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Collaborative exercise on mitochondrial DNA by laboratories in the GEFI group ☆

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Abstract. This work presents preliminary results concerning a collaborative exercise on mitochondrial DNA by laboratories of the GeFI group. © 2003 Published by Elsevier B.V.

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1. Introduction

Since 1991, the Italian Group of Forensic Genetists (GeFI) has carried out several collaborative exercises aiming at study of DNA polymorphisms, in order to standardize analytical procedures and to set up Italian databases for use in forensic cases of personal identification and controversal paternity. The present work illustrates results obtained during such an exercise on analysis of hypervariable regions HVR1 and HVR2 of mitochondrial DNA. The exercise consisted of typing three blood stains, of which one was used as a positive control, and samples from at least 50 subjects, locally resident and not related to each other, for each participating laboratory. A satellite project was also linked to the exercise, involving analysis of at least 20 mother–child pairs.

2. Materials and methods

Eleven laboratories, all belonging to centres for forensic genetics in Northern and Central Italy, participated in the project. The sample population was composed of 593 subjects, distributed as follows: Northern Italy: 50 Bologna (BO), 50 Brescia (BR), 51 Florence (FI), 56 Genova (GE), 50 Modena (MO), 50 Padova (PD), 50 Pavia (PV), 50 Turin (TO); Central Italy: 83 Ancona (AN), 53 Rome (RM), 50 Terni (TR). Seven laboratories participated in the satellite project, analysing 173 mother–child pairs: 23

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Table 1
Materials and methods used by 11 participating laboratories

DNA extraction	Phenol–chloroform (AN-BO-GE-PV-RM-TO)	Chelex (AN-BO-BR-MO-RM)	Other (FI-MO-PD-TR)
DNA quantification	Quanti-blot (AN-BO-BR-MO-TO)	Agarose minigel (AN-GE-RM-TR)	Spectrophotometer (AN-FI-PD-PV-TR)
PCR conditions	Holland '94 [1] with minor modifications		
Sequencing			
Dye terminators	ABI 310(AN-BO-BR-PD-PV-RM-TR-TO)	ABI 377 (MO-GE)	
Dye primers	LICOR 4200 (FI)	ABI 377 (MO-GE)	ALFexpress (RM)

Ancona, 50 Bologna, 20 Modena, 20 Padova, 20 Pavia, 20 Rome and 20 Turin. Table 1 shows materials and methods used by the participating laboratories.

3. Results

Of the 11 laboratories taking part in this exercise, 2 did not correctly type the control stains. Observed mutations (Table 2) included substitutions, with transitions prevailing over transversions in both polymorphic regions, a well-known phenomenon in mitochondrial DNA. Heteroplasmic positions were observed and related to: the HVR1 and HVR2 of one sample of the study population study, i.e., 16366 C (T-70%; C-30%) and 199 T (T-70%; C-30%); the HVR2 of three mother–child pairs: 199 T (Mother T-70%/C-30%; Child T-50%/C-50%); 251 g (Mother A-70%/g-30%; Child A-40%/g-60%); 204 T (Mother T; Child T-60%/C-40%). Heteroplasmy was observed at a frequency—both in study population and mother–child pairs—which was lower than that found in similar

Table 2
Observed mutations in an Italian sample population of 593 individuals

Mutations	HVR1		HVR2	
	Number of positions	Total number of mutations	Number of positions	Total number of mutations
Transitions				
C–T	65	568	23	156
T–C	34	617	25	382
A–g	34	102	23	957
g–A	14	96	13	106
Total	147	1383	84	1601
Tranversions				
C–A	11	15	4	4
A–C	8	36	3	5
C–g	6	12	–	–
g–C	2	4	–	–
T–g	1	1	1	2
g–T	1	1	1	1
A–T	7	9	1	1
T–A	–	–	3	3
Total	36	78	13	6
Insertions				
+1C	1	2	4	826
+2C			1	56
+1T			2	3
+1A			1	1
Total	1	2	8	886
Deletions				
−1A			1	1
Total			1	1

Table 3
HVR1/HVR2 haplotype frequency spectrum of 593 individuals from 11 Italian regions

Number of individuals per haplotype	North								Center		
	BO	BS	FI	GE	MO	PD	PV	TO	AN	RM	TR
7									1		
4											1
3	1		1					1		1	
2	3		2		1		3		2	2	3
1	41	50	44	56	48	50	44	47	72	46	40
% Unique haplotypes	82.0	100	86.3	100	96.0	100	88.0	94.0	86.7	86.8	80.0
Number of different haplotypes (*H*)	45	50	47	56	49	50	47	48	75	49	44
%	90.0	100	92.2	100	98.0	100	94.0	96.0	90.4	92.5	88.0
Number of individuals (*N*)	50	50	51	56	50	50	50	50	83	53	50
H/*N*	0.90	1	0.92	1	0.98	1	0.94	0.96	0.90	0.92	0.88

previous studies [2]. Table 3 shows a summary of the diversity found in each sample in terms of the number of haplotypes that have been observed in one individual, in two individuals,. . ., in *k* individuals (the frequency spectrum of the haplotypes).

A simple parameter related to haplotype diversity is the rate (*H*/*N*) of the total number of different haplotypes, *H*, to the total number of individuals in a sample. One sample only showed a value of this parameter lower than 90%, whereas all sequences were unique in three subsamples (*H*/*N* = 100%). All the six instances of haplotypes observed in three or more subjects (top three data rows in Table 3) included the two most frequent haplotypes observed in the global sample, namely ''CRS,263G,309insC,315insC'' and ''CRS,263G,315insC''. These two haplotypes were observed in 25 and 13 individuals of the global sample, respectively, and together represented 6.4% of it. The ratio *H*/*N* in the total sample was 460/593, or 84.7%, and the percentage of unique haplotypes was 74.6%, testifying a situation very far from sample saturation (when practically all haplotypes existing in a population are present in a sample).

4. Discussion

Most of the errors in published mtDNA sequences have been noted [3], with grave consequences for the purposes at which forensic investigations aim. For these reasons, the results of this exercise emphasize the need to continue collaboration and quality control exercises, for: (a) retesting of original pherograms by each laboratory; (b) establishing a task force for centralized collection and checking of pherograms; (e) verification of whether haplotypes are in line with European haplogroups.

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