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FOR THE RECORD

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Distribution of mtDNA Haplogroups in a Population Sample from Poland

POPULATION: South part of Poland population sample ($n = 111$).

KEYWORDS: forensic science, DNA typing, population genetics, mitochondrial DNA; European haplogroups, Southern Poland, Caucasian

16 SNP positions localized in the coding region of human mtDNA were analyzed with the procedure described by Brandstätter et al. (1) on the 111 samples from unrelated individuals

$$\left(\mathrm{P(M)} = \frac{1}{n^2}\sum_{i=1}^{m} x_i^2(2) = 10.56\%\right)$$

DNA was isolated using standard organic method followed by double phenol/chloroform/isoamyl alcohol extraction and its concentration was determined fluorescently with PicoGreen on Fluoroscan Ascent Fl. Amplification was performed in two 8-plex reactions. PCR mixture consisted of 5 μL of Qiagen Multiplex PCR Master Mix, 1 μL of premixed primers (1), 1–4 μL of DNA extract (about 0.5 ng of DNA) and distilled water up to 10 μL. Reaction conditions were as follows: pre-incubation step: 95°C for 15 min; denaturation: 94°C for 30 s, annealing: 60°C for 90 s, extension: 72°C for 90 s; 30 cycles; final extension: 72°C for 10 min. The samples were amplified using GeneAmp PCR System 9700 or 9600 (PE Applied Biosystems). PCR products were detected by 3% agarose gel electrophoresis. PCR primers and unincorporated dNTPs were removed by Microcone YM-100 Centrifugal Filter Devices (Millipore) according to manufacturer's protocol. SNPs were detected in two multiplex minisequencing reactions. Each reaction mixture contained 5 μL of SNaPShot Multiplex Ready Reaction Mix (Applied Biosystems), 1 μL of pooled extension primers (1), 2 μL of PCR product and distilled water up to 10 μL. Thermal cycling and post-extension treatment were conducted following manufacturer's directions. Unincorporated ddNTPs were removed with SAP (shrimp alkaline phosphatase, MBI Fermentas). Electrophoresis and data analysis was carried on ABI 3100 Avant Genetic Analyzer (Applied Biosystems). Each well contained 20 μL of formamide, 2 μL of GeneScan-120-LIZ Size Standard (Applied Biosystems) and 0.5 μL of SNaPShot product. Data was analyzed with GeneScan and Genotyper software (Applied Biosystems). The Genotyper macro was kindly supplied by Walther Parson (1).

One hundred and six out of 111 samples can be assigned to nine of major European haplogroups and 5 do not fit into these predefined groups. Single heteroplasmic position was detected in one sample. The most common haplotype concordant with rCRS can be found in 21.6% of population. Totally 26 different lineages were revealed among 111 samples. Fourteen of them are represented only by one sample. The frequencies characteristic for particular haplogroups revealed during this study are concordant with data for other European population samples (1,3–6). We have also successfully tested that method when examining problematic material such as degraded bone samples and single hair shafts. Therefore it can be said that the method proved to be useful in real casework.

Results are listed in Table 1. Access to data—The complete dataset is available to any interested researcher upon request at e-mail: wbranic@ies.krakow.pl.

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TABLE 1—*mtDNA haplotypes in a population of Southern Poland (n = 111).*

Haplogroup	Haplotype	No. of Samples	%
H	7028C (no difference with rCRS)	24	
H	7028T	3	
H	709A – 7028C	1	37.8
H	709A – 7028C – 8251 A	1	
H1	3010A – 7028C	12	
H1	3010A – 7028C – 14766T	1	
I	1719A – 7028T – 8251A – 14766T	2	1.8
J	7028T – 11251G – 13708A – 14766T	1	
J	3010A – 7028T – 11251G – 13708A – 14766T – 14798C	4	7.2
J	709A – 3010A – 7028T – 11251G – 13708A – 14766T – 14798C	2	
J	3010A – 7028T – 11251G – 13708A – 14766T	1	
K	1811G – 7028T – 9055A – 12372A – 14766T – 14798C	8	7.2
T	709A – 7028T – 8697A – 11251G – 14766T	12	10.8
U	7028T – 12372A – 14766T	15	
U	1811G – 7028T – 12372A – 14766T	9	22.5
U	7028T – 9055A – 12372A – 14766T	1	
V	7028T – 15904T	4	4.5
V	7028T – 8697A – 15904T	1	
W	709A – 7028T – 8251A – 14766T	2	1.8
X	1719A – 7028T – 14766T	1	1.8
X	1719A – 7028T – 13708A – 14766T	1	
Others	1811G – 7028T – 9055A – 14766T – 14798C	1	0.9
Others	1811R – 7028T – 14766T	1	0.9
Others	7028T – 14766T	1	0.9
Others	3010A – 7028T – 14766T	1	0.9
Others	7028T – 12372A	1	0.9
Total		111	100

Note: Individuals were assigned to haplogroups according to the bifurcating decision tree presented in (1). Determined differences according to the rCRS are specified. In case of position 7028 both allelic variants are included. Examined individuals that did not fit to none of the European haplogroups were classified as "others." Heteroplasmic position 1811 detected in one sample is marked with R.

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