PROFILING A NORTH-EAST ITALIAN POPULATION BY FOUR HIGHLY POLYMORPHIC DNA PROBES

P. Fattorini*, F. Florian§, S. Tafuro§, F. Cossutta*, B.M. Altamura* and G. Graziosi§

*Istituto di Medicina Legale e delle Assicurazioni dell'Universita' di Trieste, Trieste (Italy)
§Dipartimento di Biologia dell'Universita' di Trieste, via Giorgieri 5, 34100, Trieste (Italy)

INTRODUCTION

After the forensic validation of the SBA (Southern Blot Analysis), the use of SLPs (single locus probes) has been used in the forensic laboratory for personal identification and paternity testing since the end of the 1980s. Even if expensive and time consuming, the SBA remains the most informative approach in some circumstances, for example in those paternity cases where either the alleged father is unavailable or the paternity index achieved by PCR-based polymorphisms is too low to prove paternity. In this work the allele frequenciy distributions at four highly polymorphic loci (D1S7, D7S21, D12S11 and D7S22) are described in a population living in the Trieste and Gorizia areas (North-East Italy).

MATERIALS AND METHODS

Profiling the samples High molecular weight DNA was purified from Na_2EDTA blood samples from randomly selected donors living in North-East Italy following standard procedure. About 2 µg of each DNA was fully digested with Hinf I and the samples were then electrophoresed according to the following conditions: 20 cm 0.7 % agarose gel in 1xTBE buffer (134 mM Tris, 75mM H_3B0_3, 2.5 mM Na_2EDTA) at 40 V until all fragments <2 Kb had run off the gel. Southern blot, hybridisation and washing were carried out following established conditions; the purified fragments of the clones λMS1 (locus D1S7), λMS31 (locus D7S21), λMS43 (locus D12S22) and pλg3 (locus D7S22) were P^{32} labelled and used as probes (Wong, 1987). All the samples showing only one fragment were redigested and run through a 20 cm 0.85 % agarose until the 0.5 Kb marker was at 3 cm from the end of the gel. This procedure was introduced both to avoid bands running off the gels and to obtain a good resolution of the low molecular weight fragments. The samples showing only one band even after the second electrophoresis were run again through a 25 cm 0.6 % agarose gel until the 3 Kb marker was at the end of the gel. This last precaution was taken to show the possible coalescence of the bands (or pseudohomozygosity condition). The DNA of the cell line K562 was used as a reference standard.

Sizing the DNA fragments The films were scored independently by two operators and the migration of the DNA fragments was measured by a ruler following conventional methods (Gill, 1991; Pascali, 1992). The P^{32} 1Kb ladder was used as reference molecular weight marker. The fragment size was determined by he method of Elder and Southern (1987).

Creating the database For each locus studied, two databases were created. The first contains the observed genotypes and the molecular weight of the alleles expressed in bp. In order to estimate the allele frequency distributions, a second database was created and the fixed bin method was adopted (Bubowle, 1991) with bins of 0,1 Kb.

Testing for Hardy-Weinberg Equilibrium The number of expected homozygotes (EH) at each locus was calculated by $EH= X1^2+ X2^2+ X3^2+ ...+ Xn^2$ where X1 is the frequency of the alleles at bin 1, X2 is the frequency of the alleles at bin 2, etc (Weir and Hill, 1993).

Comparison between the expected and observed values was carried out by χ^2 test when the expected frequencies were more than four.

RESULTS

The mobility of the fragments of the standard DNA K562 differed by less than +/- 2.5 % on different gels and therefore the molecular weight of the alleles detected in this study should fall in this range. The final results are summarised in Tab. 1. The allele frequency distributions at loci studied are shown in the Figures. These data refer to the results after the third cycle of electrophoresis and the number of "single band profiles" after each cycle of electrophoresis is reported in Table 2. However, no divergences from H-W equilibrium were observed at loci D7S21 and D7S22 while a low discrepancy was shown at locus D12S11 (Table 2).

DISCUSSION

The data reported here do not differ from those of others reports on the Caucasian populations as far as heterozygosity and allele frequencies are concerned. This fact is even more relevant if we take in account that the population studied is composed of a majority of Italians, a minority of Slovenians and minor contributions by Croats, Serbs, Greeks, Germans and Hungarians. These ethnic groups moved to the region centuries ago and, apparently, as reported both by historical studies and blood group analyses, did not give rise to apparent population stratification. However, in the first years after the second World War, about 300.000 Italians moved from Istria and Dalmatia to our area and not less than 50-60.000 refugees settled in the Trieste area (more then 25 % of the inhabitants of Trieste). Therefore they would be expected to have contributed to modify the genetic structure to some degree. Nevertheless, our results, as well as those reported for the locus D2S44 (Fattorini, 1991), do not show stratification or subpopulation phenomena and suggest genetic homogeneity between our local population and other Caucasian populations. However, no deviation from H-W equilibrium could be demonstrated with the exception at the locus D12S11 but the reasons for this apparent excess of homozygosity have been pointed out (Devlin, 1990; Weir and Hill, 1993).

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Table 1. n: number of blood samples tested; Ht: heterozygosity; Kb: allele range.

locus	n	Ht	Kb
D1S7	332	0,9879	0,4-27
D7S21	340	0,9529	2,5-20
D12S11	339	0,9471	1,7-13
D7S22	332	0,9518	1,3-17

Table 2. Number of "single band profiles" observed after the 1st, the 2nd and 3rd cycle of electrophoresis (frequency in brackets). The results of the χ^2 test are reported in the last column; *: not carried out.

locus	1st	2nd	3rd	χ^2
D1S7	35 (0,105)	6 (0,018)	4 (0,012)	*
D7S21	19 (0,056)	19 (0,056)	16 (0,047)	3,239 (p>0,05)
D12S11	25 (0,073)	24 (0,071)	18 (0,053)	5,950 (p>0,05)
D7S22	96 (0,289)	17 (0,051)	16 (0,048)	0,201 (p>0,05)