APPLICATION OF SINGLE LOCUS PROBES IN CASES OF DISPUTED PATERNITY - THE USEFULNESS OF THE COMBINATION OF HLA AND DNA.

C.Seidl, U.Rabold, B.Brüggemann, M.Kilp, D.Teixidor, E.Seifried.

Red Cross Donor Service Hessen, Frankfurt, Germany.

Introduction

Genetic polymorphism analysis has been a classical technique to identify individual-specific patterns of in heritage in paternity testing. Blood group antigens, plasma protein and erythrocyte enzyme polymorphism's are the conventional systems used for paternity testing. Phenotypic gene frequencies are particularly significant in various populations but most of the systems exhibit only a limited polymorphism. Thus several systems (e.g. about 21) have to be used for accurate calculations of paternity probability. However, in some cases probability values are still not conclusive. Therefore, genetic systems with high polymorphism are of particular interest. In this respect, the human leukocyte antigens (HLA-system) demonstrating a high number of alleles have been used (1). Due to linkage disequilibrium and uneven distribution of haplotypes the W values (Essen Möller) of alleged men are not always sufficient. In recent years variable numbers of tandem repeats (VNTR's) have been analysed by single locus probes (SLP's) (2). We have compared the sole application of HLA and the combination of HLA and SLP's analysis. The results demonstrate that the combination of HLA - A and - B locus typing and 3 SLP's resulted in W values of more than 99,73% .

Materials and Methods

Antigen systems and enzyme polymorphisms (Conventional systems). Blood group antigens (ABO-, MNSs-, Rh-, Kell-, Duffy- and Kidd-system), plasma protein polymorphisms (PLG-, Gm-, Km-, C3-, Tf-, Pi-, Hp- and Gc-system) and erythrocyte enzyme polymorphisms (PGM-, GPT-, ACP-, AK-, ADA-, EsD- and PGD-system) were determined for each case following standard procedures.
HLA-Typing. HLA-A, HLA-B and HLA-C antigens were typed by the microlymphocytotoxicity test (LCT).
VNTR (SLP) polymorphism analysis. Genomic DNA was extracted from 8ml of peripheral blood following the salting-out method (4) and 10µg of DNA were digested with 25 units of the appropriate restriction enzyme (RE) for 16 hours. Subsequently, DNA fragments were separated by gel electrophoresis (44-62 hours at 0.8-1.0 V/cm). Electrophoresis conditions were chosen depending on the range of fragment sizes detected by the various DNA probes. Hybridisation, southern blot transfer to nylon membranes (Quiabrane from Diagen Inc, Hilden,

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg

Germany) and detection of probes were performed as recommended by the manufacturers (AlphaProbe™ from IMMUCOR/ONCOR, NICE™ from ICI/CELLMARK and GenePrint Light™ from Promega). The results were analysed by an semiautomatic computerised system (DNA-Auswertungssystem Version 2.40 from M.Muche Immucor Medizinische Diagnostik GmbH, Rödermark, Germany) and the biostatistical calculation of paternity was performed with the Essen Möller probability (W) using the allele frequencies (according to J.Henke, Institut für Blutgruppenforschung, Köln) of at least three different probes. The following table summarises the investigated probes and the corresponding heterozygosity rates (HR).

Locus	SLP	HR/RE	Manufacturer
D7S107	pS 194	0,85(PstI)	Immucor
D1S47	pl 336	0,90(PstI)	Immucor
D18S17	pl 159-1	0,74(PstI)	Immucor
D20S15	pl 355-8	0,66(PstI)	Immucor
D21S112	pl 427-4	0,94(PstI)	Immucor
D11S129	pR 365-1	0,66(PstI)	Immucor
D2S44	yNH24	0,97(HinfI)	Promega
D1S7	MS1	0,99(HinfI)	Cellmark
D7S21	MS31	0,98(HinfI)	Cellmark
D12S11	MS43A	0,97(HinfI)	Cellmark
D7S22	G3	0,96(HinfI)	Cellmark

Results and Discussion

We analysed the distribution of W values obtained by typing the HLA - A and -B locus antigens and by using 21 conventional systems (fig.1 and fig.2). In figure 1, the HLA data demonstrates, that in 6,4% of cases W values of 99,73% or more are obtained. In comparison, the analysis of conventional systems shown in figure 2 resulted in 17,4% of cases with W values of 99,73% or above. In 38,1% of cases analysed by conventional systems the W values were between 99,0% and 99,73%, whereas only 19,1% of cases analysed by HLA were in this range. Thus, in comparison to the HLA system, the conventional systems lead in twice of the cases to W values of greater than 99,0%. Since HLA typing is less time consuming and is also cost effective we have chosen to analyse the combination of HLA typing and DNA analysis by SLP's for paternity testing. Previous studies of SLP's have shown that in 23% of cases analysis by 6 SLP's did not result in W values of more than 99,73%. To further evaluate the significance and practicability of SLP's we have extended our panel by SLP's demonstrating a higher heterozygosity rate. The data are illustrated in figure 3. These data have been obtained by selecting fragments with high frequency and fragments with low frequency. Therefore the dark columns represent the W values obtained by worst case analysis whereas the light columns represent the W values by best case analysis. Regarding worst case analysis the application of 6 SLP's was necessary to reach W values of 99,73% in all cases. Application of three or four SLP's can result in W values as low as 62,0% or 82,0%

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg

respectively, whereas the application of five SLP's results in W-values of greater than 99,73% in 76,5% of cases. In all of these cases we did not include the situation that all SLP's exhibited either homozygosity of the fragments between the mother and the child ore that both fragments of the child could have been inherited from the mother. If this situation occurs it can lead to W values below 50%. In our analysis this situation did occur only in one case were 3 SLP's with HR's between 0,66 and 0,85 were used. In this instance an extended investigation with 3 additional SLP's (HR 0,94 - 0,98) did correct the W value up to 99,74%. The sole application of the HLA-system in this case resulted in an W value of 95% and the combination of HLA with the worst case DNA analysis did reach an W value of 93.8%. Besides this exception, table 1 summarises the data obtained from various cases where W values were obtained either by HLA or by the combination of HLA with 3 SLP's. The data are grouped according to the W values obtained with the HLA system. Our results demonstrate, that in all of these cases the combination of HLA and 3 SLP's leads to W values of greater than 99,73%. These values were also obtained when HLA typing alone did reach only W values of less than 90,0%. In cases of exclusion (N=10) were probes with high heterozygosity rates between (0.97) and (0.99) were applied (YNH24 , MS1 , MS31 , MS43A, G3) all probes indicated exclusion.

Summary

1. Single application of HLA results in only 25% of cases in W-values of 99,0% and more.
2. The combination of 3 SLP's with HLA reveals in all cases W-values of > 99,73%.
3. The application of SLP's with high heterozygosity rates reduces the number of SLP's that are required to reach sufficient W values.
4. SLP's with a high heterozygosity rate result in high exclusion rates.
5. In all cases were exclusion was found by conventional or HLA-system , the SLP's did also exhibit exclusion patterns.

References

1. Spielmann,W. and Seidl,S.: The application of the HLA-system in cases of disputed paternity. Z.Rechtsmedizin 74, 121-140 (1974).
2. Henke,L.; Paas,H,; Hoffmann,K. and Henke,J.: Applying DNA-polymorphisms to paternity testing. Z.Rechtsmed.103: 235-248 (1990).
3. Hummel,K.: Biostatistical Opinion of Parentage. Vol.I, ed. G.Fischer, Stuttgart (1971).
4. Miller SA, Dykes D, Polesky HF.: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleid Acids Research 16: 1215 (1988).
5. Brüggemann B., Teixidor D., Kilp M., Seidl S.: Experiences with six single locus probes in paternity testing. Advances in Forensic Haemogennetics 4. ed. Rittner and Schneider, Springer-Verlag, p 246-248, (1992).

Advances in Forensic Haemogenetics 5 (c) Springer-Verlag Berlin Heidelberg

Tab.1: Comparison of W - values according to data obtained by HLA-. DNA- and HLA + DNA analysis

W - values according to HLA data	HLA	3 SLP's	HLA + 3 SLP's
W(%) 95,0 - 98,5	98,407	99,913	99,999
	98,386	99,983	99,999
	97,736	99,680	99,994
	97,200	99,899	99,997
	96,150	99,964	99,998
	95,225	99,733	99,987
W(%) 90,0 - 94,5	94,770	99,896	99,994
	94,580	99,987	99,999
	94,284	99,692	99,980
	91,400	99,942	99,995
	91,463	99,932	99,994
	91,883	99,901	99,991
W(%) < 90,0	88,579	99,950	99,994
	87,740	98,051	99,730
	87,653	99,842	99,978
	87,290	99,975	99,996
	87,100	99,896	99,983

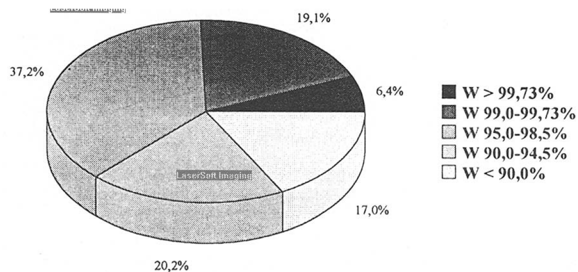

Fig.1: Biostatistical calculation of W values (Essen Möller) using HLA - A and - B frequencies in cases of disputed paternity (n=96)

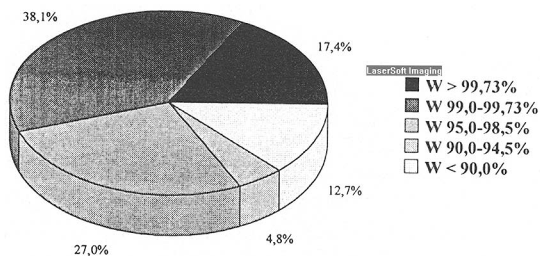

Fig.2: Biostatistical calculation of W values (Essen Möller) using 21 conventional systems in cases of disputed paternity (n=63)

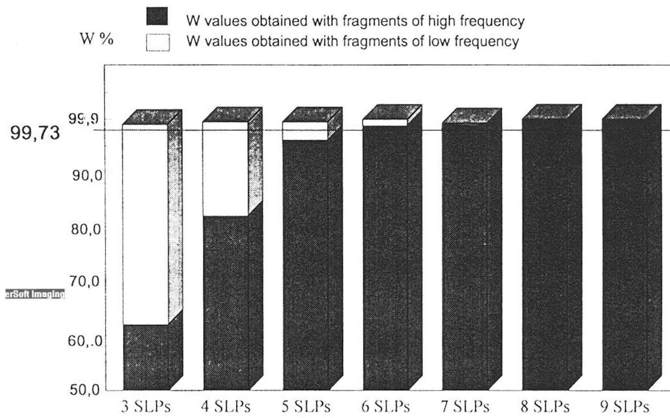

Fig.3: **Comparison of W values (Essen Möller)obtained with variable numbers of VNTR's (SLP's) in cases of disputed paternity (n=17)**