Comparison of Population data using 3 AMPFLP systems

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INTRODUCTION AND AIMS

The so-called AMPFLP's (amplified fragment length polymorphism) generally have fragment lengths of up to 1000 bp. With a good electrophoretic set up the alleles can be defined. The advantages are as follows: - discrete alleles are definable - amplification of alleles can be reliable carried out because the allele length is relatively small (1 kb) - amplification is also possible with highly degraded DNA.

Population data has been carried out for 3 different AMPFLP - systems in a north German population. The aims of investigation were: - to obtain allele frequencies in a German population sample - to perform family studies for preliminary information about possible new mutations - a comparison with other population studies to show whether differencies could be seen - to test the Hardy-Weinberg equilibrium.

MATERIALS AND METHODS

Blood samples were obtained from routine paternity cases whereby results from unrelated individuals (i. e. mother and putative fathers) were included in the population studies. DNA was extracted from EDTA blood as previously described (Brinkmann et al 1991). Each amplification sample contained:
100ng DNA, 2 Units taq-Polymerase (Promega), 0,5 mmol each Primer, 200 mmol each NTP, 5 μl PCR-Buffer (Promega).
The following primers were used: Apo B (Boerwinkle et al, 1989)
pMCT 118 (Budowle et al, 1991)
YNZ 22 (Budowle pers. com.)
5'-AAA CTG CAG AGA GAA AGG TCG AAG AGT GAA GTG-3'
5'-AAA GGA TCC CCC ACA TCC GCT CCC CAA GTT-3'
The electrophoretical separation of the amplified fragments was carried out in short polyacrylamide gels (8 cm, YNZ 22; Budowle et al 1991, Sambrook et al 1989) or long polyacrylamide gels (18-20cm, pMCT 118, Apo B) using a discontinuous buffer system (Allen et al 1989). Visualisation of the bands was carried out by silver staining (Budowle et al 1991). Allele determination was carried out by comparison with an allele cocktail in addition to the size marker (123Bp-Ladder).

RESULTS

I. Population data

In pMCT there are 2 fragments with a frequency of more than 20%, allele Nr. 7 has a frequency of nearly 40%. In Apo B there are two fragments with frequencies of approximately 20% can be found. YNZ 22 also has 3 fragments with frequencies between 15-25%. From the allele frequencies a mean exclusion chance for the 3 systems of 0,58 - 0,7 was calculated and the combined exclusion chance is approximately 93%. For the 3 AMPFLP systems the value of the discrimination index ranged from 0,29 - 0,05 with a combined value of $1,6 * 10^{-3}$
As the AMPFLP systems shown here have many alleles it is possible to construct a variety of allele groups which can then be treated as one allele. Chi square tests can then carried out on these allele groups to test for Hardy-Weinberg-equilibrium. Hardy-Weinberg calculations have been carried out using a 4- allele model (pMCT 118, Apo B)or a 5-allele-model (YNZ 22). As the combined chi-square value shows that there is no significant deviation between expected und observed values the population is assumed to be in Hardy Weinberg equilibrium: For Apo B x = 12,37, p = 0,1-0,2 (df 9); for pMCT x = 5,51, p = 0,7-0,8 (df 9); for YNZ x = 4,73, p = 0,99 - 0.97 (df 4,73).
Although the repeat lengh of pMCT is assumed to be 16bp, two additional intermediate bands were found in the range of 369 - 492 bp.

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II. Comparison of different population studies

A comparison of different population studies using smoothed curves for convenience showed clear conformity for all systems. With Apo B a comparison of 2 European studies shows a very similar profile. A shift can be seen in the lower frequency region between repeats 27 - 29 and between repeats 45 - 49. With pMCT there are only 2 peaks with obvious differences in the peak height. Alleles 6 and 7 give a combined allele frequency of approximately 43% in the German study and 35% in the American study. With YNZ the smoothed curves show good correlation. In the German study a large peak can be seen in the range of the 2nd -4th alleles which is much smaller in the American study. The peak between 8-10 alleles is similar in both studies.

III. Family studies:

The results are in Fig. 3. In Apo B 76% were in agreement with the basic investigation, for YNZ 89% and for pMCT 118 86%. As 24% for Apo B, 11% for YNZ, 14% for pMCT of investigated families show exclusion in the basic investigations and no exclusion in PCR. There was no evidence of new mutations found.

DISCUSSION

Population studies should be carried out by each laboratory for the calculation of population specific allele frequencies. Deviations between various populations seem to be relatively small but it must be taken into consideration that the sample sizes of these studies are as yet too small for a reliable comparison. The classical method for testing if a population sample is in Hardy-Weinberg equilibrium is impractical and not statistical suitable for systems containing large numbers of alleles as suggested by Brenner and Morris (1989) for a quasi continuous distribution of alleles. No significant deviations from Hardy-Weinberg equilibrium could be found in all 3 AMPFLP systems.

This paper is an updating from Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B: Population genetics and forensic efficiency data of 4 AMPFLP's (in press: Int. J. Leg. Med.).

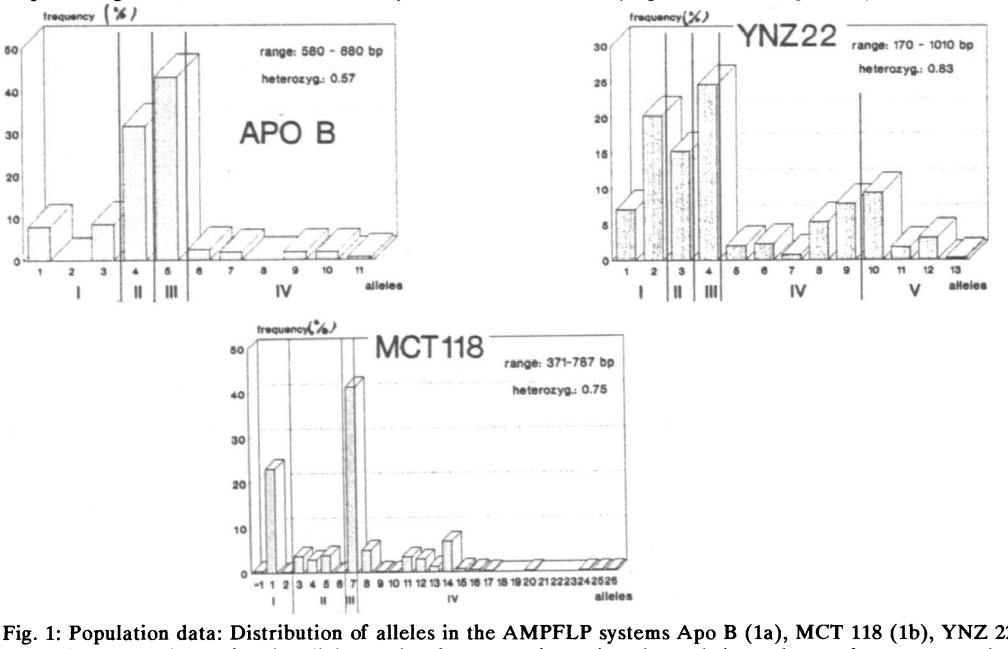

Fig. 1: Population data: Distribution of alleles in the AMPFLP systems Apo B (1a), MCT 118 (1b), YNZ 22 (1c). The nomenclature for the allele number for Apo B is as given by Ludwig et al 1989, for pMCT as given by Budowle et al 1991, for YNZ as given by Horn et al 1989. I-V indicate the groups of alleles used to estimate the Hardy Weinberg equilibrium for APO B, MCT, YNZ

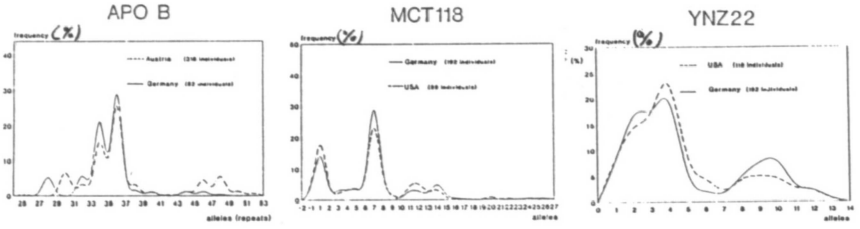

Fig. 2: Comparison of data from Münster with data from Ludwig et al 1989, for Apo B, for pMCT 118 with Budowle et al 1991, for YNZ 22 with Bataninan et al 1990

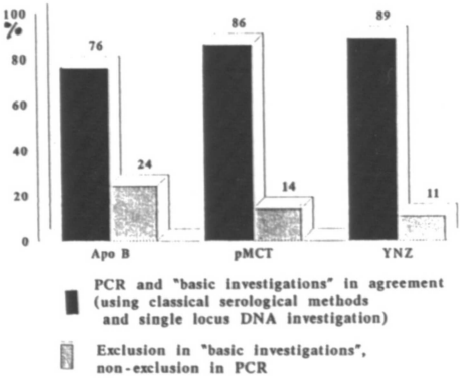

Fig. 3: Family studies for Apo B, pMCT 118, showing a comparison of the results from PCR and basic investigation. Family studies have been carried out for different AMPFLP systems and the results habe been compared to the "basic paternity investigation" using classical serological methods and a single-locus DNA investigation

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