PCR based analysis of HLA-DQα, D1S80 and Apo B loci in paternity testing.

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Introduction

The development of the polymerase chain reaction (PCR) has considerably simplified the analysis of locus specific DNA polymorphisms, generated both by VNTR regions and DNA sequence variation. These kinds of allelism represent informative genetic marker systems which may also be useful in paternity determination. We have focused our interest on the well established HLA-DQα, D1S80 and Apo B amplifications systems (1).
For practical application data bases have been set up with a large german population sample. Observed allele frequencies were tested for Hardy-Weinberg equilibrium and compared to published data. This report further presents forensic efficiency data as derived from extensive family studies and case work.

Material and methods

DNA was extracted from ACD blood samples using a standard salting out procedure (2). Amplification of HLA-DQα and D1S80 alleles was performed with commercially available typing kits (Perkin Elmer Cetus) according to the manufacturer's protocols with the following modifications: input DNA 25 ng (DQα) and 20 ng (D1S80). Amplified DQα alleles were analyzed by a reversed dot blot assay; D1S80 alleles were run on 5% polyacrylamide gels (1 x TBE, 2 V/cm, 16 h) and subsequently stained with ethidium bromide.
Amplification of Apo B alleles was carried out with primers described by Ludwig (3) under the following conditions: 94°C for 2 min, 62°C for 3 min, 3 cycles; 94°C for 1,5 min, 61°C for 3 min (auto extension 10 sec/cycle) 28 cycles. Analysis of amplified fragments was performed on 1.8% "MetaPhor" agarose gels (1xTBE, 4 V/cm, 8 h). Apo B alleles were identified by coelectrophoresis with an allelic ladder, composed of 9 discrete alleles.

Table 1 **DISTRIBUTION OF HLA - DQα ALLELES**

DQα - ALLELE	Lab Dr. Arnold (%) n=604	Schneider et al (4) (%) n=318	Helmuth et al (5) (%) n=826
1.1	14.0	15.41	13.7
1.2	21.0	22.96	19.7
1.3	9.3	6.92	8.5
2	13.6	10.38	10.9
3	11.2	13.52	20.1
4	30.8	30.82	27.1

(4) south-western Germany, (5) US Caucasians

Results

1. Allele frequency distributions

Frequency estimates for HLA-DQα, D1S80 and Apo B amplification systems were based on a population sample originating from southern and middle Germany.
At the HLA-DQα locus six alleles can be typed by hybridisation of the amplified product to allele specific oligonucleotide (ASO) probes. Identification of the correct genotypes is fast and unequivocal provided that the recommended hybridisation and washing temperature (55°C) is correct within the

limits of +/- 2°C. For the DQα locus allele frequencies ranged from 9.3 - 30.8% which is in close agreement with published data (Table 1). All 21 possible genotypes were observed, heterozygosity rate was 82.1% (79.8% expected). There was no deviation from Hardy-Weinberg equilibrium when expected and observed genotype frequencies were applied in a chi² test (Table 2).
With the D1S80 AmpFLP system a total of 23 different alleles could be analyzed, representing 16 - 40 tandemly arranged copies of the 16 bp repeat unit (n = 484). One single allele with approximately 60 - 65 repeats (judged by agarose gel electrophoresis) was observed. The two most common alleles exhibit frequencies of 32.8 and 23.3%, all the other alleles were found at or below 7.6% (Fig 1a). These findings compare favourably to data bases obtained with german (6) and US caucasian samples (32.0 and 26.5% [7]). Chi² - tests were carried out as proposed (6) by binning the alleles into 4 groups. Again no significant deviation from the assumed Hardy-Weinberg equilibrium was revealed (Table 2). Observed heterozygosity was 81.8% (81.9% expected).

Fig. 1a

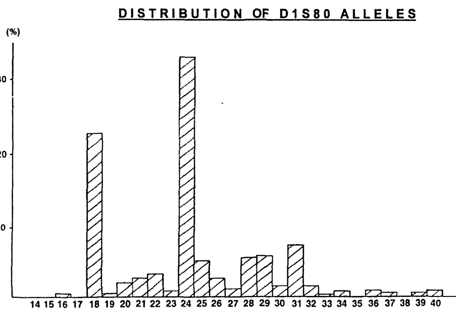

Fig. 1b

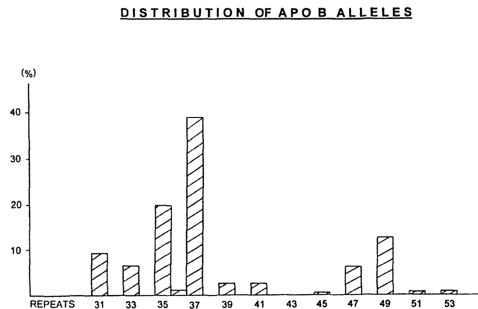

Fig. 1a,b. Allele frequencies at the D1S80 (Fig. 1a) and Apo B loci (Fig. 1b) in a sample of unrelated german individuals

Analysis of the amplified Apo B fragments (n = 240) revealed 12 different alleles representing 31 - 53 repeat units. One single allele with a putative length of 27 repeats was detected in a blood sample of a libanesian man.
Comparison of the allelic data base (Fig. 1b) with results obtained by Schnee-Griese (8) demonstrate a similar distribution, the most common alleles reaching frequencies of 38.5 (37.0), 20.0 (25,4) and 12.5% (8.8%) respectively (values from (8) in parenthesis). As both estimates are based on population samples of similar origin, the observed differences may be due to different sample size. Hardy-Weinberg equilibrium was obviously reached in the Apo B amplification system (Table 2).

Table 2 **Hardy - Weinberg equilibrium at three amplified loci**

locus	$\chi 2$	probability	df
HLA-DQα	6.92	$p > 0.99$	20
D1S80	3.52	$0.95 > p > 0.90$	9 *
Apo B	4.22	$0.90 > p > 0.80$	9 *

* alleles binned into 4 groups

2. Family studies

For this report we carried out extensive family studies, consisting mainly of paternity cases which had been previously investigated with classical blood group markers (24 systems).

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Alltogether 179 mother - child - putative father trios were investigated. In 42 trios the men had been excluded from paternity by conventional methods. These findings could be confirmed by PCR analysis with one single exception. In this case, the non-excluded man was the brother of the true father.
On the other hand, two father - child exclusions were revealed by PCR but not by the classical marker systems. Based solely on the 24 blood group systems, the probability of paternity had been calculated at 85 and 94% respectively.
The results of all other cases were consistent with the classical genetic systems.

Numbers of analyzed meiosis are: 281 for HLA-DQα, 220 for D1S80 and 106 for Apo B. Concerning the HLA-DQα and Apo B loci, there was no evidence for new mutations; with D1S80 one single new mutation was detected by a mother - child incompatibility. This finding corresponds to a mutation rate of 0.45%. We conclude, that the described loci are genetically relatively stable compared to the more polymorphic VNTR loci detected by Southern blot analysis.

3. Forensic efficiency data

The power of exclusion (PE) of HLA-DQα, Apo B and D1S80 amplification systems was calculated at 0.595, 0.557 and 0.635; the combined value is approximately 0.935. DI - values (discrimination index) estimating the individual specifity of genotypes are 0.07, 0.09 and 0,06, respectively (Table 3). To validate the calculated PE - values, 30 paternity cases with putative father - child exclusions were re - analyzed with the three PCR systems. Exclusions could be verified in all cases at the following rates: 53% (DQα), 63% (Apo B) and 73% (D1S80). Single exclusions were observed with 7 trios, two exclusions with 18 trios and three exclusions with 5 trios. This result underlines the resolving power of these 3 systems which is comparable to the combination of approximately 20 commonly used blood group systems.

Table 3 **Discrimination power of three amplification systems**

amplification system	heterozygosity (calculated, %)	power of excl. PE (%)	discrim. index DI	frequency of most common genotype
HLA-DQα	79.8	59.5	0.07	0.142
Apo B	77.7	55.7	0.09	0.155
D1S80	81.9	63.5	0.06	0.153
combined		93.5	3.8×10^{-4}	3.4×10^{-3}

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