2.1 General

PATERNITY INVESTIGATIONS BASED ON DNA-ANALYSIS ONLY

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1. Introduction

Innumerable investigations have shown that conventional blood group typing is a powerful and reliable means to solve cases of disputed paternity in simple trio cases as well as in some of the more complicated deficiency cases. The benefit of the introduction of additional systems and of the sophistication of methods, e.g. isoelectric focusing was a constant increase of the so-called positive proof of fatherhood, but the chance to find a case not already showing exclusions in other systems remained extremely small. Inherently, the plethora of methods and systems also increased almost unnoticed the chance of error. Furthermore, the training time of new technical staff considerably lengthened. The appearance of the highly polymorphic DNA systems (Jeffreys et al., 1985, Smith et al., 1990) quickly unsecured a typing system that seemed to be well established forever. Bär (1988) compared the results and the practical handling of the so-called multi-locus probes in paternity investigations as well as in stain analysis. Many reasons, e.g. the poor statistical evaluation of the results of multi-locus probes and the delicate working conditions of low stringency showed that in the hands of ordinary technical staff the work with single locus probes gives more reproducible results. Furthermore, well known and accepted statistical approaches like the one of Essen-Möller or the calculation of a paternity index are possible. The easiness of the statistical combination of DNA results with those of conventional techniques is an additional strong argument for the use of single locus probes, not unimportant in difficult cases of severe deficiency(Bär and Hummel, 1991). Based on the results of many supplementary expertises in paternity cases showing low values of probabilities , single exclusions and opposite homozygosities after conventional blood grouping, we decided in March of 1991 to completely abandon the conventional systems and to exclusively use DNA single locus probes.

2. Material and Methods

DNA analysis is independently performed by two technicians which extract high-molecular DNA from 300 µl EDTA-blood. After restriction with Hinf I band separation is done on 4 gels of 1% (2 per technician). Electrophoresis is performed over the weekend (running time 60 hrs !). Probes MS43a, MS31 and g3, yNH24 respectively are applied. Labelling is done according to Feinberg and Vogelstein (1986) using P32. Hybridization condition are of high stringency. One of the two blots per technician is cross-hybridized. Exclusions are extracted and hybridized a third time. Band length is measured with a BioImage Visage System from Millipore. In theory, after two and a half week the report to the parties can be established. However, in praxis the mean handling time per case is longer, 33 days for cases of non-exclusion and 39 days for cases of exclusion.

3. Interpretation

3.1. Non-Exclusions

The interpretation of a match is based on a visual side-by-side comparison by two staff members. In cases of shifts, the analysis is repeated from the beginning. The visual match is backed-up by the computerized band length measurement. A match window of 2.5 % proposed by others (Gill, pers. communication) was found to be too large and too rigid taking into account that the standard deviation positively correlates with the band length. Looking at the real size of the observed band length differences based on the measurements of visually matched bands in cases of non-exclusions for the two probes MS43a and yNH24 the following observations can be reported. Probing with MS43A, of 115 pairs of mother-child and 96 child -putativfather duos about 45 % showed no difference in band length at all. The highest difference observed was 1.7 %. There was no significant difference between the samples of Mother-Child and Child-Father comparisons. If a window of 1.1 % was chosen, more than 95 % of the cases were included. Therefore our visual matches fulfilled in fact much more rigid criteria than those proposed (fig. 1). Probing with yNH24, of 109 pairs of mother-child and 90 child -putativfather duos about 50 % of the cases showed no difference in band length at all. The highest difference observed was 1.8 % of the band length. There was no significant difference between the distribution of the Mother-Child and Child-Father comparisons. If a window of 1 % was chosen, about 98 % of the cases were included. Therefore our visual matches fulfilled in fact much more rigid criteria than those proposed (fig. 2).

3.2. Band Frequency Estimation and Biostatistical Evaluation

Frequency distributions for a Swiss population sample larger than 200 unrelated persons were established for the four probes. Fixed bin sizes for yNH24 was 150 bp, for the others probes 200 bp. These bin sizes of 200 bp and 150 bp respectively which correspond to 2.5% of the mean band length for MS43A and 4.4 % for yNH24, are largely conservative taking into account the above mentioned visual match criteria which actually respect a window of about 1%. In each case of non-exclusion, the biostatistical evaluation is done according to Essen-Möller (1938), a method widely used by paternity labo-

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ratories and well-known by the judges. In cases of deficiency, a more common algorithm, e.g. the one of Ihm and Hummel (1975) is used. The attribution of a band frequency follows a modified fixed bin approach. The minimum band frequency employed is always 1 %. The "real" band frequencies are chosen according to band size, but in cases where the band value is close to a bin border with the adjacent bin showing a higher frequency, this higher frequency is finally used for the calculation (conservative). The use of Caucasian databases of others, e.g. Cellmark had no influence on the final decisions. The correlation of pairwise plotted W-values based on Swiss or Cellmark databases was 100 % (fig. 3). The posteriori calculation of a hypothetical mean band frequency when applying 4 probes gave values of 4.6 % , respectively 4.4 % per band for the two databases. It can therefore be concluded that a mean band frequency of 5%, proposed by us 3 years ago when databases were not yet available, would have been conservative. The general use of a mean band frequency of 10 % in cases with Non-Caucasians is again proposed.

3.3. Exclusions

24 exclusions out of 118 cases were observed corresponding to 20% of our cases which is equal to the mean value found when using conventional blood group typing. In three quarters of the cases, exclusions were seen in all 4 SL-systems and in one quarter of the cases only a single matching situation was observed. Cases of exclusions with 2 matching situations were not observed. However, cases with only one exclusion were seen in 3 instances. These latter cases cannot be solved without extension of the investigation. Either conventional markers or additional DNA single locus probes can be used to either exclude or confirm an underlying situation of a non-excluded first order relative or the occurrence of a recombination (mutation). It is worth emphasizing that when using 4 single locus probes the exclusion chance for a brother is only 1: 256 if the parents are heterozygous in the 4 systems. The extension of the investigation on only a few systems may therefore not necessarily show additional exclusions which would prove the exclusion.

3.4. Biostatistical Handling of Mutations

According to a proposition of Hummel (pers. communication), in all cases of single exclusions a biostatistical evaluation of the probability of a mutation should and can be made. The general formula of the calculation of a EM-value is: $\mathbf{EM_{mut}=\log c/u+10,}$ where c is the frequency of the "mutant" band and u is the mutation rate of the DNA system under consideration. The extension of the investigation on additional systems is usually mandatory. The integration of the EM_{mut}-value in the general formula of Essen-Möller is easily possible. Despite of the biostatistical proof of the mutation, it is necessary to report to the parties the possibility of a non-excluded first order relative.

4. Summary

DNA analysis based on 4 highly polymorphic marker systems proves to be an very powerful means to solve cases of disputed paternity and can completely replace conventional blood group techniques. The gain of evidence is very remarkable for the so-called positive proof of paternity. Despite the absence of discrete alleles the evaluation of the results can be done applying well-known methods. Visual matching is in fact even stricter than window matching since the visual match window respects a size of about 1% which is half of that proposed (2.5%). Computerized band size determination facilitates routine case work. Application of band frequencies from databases of different Caucasian population samples gives identical biostatistical results using 4 DNA probes (MS43A, MS31, g3, yNH24). Single exclusions must be further evaluated by extending the investigation on additional marker systems. The probability of a mutation can be calculated by a Bayesian approach and its value can be integrated in the W-value of Essen-Möller or the PI. However, the possibility of a non-excluded first order relative must be kept in mind.

5. References

Bär W (1988) DNA-Polymorphismen: Ihre Bedeutung in der Forensik. Habilitationsschrift. Med. Fakultät, Universität Zürich

Bär W and Hummel K (1991) DNA-Fingerprinting: Its Application in Forensic Case Work. DNA Fingerprinting: Approaches and Applications. Burke T, Dolf G, Jeffreys AJ & Wolff R (eds.) Birkhäuser Verlag Basel/Switzerland, 349-355

Essen-Möller E (1938) Die Beweiskraft der Ähnlichkeit im Vaterschaftsnachweis. Theoretische Grundlagen. Mitteil Anthopol Gesell Wien 68:9-53

Feinberg AP and Vogelstein B (1983) A Technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6-13

Ihm P and Hummel K (1975) Ein Verfahren zur Ermittlung der Vaterschaftswahrscheinlichkeit aus Blutgruppenbefunden unter beliebiger Einbeziehung von Verwandten. Z Immun Forsch 149:405- 416

Jeffreys A, Brookfield JFY, Semeonoff R (1985) Positive identification of an immigration test-case using human DNA fingerprints. Nature 317: 818-819

Smith JC, Anwar R, Riley J, Jenner D, Markham AF, Jeffreys AJ (1990) Highly polymorphic minisatellite sequences: allele frequencies and mutation rates for five locus-specific probes in a Caucasian population. J Forensic Sci Soc 30:19-32

Visual Match-MS43A: Distribution of Bandlength Differences in % of the Bandlength in 115 Mother-Child and 96 Child-Putativfather Duos

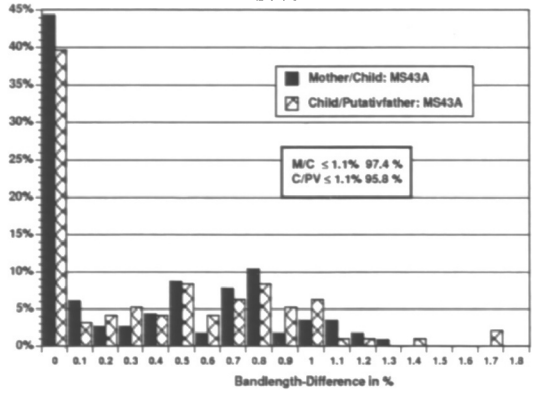

Figure 1

Visual Match-yNH24: Distribution of Bandlength Differences in % of the Bandlength in 109 Mother-Child and 90 Child-Putativfather Duos

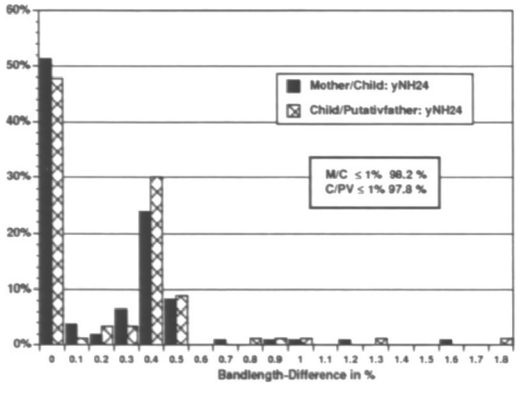

Figure 2

Simple Regression of W-Values in 60 Paternity Cases based on Cellmark and Swiss Databases (MS43A, MS31, g3 and yNH24)

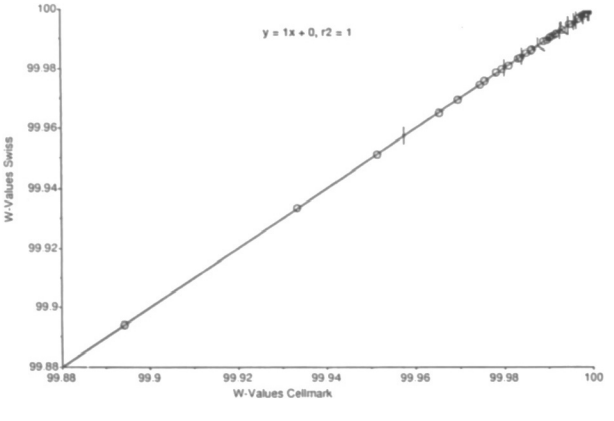

Figure 3