TYPING FOR STR-LOCI BY ELECTROPHORESIS ON REHYDRATABLE POLYACRYLAMIDE GELS: PHENOTYPE AND ALLELE FREQUENCIES OF SE33 AND TC11 IN AN AUSTRIAN POPULATION SAMPLE

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Introduction

Short tandem repeat (STR) microsatellite loci (Edwards et al 1991) are becoming increasingly important in forensic haemogenetics. Like the VNTR-AMPFLP microsatellite loci they represent highly polymorphic markers for genetic linkage studies, human identification and paternity testing and can be typed by direct high resolution polyacrylamide gel electrophoresis (PAGE). Because of their short overall size (100-500bp) STR loci may still be successfully amplified by PCR from highly degraded material (stains) when other methods fail to produce a typeable result. In order to establish procedures and references for reliable typing of the STR-loci SE33 (Polymeropoulos et al 1992) and TC11 (Edwards et al 1992) we optimized conditions for high resolution PAGE with subsequent silver staining of the amplification products and employed this method for typing a local population sample.

Material and methods

Samples

DNA from healthy unrelated individuals (Austrian blood donors; SE33: n=200, TC11: 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

In initial studies, the samples were compared to each other and to a molecular weight standard to identify the different alleles and to establish the optimal electrophoresis conditions. After that phase, allele cocktails (2 for SE33, 2 for TC11) were produced by coamplification of genomic DNA of selected individuals. In comparison to these allelic ladders all samples were typed using a provisional local nomenclature. After calculation of allele frequencies this nomenclature was aligned with the nomenclature suggested by Wiegand et al (1993) for SE33 and Edwards et al (1992) for TC11. Until confirmation we will use a capital V (Vienna) to indicate that direct comparison is still lacking.

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.

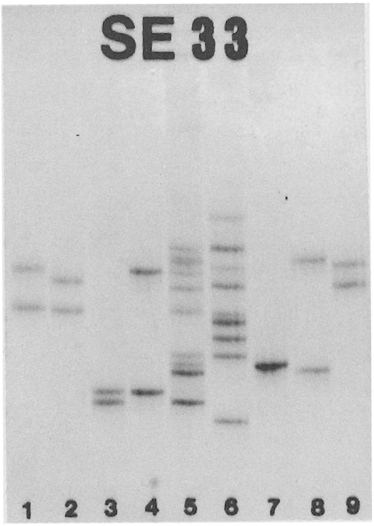
Results and discussion

Under optimized conditions, 30 alleles for SE33 and 8 alleles for TC11 were readily distinguishable. The mean range of separation between the smallest and the longest observed alleles was 23mm for SE33 (Fig.1) and 8mm for TC11. Allele frequencies obtained from our study are listed in Tab 1 and Tab 2. The phenotype frequencies (data not shown) showed good agreement with Hardy-Weinberg-expectations when using the approach of grouping alleles together in order to obtain expected phenotype frequency values of n>5. This method was repeated several times with different allelic groups and no siginificant deviation was observed. The Heterozygositiy Index was 94.8% for SE33 and 78.8% for TC11. The calculated MEC values give a combined exclusion chance of 97.7% (94.5% for SE33, 58.3% for TC11). The distribution of alleles was compared to the results of other workers after provisional alignment of the alleles as described and showed good overall agreement (Fig.2,Tab.2)

Tab 1 SE33 Allele Frequencies (n=200)

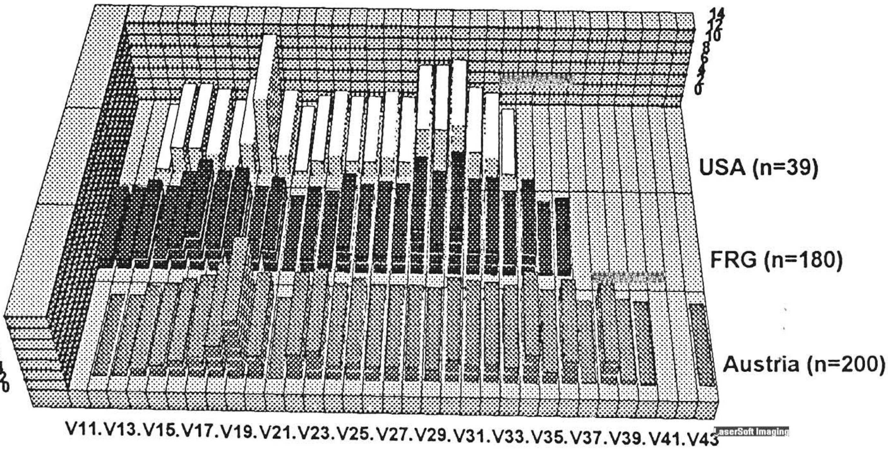
V11	0.0	V28	3.0
V12	0.2	V29	2.5
V13	0.2	V30	4.5
V14	2.0	V31	4.0
V15	2.5	V32	3.8
V16	3.5	V33	3.2
V17	4.3	V34	5.8
V18	6.5	V35	2.5
V19	11.2	V36	4.5
V20	4.7	V37	0.5
V21	0.2	V38	4.7
V22	5.0	V39	1.0
V23	5.8	V40	0.5
V24	3.0	V41	0.0
V25	4.0	V42	0.0
V26	3.0	V43	0.2
V27	3.0		

Fig 1 SE33 Typing by PAGE



Lane 5: Allelic Cocktail: V04, V07, V08, V09, V15, V19, V22, V24, V25, V27 Lane 6: Allelic Cocktail: V02, V09, V12, V14, V15, V20, V23, V27, V32 Lane 1: V16-V22, Lane 2: V15-V20, Lane 3: V04-V05, Lane 4: V05-V20, Lane 7: V08-V08, Lane 8: V07-V24, Lane 9: V19-V23

Fig 2 Comparison of SE33 allele frequencies from our study with data of Edwards et al (1992) and Wiegand et al (1993)



However, for SE33 the bimodal frequency distribution shows a marked shift of the high molecular weight alleles and we recognized a considerable number of new high frequency alleles in this range. We suppose that the enhanced electrophoretic separation that especially effects the high molecular weight alleles accounts for this phenomenon. Therefore this alignment is only speculative and sequencing studies are needed to establish a final nomenclature for SE33. For TC11, allele assignment was easily possible when comparing to the frequency values of other authors (Tab 2). Two new low frequency alleles (V04,V05) could be identified. However, we will still use our own nomenclature since we feel that a definitve nomenclature for any VNTR-marker should be based on sequencing data. Reamplifiable allelic cocktails, composed of alleles with known sequence as well as standardized electrophoretic separation conditions for each marker system are needed to unambigously type for VNTR loci. Under these prerequisites, we think that typing for STR loci by high resolution PAGE will become a reliable and effective method in forensic haemogenetics in the future.

Tab 2 TC11 Allele Frequencies

	AUSTRIA (n=206; this study)	FRG (n=110; Wiegand et al 1993)	USA (n=40; Edwards et al 1992)
V04	0.2	0.0	0.0
V05	0.2	0.0	0.0
V06	23.1	20.7	26.2
V07	16.3	18.0	8.8
V08	11.2	12.6	11.3
V09	19.2	17.1	16.2
V10	28.9	30.2	36.2
V11	1.0	1.4	1.3

Acknowledgements

The authors are grateful to B.Brinkmann and P.Wiegand for providing useful information.

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