Report of three further collaborative exercises on STR loci by the Italian Group of Forensic Hematology

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Allele frequencies at the CD4, TPOX, and CSF1PO loci are determined in Italy by a collaborative study involving 19 laboratories and about 2,000 individuals.

1.INTRODUCTION

Previous collaborative exercises conducted by the GEFI (the Italian forensic hematology group) considered the DQA1, FES, VWA31/A, TH01, and D1S80 loci. We report here the results of three further studies, involving the CD4, TPOX, and CSF1PO loci (the last two were co-amplified). The objectives of the exercises were: (1) to determine the allele frequencies in Italy, (2) to assess the presence of gene frequency heterogeneity in different regions, (3) to verify the suitability of these systems in forensic analysis, and (4) to validate and homogenize the analytical methods used by different laboratories throughout Italy.

2. MATERIAL AND METHODS

Participant laboratories were required to genotype at least 50 subjects born in their region, and two blind controls. The choice of the analytical method (automated or manual) was left to each laboratory, with the only restriction that TPOX, and CSF1PO had to be co-amplified.

Heterogeneity between phenotype and allele frequencies was assayed by Pearson chi-square (two-way contingency tables); small-expectations were summed together to avoid cell values lower than 3.0. Hardy-Weinberg equilibrium (HWE) and homozygosity were tested in each laboratory and in the total sample by Pearson chi-square. In the case of HWE, genotypes with expected values <3.0 were pooled (the number of degree of freedom was F

– A, F being the number of phenotypic classes and A the number of allele frequencies remaining after pooling). Italian allele frequencies were calculated by summing the absolute frequencies in each region, and weighting the relative regional frequencies by population size (data source: ISTAT 1998).

3. RESULTS

3.1 CD4

Nineteen laboratories sent results, pertaining to 2,027 individuals. They included 31 different phenotypes and 10 alleles. 88% of the entire sample was composed by 6 high-frequency phenotypes (>10%), whereas the other phenotypes were rare (>2.5%). The overall chi-square for phenotype heterogeneity was not significant. However, the Sardinian sample gave the highest contribution and was significant of heterogeneity, when assayed against continental Italy (χ^2 = 17.2, 6 d.f., P = 0.009). HWE was tested in each laboratory and in the total sample, considering the six most frequent phenotypes (including three alleles) and the cumulative group "others". Four laboratories were out of equilibrium with P<0.05, and another three with P<0.01. The global sample was near the level of 0.05. The homozygosity test was positive for two samples at P<0.05, and for another two at P<0.01. The total sample showed a clear excess of homozygotes (χ^2 = 7.64, 1 d.f., P = 0.006). Heterogeneity of allele frequency was assayed on the four more frequent alleles, including 97% of all sampled chromosomes) and the cumulative group "rare alleles". The overall value was marginally significant (P=0.085). The Sardinian sample was again the most differentiated.

3.2 CSF1PO

Seventeen laboratories sent results, for a total of 1,983 individuals (32 different phenotypes and 10 alleles). The distribution of phenotype frequency was smooth and there was no clear-cut distinction between phenotypes with high and low frequency. The overall chi-square for phenotype heterogeneity was not significant, but the Sardinian sample was remarkably distinct (χ^2 = 17.5, 6 d.f., P = 0.008). HWE was satisfied in each laboratory, as well as in the total sample. The homozygosity test was significant in one sample (P=0.004). but not after Bonferroni's correction. There was significant heterogeneity of allele frequency (χ^2 = 90.02, 64 d.f., P = 0.018). The Sardinian sample was the most differentiated.

3.3 TPOX

Seventeen laboratories sent results, for a total of 1,996 individuals, including 21 different phenotypes and 8 alleles. 90% of the sample included the 7 most frequent phenotypes, determined by 4 alleles. Only the test on allele frequencies was significant for heterogeneity (χ^2 = 86.47, 64 d.f., P = 0.032).

Table 1
Allele frequencies (x 10,000) in Italy

Allele	CD4	CSF1PO	TPOX
5	3,233		
6	3,165		8
7	25	12	10
8	22	55	5,072
9	95	421	1,214
10	2,995	2,681	786
11	311	3,032	2,535
12	144	3,146	363
13	4	586	12
14	6	59	
15		7	
16		1	
Total	10,000	10,000	10,000

3.4 Allele frequencies in Italy

Table 1 shows the allele frequencies (x 10,000) computed in Italy on the basis of the present study. The Sardinian sample was excluded.

3. CONCLUSIONS

The TPOX and CSF1PO loci are validated for use in forensic medicine in Italy. The CD4 locus is also validated, although the possible high level of population structure that emerges from data may justify further population genetics analyses.