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Population genetics and forensic efficiency data of 4 AMPFLP's

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Summary. Family studies were carried out in a population sample from north west Germany using 4 amplifiable VNTR polymorphic systems D1S80 (MCT118), ApoB, D17S30 (YNZ22) and COL2A1. Separation was carried out in polyacrylamide gels and visualised using silver staining. In family studies ($n = 30$) no evidence of new mutations was found. The population study of unrelated individuals (mothers and putative fathers) showed that all 4 systems were highly polymorphic and similar to other population studies. The combined exclusion chance was calculated to be approximately 99% and the combined discrimination index $1.5 \cdot 10^{-4}$. The Hardy-Weinberg equilibrium was checked by forming groups of alleles and no significant deviations could be found in all systems.

Key words: AMPFLP's – PCR – Allele frequencies – Discrimination index – Hardy-Weinberg equilibrium

Zusammenfassung. Untersucht wurden Familien einer nordwestdeutschen Bevölkerungsstichprobe mit 4 amplifizierbaren VNTR-Polymorphismen (MCT118, ApoB, YNZ22, COL2A1). Die Darstellung der Systeme erfolgte in Polyacrylamidgelen durch Silberfärbung. Es fand sich kein Hinweis auf Neumutationen. Anhand der typisierten Unverwandten (Mütter und Putativväter) konnten für jedes System Allelfrequenzen ermittelt werden, die im Vergleich zu anderen Bevölkerungsstichproben relativ gute Übereinstimmungen aufwiesen. Die Berechnung der kombinierten Ausschlußchance für die 4 Systeme führte zu einem Wert von etwa 99%. Der dazu korrespondierende Diskriminationsindex betrug $1{,}5 \cdot 10^{-4}$. Zur Überprüfung des Hardy-Weinberg-Gleichgewichts wurden Allelgruppen gebildet. Für alle 4 Systeme konnte das Hardy-Weinberg-Gleichgewicht nachgewiesen werden.

Schlüsselwörter: AMPFLP's – PCR – Allelfrequenzen – Diskriminationsindex – Hardy-Weinberg-Gleichgewicht

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Introduction

The first relevant application of the polymerase chain reaction (PCR) methods for identity testing were investigated on sequence polymorphisms (Saiki et al. 1985, 1988; Higuchi et al. 1989) and on fragment length polymorphisms (Jeffreys et al. 1988). The so-called AMPFLP's (amplifiable fragment length polymorphisms) belong to the latter group of polymorphic systems and include the following 4 systems:

D1S80	– MCT118 (Kasai et al. 1990; Budowle et al. 1991)
2p24-p23	– ApoB (Boerwinkle et al. 1989; Ludwig et al. 1989)
D17S30	– YNZ22 (Wolff et al. 1988; Horn et al. 1989)
12q13.1	– COL2A1 (Wu et al. 1990; Priestley et al. 1990)

These systems are characterized by the following criteria: 1) discrete alleles are definable 2) amplification of alleles can be reliably carried out because the allele length is relatively small (≥ 2 kb) and 3) because of the size of the genetic markers amplification is also possible with highly degraded DNA.

The aims of this investigation were:
1) to obtain preliminary allele frequency estimates for a German population sample
2) to perform family studies for preliminary information about mutations
3) to compare preliminary results with other population samples
4) to test whether or not the allele frequencies conform to Hardy-Weinberg expectations

Materials and methods

Blood samples were obtained from routine paternity and criminal cases from the Münster area, whereby only results from unrelated

individuals (i.e. mothers and putative fathers) were included in the population studies ($n = 81–192$; see Fig. 3). Family studies were carried out on 30–40 families (68–89 meioses). DNA was extracted from EDTA blood as previously described (Brinkmann et al. 1991).

The amplification was carried out with the following primer sequences and conditions:

MCT 118 (Budowle et al. 1991)
5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′
5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′

temp.: 94/65/72°C
time: 1 1 8 min
cycles: 25

YNZ22 (B. Budowle, pers. com.)
5′-AAACTGCAGAGAGAAAGGTCGAAGAGTGAAGTG-3′
5′-AAAGGATCCCCCACATCCGCTCCCCAAGTT-3′

temp.: 94/63/72°C
time: 1 1 6 min
cycles: 27

ApoB (Boerwinkle et al. 1989)
5′-ATGGAAACGGAGAAATTATG-3′
5′-CCTTCTCACTTGGCAAATAC-3′

temp.: 94/58/72°C
time: 1 1 4 min
cycles: 27

COL2A1 (Wu et al. 1990)
5′-CCAGGTTAAGGTTGACAGCT-3′
5′-GTCATGAACTAGCTCTGGTG-3′

temp.: 94/60/72°C
time: 1 1 2 min
cycles: 25

Each amplification sample contained 100 ng DNA, 10 mmol *Tris*HCl, pH 8.3, 50 mmol KCl, 1.5 mmol $MgCl_2$ and 0.01% gelatine; 2.5 U Amplitaq DNA-polymerase (Perkin-Elmer Cetus), 0.5 µM each primer and 200 µM each dNTP. The total volume was 50 µl with the addition of 30 µl oil for an overlay. Amplification was carried out using the thermal cycler: Triothermoblock (Biometra, FRG).

Electrophoretical separation of the amplified fragments was carried out in polyacrylamide gels (6% T, 3% C; thickness 400 µ) with piperazine diacrylamide as cross-linker (Budowle et al. 1991) using a discontinuous buffer system (Allen et al. 1989). The separation distance was 10 cm and electrophoresis was stopped when the bromophenol blue front had reached the anodal end of the gel. Bands were visualised using the silver staining method of Budowle et al. (1991). Allele determination in each polymorphism was carried out by comparison with an allele cocktail in addition to a size marker (123 bp BRL-ladder). The cocktail was constructed from alleles observed during this study and contained approximately every other allele of the polymorphism, so that it was possible to define the unknown alleles by side-to-side comparison with the allele cocktail (Fig. 1a, b, d). This procedure was not necessary for

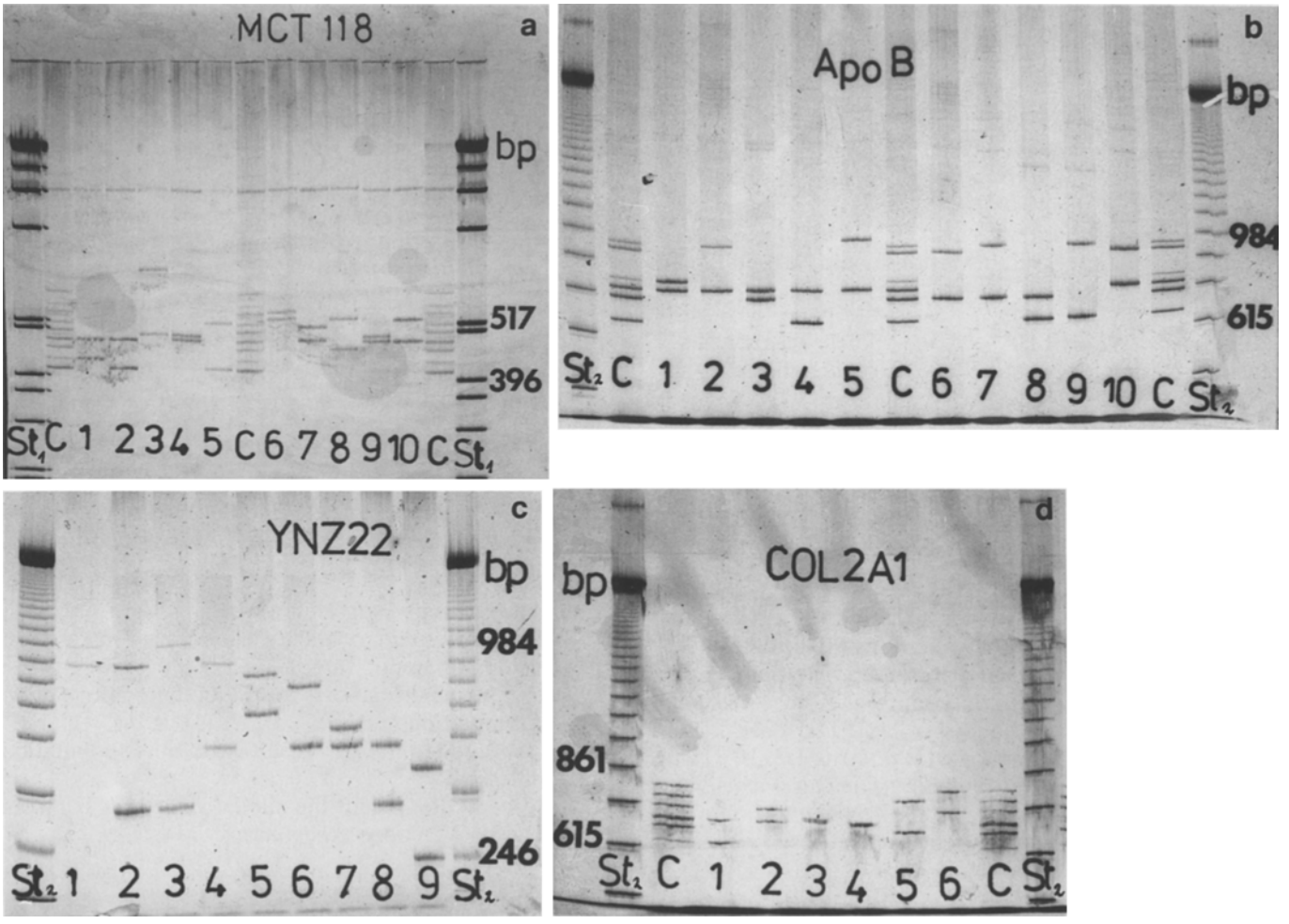

Fig. 1a–d. Examples of unrelated individuals to demonstrate the allele distributions in the AMPFLP systems MCT118 (a), ApoB (b), YNZ22 (c) and COL2A1 (d). Visualisation in PAG after silver staining. St_1 = 1 kb ladder (Gibco, BRL) for MCT118; St_2 = 123 bp ladder (Gibco, BRL) for ApoB, YNZ22 and COL2A1; C = Allele cocktail

YNZ22 (Fig. 1c), because the alleles in this system have a repeat length of 70 bp and could be determined by comparison with the size marker only.

Results

For D1S80, a total of 20 different alleles was observed in 192 individuals. Two alleles were found to be relatively common in the German sample having frequencies of 0.232 and 0.414, respectively. Similarly, for ApoB there were 14 alleles observed in 81 individuals with the 3 most common alleles occurring at a frequency greater than 0.160. For D17S30 there were 11 alleles found in 88 unrelated people with 4 alleles occuring at a frequency greater than 0.100. There were 10 alleles in 84 people observed for COL2A1 with two alleles being relatively common.

Based on the allele frequencies a mean exclusion chance for the 4 AMPFLP systems of 0.58–0.71 could be calculated (Table 1, after Krüger et al. 1968) and the combined exclusion chance is approximately 99%. This value is equivalent to the exclusion chance of approximately 20 conventional blood group systems.

The discrimination index is another important factor which can be used to estimate the forensic efficiency of a system (Table 2). For these 4 AMPFLP systems the DI values ranged from 0.07–0.16 with a combined value of $1.5 \cdot 10^{-4}$ (calculated after the method of Wong et al. 1987).

Hardy-Weinberg equilibrium

It is important for the application of any genetic marker that the system be tested for deviation from Hardy Weinberg expectations or other suitable biostatistical methods. If a deviation is observed the possible causes should be addressed, as well as the forensic significance. The AMPFLP systems investigated here have many alleles and therefore an extremely high number of possible genotypes. For example, for D1S80, 22 alleles were observed in our German sample which means there are 253 possible genotypes. It is obvious that an exceedingly large population sample will be required to observe all possible genotypes. Because of the relatively small population samples, a reliable estimation of deviations from Hardy-Weinberg equilibrium is not possible using each separate allele (Fig. 2). A less sensitive but informative approach for testing for deviations for preliminary data might be by binning alleles together and then by retesting with reshuffled allele categories. Calculations have been carried out using a 4-allele model (MCT118, YNZ22) or a 3-allele model (ApoB, COL2A1). The combined chi^2 values show no significant deviation between expected and observed values. There was no evidence of deviations from expectations using this binned allele approach (Table 3). The validity of this test is supported by repeating the chi^2-test after regrouping of alleles into new subdivisions. Again no evidence of significant differences was observed.

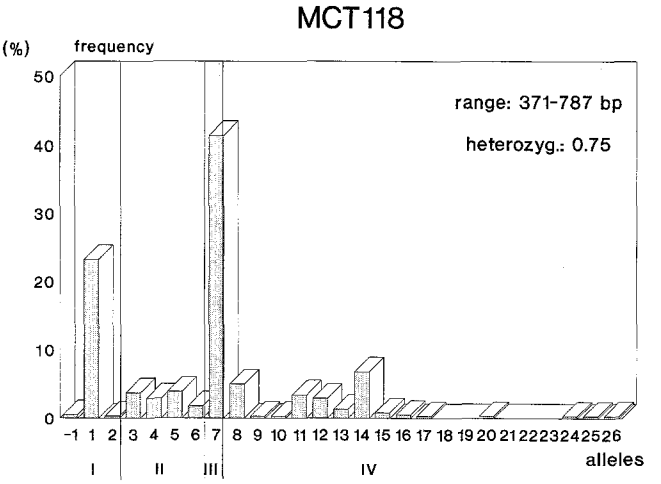

Fig. 2. I–IV indicate the groups of alleles used to estimate the Hardy-Weinberg equilibrium for MCT118

Table 1. Mean exclusion chance of the individual AMPFLP systems calculated after Krüger et al. (1968) and the combined value

System	Mean exclusion chance
MCT118	0.58
YNZ22	0.67
COL2A1	0.58
ApoB	0.71
Combined	0.99

Table 2. Individual discrimination indices for AMPFLP systems calculated after Wong et al. (1987)

System	DI
MCT118	0.11
YNZ22	0.07
ApoB	0.16
COL2A1	0.12
	$1.5 \cdot 10^{-4}$

Table 3. Chi-square values for AMPFLP systems using a binned 4 allele system (MCT118 and YNZ22) and a 3 allele system (COL2A1 and ApoB)

System	χ	P
MCT118	5.51	0.70–0.80 ($df = 9$)
YNZ22	5.08	0.80–0.90 ($df = 9$)
COL2A1	0.91	0.95–0.98 ($df = 5$)
ApoB	6.98	0.20–0.30 ($df = 5$)

Comparison of different population studies

A qualitative comparison of our data with other population samples (Budowle et al. 1991; Ludwig et al. 1989; Batanian et al. 1990; Priestley et al. 1990) shows that the distributions of alleles are similar (Fig. 3a–d). However, quantitative comparisons should be carried out cautiously.

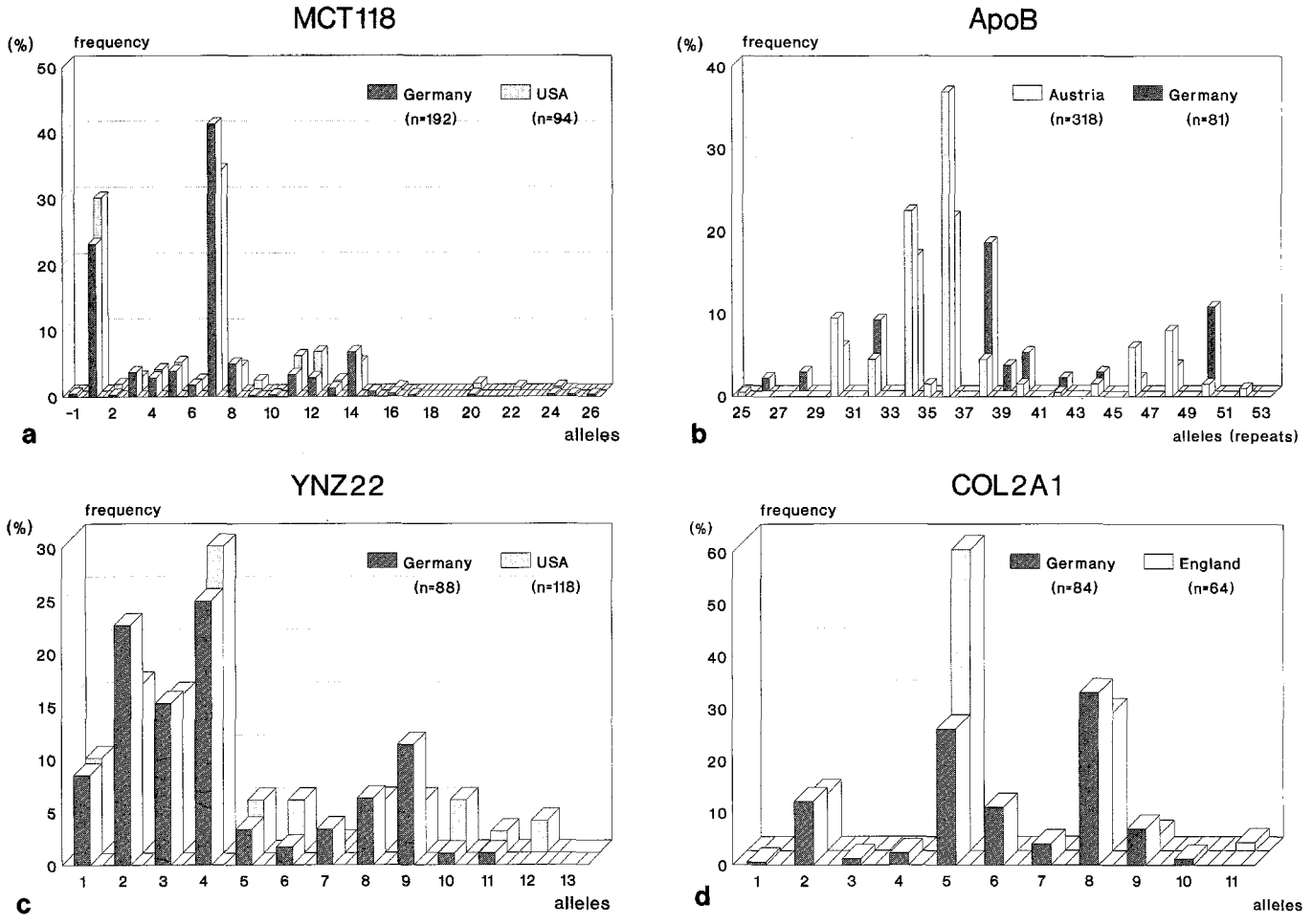

Fig. 3a–d. Comparison of data from Münster for MCT188 with Budowle et al. (1991), for YNZ22 with Batanian et al. (1990), for ApoB with Ludwig et al. (1989) and for COL2A1 with Priestley et al. (1990) (n = number of unrelated individuals)

Only quantitative comparisons of allele distributions between the German and USA samples for D1S80 and D17S30 were made to test whether or not the population samples are statistically similar. Similar high resolution electrophoretic systems were used for typing D1S80 in our German sample and the USA sample (Budowle et al. 1991). Therefore, D1S80 allele classifications can be anticipated to be similar. Additionally, since D17S30 has a 70 base pair repeat sequence size (Wolff et al. 1988) allelic data from electrophoretic systems even with different resolving capabilities should still be compatible using a 2-way RXC contingency table test comparing allele distributions for population sample homogeneity (kindly provided by G. Carnody) (Sajantila et al. 1991). The German and USA samples appear to be statistically similar for D1S80 and D17S30 ($chi^2 = 24.6875$, $P = 0.3220 \pm 0.0148$; G statistic $= 27.2581$, $P = 0.4110 \pm 0.0156$, $P < 0.000$; respectively). The results for YNZ22 were to be expected since the data of Batanian et al. (1990) was a heterogeneous mixture of caucasian, black, hispanic and other racial groups.

In contrast, the COL2A1 and ApoB data were not compared statistically at this time. With different analytical approaches allele resolution, and hence classifications, may differ. This is particularly apparent for COL2A1 where alleles 3, 4, 6 and 7 were detected in our sample but not in the English sample (see Fig. 3d). More work is needed to establish an effective comparison of allelic data between laboratories (similar to SLP and EDNAP – see Schneider et al. 1991) for some genetic markers and sample sizes should be increased before meaningful comparisons can be made.

Family studies

In this study 30–40 families (68–89 meioses) were analysed with all 4 systems. These families had been previously validated with classical blood group systems. No evidence of new mutations was found although the sample size was too small for establishing a conclusive mutation rate.

Discussion

The necessary prerequisites for the application of these AMPFLP systems in forensic practice are that some population studies should be carried out by laboratories for

the calculation of population specific allele frequencies. With AMPFLP's, however, deviations between various populations in our study seem to be small. However, it must be taken into account that the sample sizes in these studies are too small for significant comparisons. The small differences that are observed may be population specific, due to sample size and/or the resolution of the analytical method.

The classical method for testing if a population sample is in Hardy-Weinberg equilibrium (chi^2 test) is impractical and not statistically suitable for systems containing large numbers of alleles. However by "binning" alleles as suggested by Brenner and Morris (1990), for a quasi continuous distribution of alleles, no significant deviations from Hardy-Weinberg equilibrium could be demonstrated in any of the AMPFLP systems. The value of this test was supported by regrouping the alleles into new "bins" and again no significant deviations could be demonstrated (data not shown).

Methods for testing the effective use of the product rule, as described by Budowle et al. (1991), will be considered when sample sizes are large enough. A true estimation of the mutation rate is only possible after extensive family studies and is desirable to consider for applications in paternity diagnostics. The combined application of all 4 systems (Tables 1 and 2) leads to a high forensic efficiency. An important factor is that the method is very economical when compared with single and multi locus VNTR systems detected by RFLP analysis. Irrespective of these advantages it is necessary that each laboratory should obtained extensive experience with PCR/AMPFLP's before using these methods in casework. Furthermore a minimisation of the risk of artefacts is important, inter alia using known laboratory DNA controls for amplification, a positive control of the DNA and a blank control for elimination of possible contamination by the operator.

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