position of mutant nucleotides

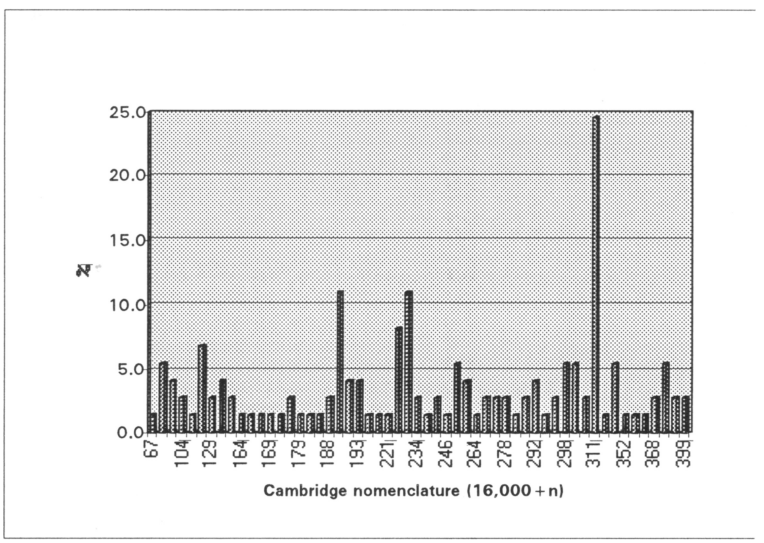

Table 1. PCR primers for the amplification and the sequencing reaction

Primers set	Forward primer L strand	Reverse primer H strand
I round I set	L15990: TTAACTCCACCATTAGCACC	H16239: TGCCTTTGGAGTTGCAGTTG
I round II set	L16159: TACTTGACCACCTGTAGTAC	H16401: TGATTTCACGGAGGATGGTG
Sequencing I set	L15997: CACCATTAGCACCCAAAGCT	H16236: CTTTGGAGTTGCAGTTGATG
Sequencing II set	L16163: TGACCACCTGTAGTACATAA	H16395: CACGGAGGATGGTGGTCAAG

Table 2. Most common haplotypes in 74 Italians

position	obs	%	16001	16224	16260	16304	16311	16410
Cambridge			A	T	C	T	T	A
hapl. 1	9	12.2	-	-	-	-	-	-
hapl. 2	6	8.1	-	C	-	-	C	-
hapl. 3	5	6.7	-	-	-	-	C	-
hapl. 4	2	2.7	-	-	T	-	-	-
halpl. 5	2	2.7	-	-	-	C	-	-
others	1 x 50	1.35 X 50						