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Analysis of SNP-variation in the coding region of mitochondrial DNA

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Abstract. A multiplex-PCR procedure was developed for the analysis of single nucleotide polymorphism (SNP) variation defining the European mtDNA-haplotypes in the coding part of the mitochondrial genome. The obtained PCR-products were genotyped for 26 SNPs in four mini-sequencing (SNaPshot™) reactions and analyzed on the ABI PRISM® 3100 Genetic Analyzer. A population study of 157 individuals of Belgian descent revealed 25 different haplotypes belonging to 5 major haplogroups. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Mitochondrial DNA (mtDNA) has been used extensively in the study of the origin and dispersal of modern humans. The geographical distribution of the lineages obtained from SNPs in the coding region of mtDNA and/or control region sequences (HV1) can detect prehistoric movements from one region to another [1]. SNP-typing in the coding region has been based on digestion of PCR products with a battery of 14 different restriction enzymes (RE), which is a tedious method when a large number of samples must be typed. In contrast, primer-extension or mini-sequencing with analysis on automated DNA sequencers provides the possibility for rapid and automated typing of several SNPs in one reaction [2]. In this study, a SnaPshot™ assay was developed for the analysis of mtDNA SNPs characteristic for the West-Eurasian lineages.

2. Materials and methods

The regions containing the SNPs were amplified in four multiplex PCR-reactions, each with four to six amplicons (between 233 and 611 bp; Table 1). The amplification was done in a 25-μl reaction containing 1.5 mM $MgCl_2$, 200 μM dNTPs, 2.5 U AmpliTaq Gold DNA-polymerase, 1 × PCR-Gold-buffer, primers (Table 1) and 1 ng DNA, with denaturation for

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Table 1
Haplogroup motifs and SNPs defining the most important West-Eurasian clusters

Haplogroup	Subgroup	RFLP motif[a]	SNP	SNaпShot	PCR fragment[b]
H		− 7025*Alu*I	T7028C	4	H6764/L7228
		− 14766*Mse*I	T14766C	2	H14293/L14905
V		− 4577*Nla*III	G4580A	1	H4147/L4719
		− 14766*Mse*I	T14766C	2	H14293/L14905
U		+ 12308*Hin*fI	A12308G	3	H12270/L12765
	K	− 9052*Hae*II/ − 9053*Hha*I	G9055A[c]	2	H8726/L9163
		+ 10394*Dde*I	A10398G	3	H9952/L10504
	U1	− 4990*Alu*I	G4991A[c]	3	H4752/L5201
		–	A13104G	1	H13049/L13434
		13103*Hin*fI/ + 13104*Mbo*I	A14070G	4	H13595/L14102
		+ 14068*Taq*I			
	U2	+ 15907*Rsa*I	A15907G	2	H15511/L15972
	U4	+ 4643*Rsa*I	T4646C	1	H4147/L4719
		+ 11329*Alu*I	C11332T	3	H11175/L11409
J		+ 4216*Nla*III	T4216C	1	H4147/L4719
		+ 10394*Dde*I	A10398G	3	H9952/L10504
		− 13704*Bst*OI	G13708A	4	H13595/L14102
T		+ 4216*Nla*III	T4216C	1	H4147/L4719
		+ 4914*Bfa*I	A4917G	3	H4752/L5201
		+ 13366*Bam*HI	G13368A	1	H13049/L13434
		+ 15606*Alu*I	A15607G	2	H15511/L15972
		− 15925*Msp*I	G15928A	3	H15511/L15972
	T1	− 12629*Ava*II	C12633A[c]	3	H12270/L12765
I		− 4529*Hae*II	A4529T	2	H4147/L4719
		+ 8249*Ava*II/ − 8250*Hae*III	G8251A	1	H8102/L8575
		+ 10032*Alu*I	T10034C	2	H9952/L10504
W		+ 8249*Ava*II/ − 8250*Hae*III	G8251A	1	H8102/L8575
		− 8994*Hae*III	G8994A[c]	2	H8726/L9163
X		+ 14465*Acc*I	T14470C	4	H14293/L14905
M		+ 10394*Dde*I	A10398G	3	H9952/L10504
		+ 10397*Alu*I	C10400T	1	H9952/L10504
C		+ 10394*Dde*I	A10398G	3	H9952/L10504
		+ 10397*Alu*I	C10400T	1	H9952/L10504
		− 13259*Hin*cII/ + 13262*Alu*I	A13263G	4	H13049/L13434

[a] Macaulay et al. [3].
[b] Position of the primers refer to the 5′ end (H = heavy chain, L = light chain); PCR and SNaпShot™ primer sequences are available upon request.
[c] SNPs defined in this study.

10 min at 94 °C, 25 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and final extension of 10 min at 72 °C in a GeneAmp® PCR System 9700.

The primers for genotyping the 26 SNPs were selected with the READIT® Interrogation Probe Design software from Promega (http://www.promega.com/readit/probedesign/default.asp). The manual of the ABI PRISM® SNaпShot™ Multiplex Kit (Applied Biosystems) was followed for primer-extension. Sizing of the extension products was done in POP-6™ on an ABI PRISM® 3100 Genetic Analyzer and the results were analyzed with GeneScan® v3.7 and GenoTyper™ v3.7. A reduced median network (Fig. 1) was computed with the program NETWORK 3.1 (http://www.fluxus-engineering.com/sharenet.htm) after star contraction [4].

3. Results and discussion

The mutated position could be identified for 22 SNPs (Table 1) by comparing the RFLP motifs with the ‘‘Cambridge Reference Sequence’’ (CRS). The positions of the

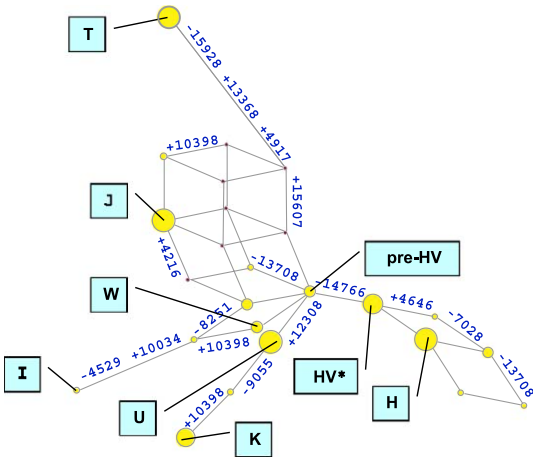

Fig. 1. Reduced median network of a Belgian population typed for 26 SNPs in the coding region of mtDNA. The position of the SNPs according to the CRS are indicated on the branches, while " + " and " – " correspond to the presence or the absence of the RE site.

remaining SNPs were determined by sequence analysis of DNA samples from individuals with a corresponding HV1 motif [3]. Validation of the developed methods in a population sample of 199 individuals revealed a result for all SNPs in 157 individuals. The remaining samples showed for at least one SNP no result indicating the need for further optimisation. In total, 25 haplotypes were identified resulting in a gene diversity of 0.81. The most common lineage present in this population was haplogroup H (42%), while the other common haplogroups were HV* (5.1%), U (including subgroup K and U4; 20.4%), J (18%) and T (8.9%). Pre-HV is identical to the CRS and is present at a frequency of 1.9%. A reduced median network of 18 different haplotypes revealed that pre-HV is the central haplogroup from which 5 clusters radiate—HV, J, T, U and IW—which is in accordance to other studies about European populations [1,3].

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