PHENOTYPE AND ALLELE FREQUENCIES OF 4 VNTR-AMPFLP´S IN AN AUSTRIAN POPULATION SAMPLE

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Introduction

Microsatellite loci (VNTR, Variable Number of Tandem Repeats), a highly polymorphic system of non-coding marker loci dispersed throughout the whole human genome with constant rates were introduced to paternity and identity testing by Jeffreys et al (1988). Initially, typing has been carried out by Restriction Fragment Length Polymorphism (RFLP). Since this method has its limitaions and moreover, is time-consuming and difficult to perform and to analyze, typing by Amplification Fragment Length Polymorphism (AMPFLP, Budowle et al 1991) represents a major improvement to the typing of VNTR-loci. Since this is a simple and reliable technique and sufficently high exclusion chances can easily be achieved, it will presumably replace conventional systems for paternity and identity testing in the near future. Population and family studies that are necessary to select and to introduce new marker systems to forensic haemogenetics have already been performed by a number of workers(see Lit.). In this paper, we present the allele and phenotype frequencies of an Austrian population sample typed for the VNTR-loci ApoB, COL2A1, D1S80 and YNZ22.

Material and methods

Samples

DNA from healthy unrelated individuals (Austrian blood donors; ApoB: n=201, COL2A1: n=204, D1S80: n=200 YNZ22: n=206) was extracted from peripheral blood samples by a salting out procedure (Miller et al 1988).

PCR, PAGE, siver stainig

Details on the amplification, electrophoresis and detection of the samples are described elsewhere in this issue (Schwartz et al: Simple and rapid Typing for VNTR polymorphisms using high resolution electrophoresis of PCR products on rehydratable polyacrylamide gels).

Allele assignment

For D1S80 and YNZ22 allele assignment was achieved by direct comparison to allelic cocktails provided by B. Brinkmann and P. Wiegand. For COL2A1 and ApoB the samples were compared to each other and to a molecular weight standard first to identify different alleles and to establish a provisional nomenclature which was converted to the nomenclature published by other authors after all observed alleles were provisionaly assigned and allele frequencies were known. For D1S80, we decided to use still a local provisional nomenclature because of the high number of interalleles observed. This nomenclature just uses a capital V (Vienna) to assign provisional status.

Statistics

For both systems the heterozygosity index (HI) and the mean exclusion chance (MEC, Krueger et al 1968) was calculated. For testing for Hardy-Weinberg equilibrium, alleles had to be grouped together because of the small sample size and the high number of possible genotypes.

Results and discussion

With all systems, typing was easily possible after optimizing the electrophoretic separation protocol. The allele frequencies are listed in Tab 1. The allele assignment was based on direct comparison with allelic cocktails from other workers (Wiegand and Brinkmann) for D1S80 and YNZ22 and on comparison of the obtained with published allele frequencies for ApoB and COL2A1. Comparison of allele frequencies showed similar distribution for all systems and populations studied, taking into account the high number of individual alleles and the relatively small sample sizes. New alleles could be identified for D1S80 (n=5), ApoB(n=9) and YNZ22 (n=3). Furthermore, we observed two distinct variants of the D1S80-22 allele which were

provisionally designated V22F and V22S. No significant deviations from Hardy-Weinberg equilibrium were observed for any locus when using the approach of grouping together alleles to obtain expected phenotype frequency values of n>5. Then Heterozygosity Indices and the Mean Exclusion Chances are listed in Tab 2. The Combined Exclusion Chance for all 4 systems was calculated to 0.9892 which is of course sufficiently high enough to suggest the only use of these 4 VNTR AMPLFP systems for routine forensic paternity and identity testing. However, there is still a lot of work to be done (typing of larger population samples of different races, segregation studies, sequencing of alleles) until VNTR-AMPLFP markers can completely replace conventional marker systems.

Acknowledgements
The authors are grateful to B.Brinkmann and P.Wiegand for providing allelic cocktails for D1S80, YNZ22 and for other useful information.

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Tab 1 Allele Frequencies (%) for 4 VNTR Marker loci

ApoB (n=201)		COL2A1 (n=204)		D1S80 (n=200)		YNZ22 (n=206)	
27	0.7	01	1.0	V16	0.0	01	6.3
28	0.5	02	9.3	V17	0.0	02	13.1
29	3.5	03	0.0	V17M	0.0	03	13.8
30	0.5	04	1.5	V18	23.1	04	30.8
31	7.7	05	12.0	V18M	0.2	05	2.9
32	1.2	06F	3.2	V19	0.9	06	3.6
33	0.7	06S	24.8	V20	3.7	07	1.9
34	0.2	07F	9.3	V21	0.2	08	4.1
35	25.4	07S	3.4	V21M	0.9	09	8.0
36	0.7	08	30.4	V22F	4.2	10	8.7
37	33.8	09	3.9	V22S	0.4	11	2.7
38	0.0	10	1.2	V23	1.1	12	1.9
39	3.5	11	0.0	V23M	0.0	13	1.2
40	0.5			V24	35.5	14	0.5
41	2.2			V24M	0.0	15	0.2
42	0.5			V25	4.4		
43	0.0			V25M	0.0		
44	0.5			V26	1.3		
45	0.2			V26M	0.2		
46	0.5			V27	0.7		
47	6.0			V28	6.4		
48	1.2			V28M1	0.2		
49	8.2			V28M2	0.2		
50	0.0			V29	4.0		
51	1.0			V30	1.5		
52	0.0			V31	7.9		
53	0.5			V32	0.2		
				V33	0.2		
				V34	0.0		
				V35	0.0		
				V36	1.3		
				V37	0.7		
				V38	0.0		
				V39	0.0		
				V40	0.2		
				V41	0.0		
				V42	0.2		

Tab 2 Heterozygosity Index (HI) and Mean Exclusion Chance (MEC) for 4 VNTR marker loci

	ApoB	COL2A1	D1S80	YNZ22
HI	80.1%	81.0%	80.3%	84.1%
MEC	64.0%	64.7%	63.9%	76.5%