DNA-Profiling with pHINS310, pMUC7, pMR24/1, pYNH24 and pMS43a for Paternity Testing

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INTRODUCTION

A huge variety of well established conventional blood group systems is available for paternity testing and still can be extended by acquiring new phenotypic polymorphisms.
Nevertheless DNA-polymorphisms widely come into increasing use as a powerful tool for paternity casework, not at least for their uniform and practicable methodical procedure.
This paper presents a comparison of the results from 12 current routine paternity cases tested with up to 30 red cell antigen & enzyme as well as serum protein polymorphisms with the results achieved by the use of up to 5 SLPs detecting high polymorphic VNTRs: pHINS310, pMUC7, pMR24/1, pYNH24 and pMS43a (Elbein et al 1985; Gendler et al 1987; Yokoi et al 1990; Odelberg et al 1989; Wong et al 1987).
The order, in which the conventional markers actually were tested, was as follows: AB0, MNS, RH, KEL, FY, HP, GC, GM, KM, ACP1, PGM1, ADA, AK1, PGD, ESD, P1, GLO1, JK, LU, TF, B, C3, PI, F13A, F13B, GPT1, C6, C8 1, HSGA, PLGN, ORM1.

METHODS

DNA of 12 triplets (child, mother, putative father) was prepared by proteolytic digestion of pelleted white blood cell nuclei, organic extraction and ethanol precipitation. The HinfI-fragments were separated in 1% agarose gels (20*20cm) at 30V/110mA for 24h and alkali blotted to charged nylon membranes. Probes were labeled with P32 dCTP by multi-primed extension, blots were exposed to Amersham MP-films with two intensifying screens at -28 degrees Celsius or to Amersham beta-max-films at ambient temperature for 5 days. Fragment lengths were calculated according to the method of Elder & Southern (1987), the plausibility of paternity ("W"-value) was calculated using the formulae of Mayr (1972) with a sliding window of +/- 3 sigma. "Allele"-frequencies for pHINS310, pMUC7 and pMR24/1 were taken from our own population data (n=207), frequencies for pYNH24 (Odelberg et al 1989) and pMS43a were provided by PROMEGA and CELLMARK.

RESULTS

The 12 cases from paternity testing revealed 10 constellations including the putative father. The corresponding values for the probability of paternity achieved by either the VNTR-markers or the conventional polymorphisms as well as both in combination are scaled up in table 1 and shown in figure 1. Profiling with only 3 probes resulted in "W"-values which only in 2 cases exceeded 99.73% whereas in 6 cases the values were ranging from 98.01% to 99.67%. "W"-values clearly beyond

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99.73% could be calculated by probing trios with 5 VNTR-markers. There also were 2 cases in which the alleged father could be excluded both with the conventional and the DNA-markers (I. all 5 probes / II. 4 probes lacking pHINS310). Figure 2 gives an example for trios tested with pMR24/1.

	pHINS310 pMUC7 pMR24/1	pHINS310 pMUC7 pMR24/1 pYNH24 pMS43a	conv. markers/ number of systems	conv. markers + 3 SLPs	conv. markers + 5 SLPs
1	96.2656		99.9200/26	99.9970	
2	99.7695		>99.9990/26	>99.9999	
3	98.0111		99.8500/29	99.9970	
4	99.9826		99.9800/26	>99.9999	
5	98.6873		99.7500/30	99.9967	
6	98.4363		99.9600/26	99.9994	
7	98.8605	99.9700	99.9920/26	99.9999	>99.9999
8	99.6064	99.9816	99.9930/26	>99.9999	>99.9999
9	95.4593	99.7967	99.9650/26	99.9983	>99.9999
10	99.6751	99.9857	>99.9990/26	>99.9999	>99.9999

Table 1 Pobability of paternity ("W"-values): Conventional polymorphisms vs DNA-profiling with 3 or 5 SLPs

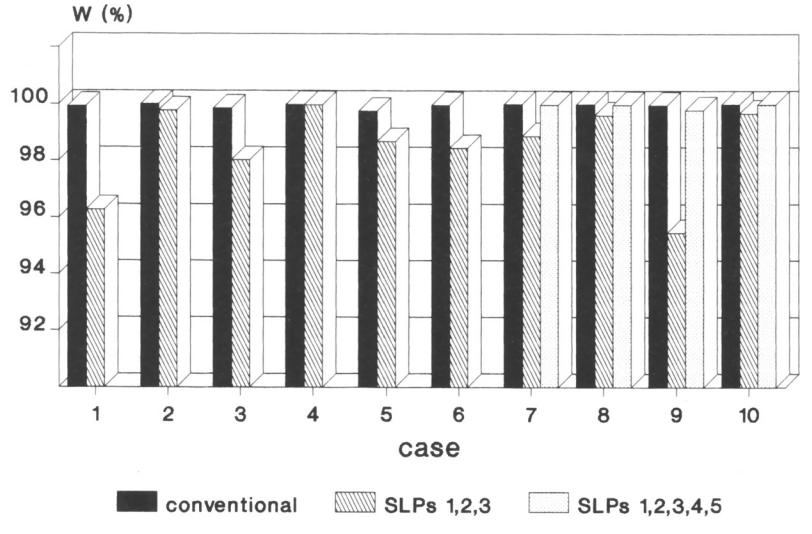

Fig. 1 Probability of paternity ("W"-values): Conventional vs DNA-markers

DISCUSSION

DNA-profiling with 5 SLPs in paternity testing seems to be sufficient to achieve "W"-values exceeding 99.73% - results which are comparable to the values calculated from a large range of conventional markers. If classical blood grouping and DNA-analysis are combined VNTRs can assist a reduced set of chosen conventional markers to lift the probability of paternity to values beyond 99.73% and, regarding their enormous exclusion potential, can help to detect falsely alleged fathers.

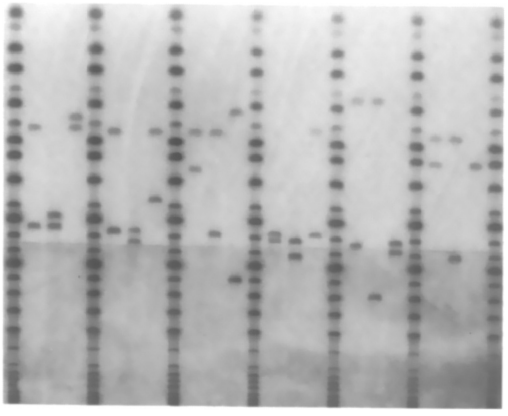

Fig. 2 Probing trios (child, mother, alleged father) with pMR24/1: (from left to right) trio 1; 2; 4; 6 reveal inclusions whereas 3 and 5 show exclusions (standard ladder = PROMEGA "wide range"; the anode is at the top)

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