Population genetic studies of six hypervariable DNA-Loci

A. Wilting, U. Hintzen, M.O.Völker, J. Bertrams

Institute of Laboratory Medicine and Microbiology, Elisabeth Hospital Essen, Germany

INTRODUCTION

Until recently, scientific investigations in cases of disputed parentage have relied upon techniques concerning gene products as HLA, red cell antigens, enzyme polymorphism, and proteins. Using recombinant DNA-techniques additional genetic polymorphism can be obtained and further exclude an alleged father from paternity. One group of genetic markers applied in disputed parentage cases refers to as DNA restriction fragment length polymorphism (RFLP). Independent of age, sex, and expression of gene products RFLPs are a helpful tool in typing new borns, children, and semen specimen. At the present time numerous genetic polymorphisms have been identified using various combinations of DNA-probes and resticton endonucleases. One obstacle in applying RFLP-techniques in forensic investigations is the lack of biostatistical evaluation of the detected polymorphism as well as gene frequency data of a given population. Here we report the allele frequency distribution of six hypervariable DNA-loci using probes pS 194, pL 159-1, pR 365-1, pL 355-8, pL 427-4, and pL 336 from ONCOR (distributed in Germany by IMMUCOR). PST I restricted probes were tested with 1200 DNA samples extracted from unrelated individuals from the German Ruhr area.

MATERIALS AND METHODS

DNA from 1200 (200 per probe) EDTA blood samples from unrelated individuals from the German Ruhr area were extracted according to the method of Miller et al. (1988) using non-toxic non-organic treatment. 10 μg of DNA sample were restricted with 140 IU PST I according to standard procedures (Maniatis et al. 1982) and electrophoresed 42 hours at 1.4 V/cm, except for probe pL 336 (66 h). Agarose concentration varied with respect to the applied probes, i.e.: 0.7% pS 194/ 355-8; 0.8% pL 159-1; 0.9% pL 427-4/ pR 365-1/ pL 336. Three biotinylated lambda size markers were loaded on the gel at the most-left, most-right and mid position. DNA samples were depurinated and denatured using 0.25 N HCl and 0.5 N NaOH/1.5 M NaCl, respectively and blotted onto ONCOR Sureblot nylon membranes using a PHARMACIA vacuum blotting device. Each sample was hybridized with six biotinylated single-locus probes in a BACHOFER rotating incubator at 42^{o}C over night. After stringend washing at 52^{o}C (probe pL 336: 70^{o}C) detection was carried out using non-isotopic alkaline phosphatase-streptavidin detection system of ONCOR. Fragment lengths were calculated by biotinylated lambda size markers restricted with Hind III, PSTE II and Sph I ranging from 23.1 to 2.2 kb.

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RESULTS AND CONCLUSIONS

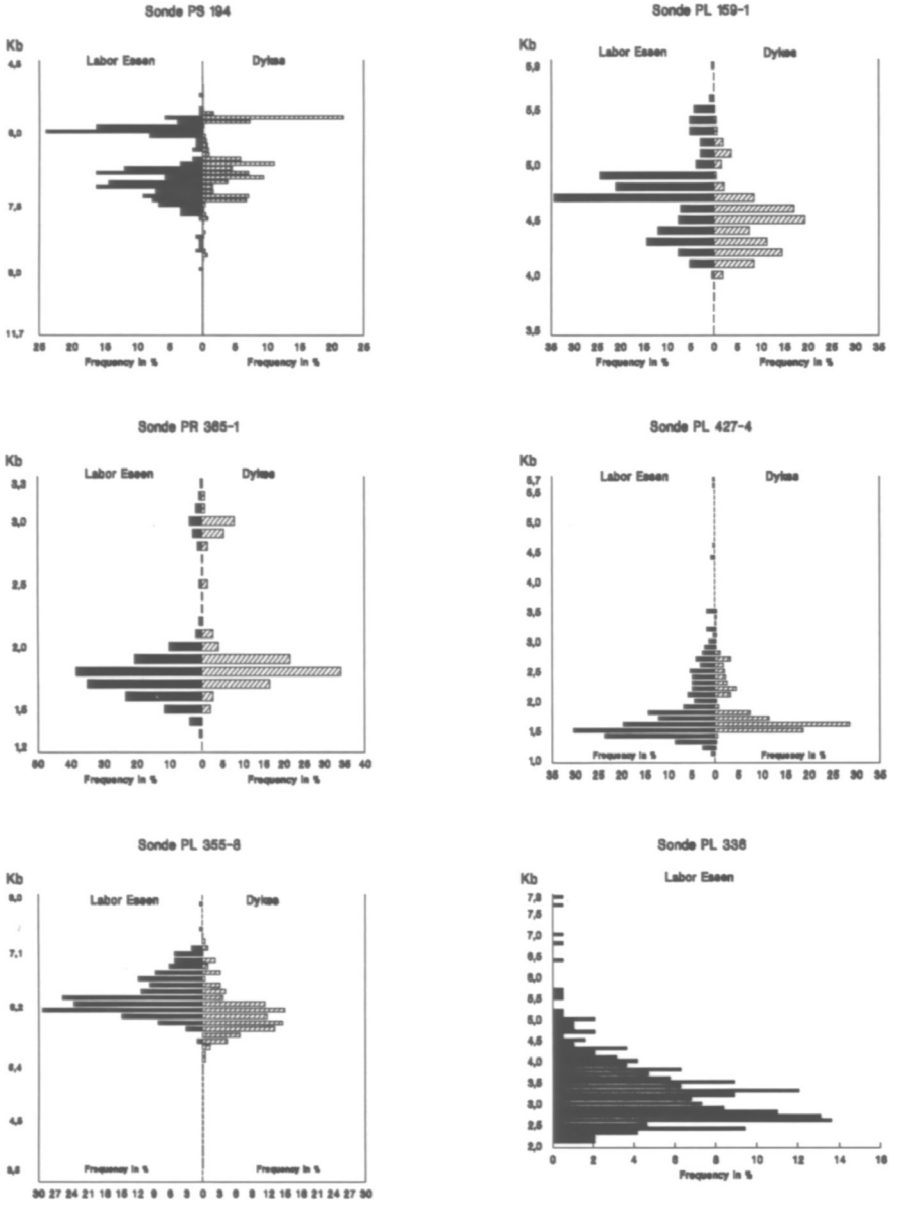
As revealed by allele frequency data plots, the 6 hypervariable single-locus probes presented here give satisfying frequency responses over a defined frequency range of 2-6 kb. The following figures show a comparison of the own data and the results obtained by Dykes (unpublished) in a Caucasian population from the United States. There are no striking differences concerning range and pattern of the frequency data produced by five single-locus probes. Some kb-shifts of frequency maxima ranging from 0.0 - 0.3 kb may be due to different evaluation procedures and are within statistical error. As can be depicted from Table 1 there are major differences in the frequency maxima of probes pL 355-8 and pL 159-1: the maximum frequency rate of the German population exceeds the correspondent of the United States by the factor of two.

Further investigations will show if this effect is due to population specificity or depends on the different population sample rate. Therefore biostatistical evaluation of data from disputed parentage cases should be based on the appropriate population allele frequency data.

Table 1. Comparison of frequency data of the tested probes obtained from two different Caucasian populations

Probe	N	Freq. range (kb)	mean freq.	Freq. max. (%)	kb	Heterozygocity
pS 194	**208**	**5.2- 9.0**	**5.8**	**24.0**	**6.0**	**0.85**
*	1908	5.0-12.5	1.5	22.0	5.7	0.85**
pR 365-1	**208**	**1.3- 3.3**	**9.4**	**38.4**	**1.8**	**0.71**
*	151	1.5- 3.3	5.35	34.0	1.8	0.66**
pL 355-8	**195**	**5.7- 7.9**	**9.8**	**29.2**	**6.2**	**0.70**
*	218	3.7- 7.4	2.64	15.0	6.2	0.83**
pL 159-1	**209**	**4.0- 5.9**	**8.9**	**34.4**	**4.7**	**0.62**
*	470	3.8- 5.5	5.52	19.0	4.5	0.74**
pL 427-4	**224**	**1.1- 5.7**	**6.2**	**30.3**	**1.5**	**0.94**
*	330	1.2- 3.5	3.9	28.0	1.6	0.94**
pL 336	**191**	**2.1- 8.9**	**4.1**	**14.6**	**2.5**	**0.97**
						0.88**

* Data obtained from Dykes (unpublished)
** Heterozygocity data published by ONCOR



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