Paternity testing using the hypervariable 3' HVR DNA probe

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INTRODUCTION

The human genome contains many polymorphic loci known as hypervariable regions (HVR) of DNA, which result from a variable number of tandem repeats of a short core sequence. There are very informative for genetic analysis since there are many alleles at each locus. These regions may occur dispersed throughout the genome, or they may be clustered on a single chromosome. Several HVRs associated with the alpha-globin gene cluster on the short arm of chromosome 16 have been characterized (Higgs et al., 1981). The alpha-globin 3' HVR (Jarman et al., 1986) is a tandem repeat array of 17 base pairs (pb), the number of which varies considerably between different alleles. Under hybridization conditions of high stringency, the 3' HVR probe reveals as highly polymorphic locus with heterozygosity approaching 1 (Higgs et al.,1986). The 3' HVR is a hypervariable region located approximately 8 kb downstream of the α1-globin gene ; the repeat segment is related to three previously identified HVRs by the sequence 5'- GNGGGG (N) ACAG - 3' ; a probe to the 3' HVR detects multiple, mendelianly inherited DNA segments, suggesting that it too may represent one member of a dispersed minisatellite family (Fowler et al., 1986).

Several possible applications of DNA fingerprinting using such probes have been explored ; however, the analysis of DNA fingerprints produced by minisatellite probes is complicated by several difficulties assessing which bands are allelic, and also in genetic linkage studies by the lack of chromosomal localisation of the bands. We described here a very usefull approach to genetic analysis which overcomes some of these problems : the method involves digestion of DNA with an enzyme and hybridization with the conventionally labelled 3' HVR probe, which detects a *Pvu* II restriction fragment length polymorphism (RFLP), whose alleles are conveniently sited on the gel ; the resulting band pattern is a simple constituent allelic band system of known chromosomal localisation. This approach to genetic characterisation would be of particular value in paternity testing (Wainscoat et al., 1987) and should prove be simple in pratice, and complementary to other methods of analysis.

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MATERIAL AND METHODS

In our experiments, blood was obtained from French families and from unrelated healthy controls. DNA was obtained from the nuclear pellet of each blood sample by phenol-chloroform extraction. DNA (10 µg) was digested to completion with 20-30 units of *Pvu* II under the conditions recommended by the manufacturer (appligène). The DNA fragments were electrophoresed in a 1 % agarose gel and transferred onto nitrocellulose filters by Southern blotting. The 4 kilobases (kb) *Hinf* I insert from the recombinant pSEA1 was used as a probe, labelled with 32 P by nick-translation. High-stringency conditions were obtained by hybridization with 50 % formamide at 42° C and post-hybridization wasking in 0.1 x SSC at 65° C. Autoradiography with Amersham Hyperfilm was carried out at -70° C with an intensifying screen (Dupont Lighting Plus) for 1 - 4 days.

RESULTS

To determine the variability parameters, DNA samples from 120 unrelated French individuals were examined. Alleles detected by the probe were grouped into 100 bp size classes ; a total of 49 different alleles have been found far ranging in size from 0.7 kb to 9 kb and more. The most common allele detected in French was 2.3 kb (gene frequency = 0.147). Observed individual heterozygosity is 0.916, and the average *a priori* probability of exclusion is about 86 %.

The paternity results are summarized for the 3' HVR probe obtained in a two-years pilot study at the INTS Institute of Paris. Before DNA analysis, all cases were tested with a full-test battery of red blood cell antigens, blood cell enzymes and serum proteins (and with HLA - A and B markers in some cases) which had a combined probability of exclusion of about 0.99. In our results on 24 families tested we found 14 cases of exclusions with the 3' HVR marker alone, 13 of them being also demonstrated with the battery of classical markers ; only family number 21 demonstrated a 3' HVR exclusion, case not excluded unambiguously by other marker systems.

CONCLUSIONS

In conclusions, we have been involved in studies aimed at evaluating the feasibility of applying RFLP analysis to routine paternity testing. DNA technology is at present clearly second to the classical serological methods concerning practicability, since DNA analysis requires more hands-on time and is

considerably more expensive. By comparison, as shown here with RFLP analysis using a proper choice of only one particular probe, the efficiency of the two procedures were about equal ; moreover, in a single case, even after the inclusion of more than 20 blood, serum and enzyme polymorphisms (including HLA - A, B), we were unable to distinguish between to potential fathers in a particular paternity analysis ; using the 3' HVR probe, it was then possible to eliminate one of the father. We conclude that RFLP analysis, if using a proper choice of probes, is extremely promising for use in future paternity diagnostics in some special situations.

REFERENCES

Higgs, D.R., Goodbourn, S.E.Y., Wainscoat, J.S., Clegg, J.B. and Weatherall, D.J. (1981), *Nucl. Acids Res., 9* : 4.213-4.224.

Jarman, A.P., Nicholls, R.D., Weatherall, D.J., Clegg, J.B. and Higgs, D.R. (1986), *EMBO J, 5* : 1.857-1.863.

Higgs, D.R., Wainscoat, J.S., Flint, J., Hill, A.V.S., Thein, S.L., Nicholls, R.D., Teal, H., Ayyub, H., Pets, T.E.A., Falusi, A.G., Jarman, A.P., Clegg, J.B. and Weatherall, D.J. (1986), *Proc. Natl. Acad. Sci. USA, 83* : 5.165-5.169.

Fowler, S.J., Gill, P., Werrett, D.J. and Higgs, D.R. (1986), *Hum. Genet., 79* : 142-146.

Wainscoat, J.S., Pilkington, S., Peto, T.E.A. and Higgs, D.R. (1987), *Hum. Genet. 75* : 384-387.

Analysis of Disputed Paternities Using Hae III/YNH24 and Hinf I/MS31 RFLPs

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ABSTRACT

The utility of the RFLPs detected by restriction enzyme Hae III and probe YNH24 and by restriction enzyme Hinf I and probe MS31 for the analysis of paternity cases was studied.
Because the differences in the size of the fragments detected by these highly polymorphic systems is much smaller than the variance due to errors of measurement, allele frequencies can not be obtained.
Based on the fragment size frequencies and the incorporation of the measurement error into the Essen-Möller version of the probability of paternity, paternity indices were calculated, and the results were compared with those of conventional bloodgroup testing. The results showed that the RFLPs Hae III/YNH24 and Hinf I/MS31 can make a major contribution to the analysis of paternity.

INTRODUCTION

Typing for 16 conventional polymorphic systems which include bloodgroup antigens, red cell enzymes, serum proteins and HLA antigens, it is possible to exclude in average 99% of falsely accused men in Caucasian paternity cases. To improve the analysis of disputed parentages in general it has been demonstrated that restriction fragment length polymorphisms (RFLPs) can be very useful (Baird et al., 1986; Kanter et al., 1986; Balasz et al., 1989).
Because the differences in the size of the fragments detected by highly polymorphic probes is much smaller than the variance due to the errors of measurement, allele frequencies can not be obtained and therefore fragment size frequencies has to be calculated. For the calculation of paternity indices the Essen-Möller version of the probability of paternity was used as a function of the measurement error of the fragment size (Essen-Möller, 1938; Gjertson et al., 1988).
In this paper the results of the analysis of 24 paternity cases, performed on two highly polymorphic RFLPs, enzyme Hae III with probe YNH24 and enzyme Hinf I with probe MS31, were compared with those of 16 conventional bloodgroup systems applied in the same cases.

MATERIALS AND METHODS

DNA preparation and Southern blotting

Genomic DNA was isolated from peripheral blood from unrelated Caucasian individuals and from 24 paternity cases according to standard procedures (Maniatis et al. 1982). DNA samples (3 ug) were digested with the

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restriction enzymes Hae III or Hinf I at a concentration of 7 U/ug DNA following the manufacturers specifications. Each sample was mixed with 5 ng digested lambda DNA.
Separation of DNA fragments was performed by electrophoresis in 0.8% agarose, followed by Southern blotting and hybridization with ^{32}P-labeled DNA probes, YNH24 (kindly given by Dr Y. Nakamura and Dr R. White, Salt Lake City, USA) and MS31 (kindly given by Dr P.G. Debenham, ICI, Cellmark diagnostics, Oxfordshire, UK), the radioactive bands were visualized by autoradiography. The filters were reprobed with ^{32}P-labeled lambda DNA and exposed to X-ray film. (Southern, 1975; Feinberg and Vogelstein, 1983).

Fragment size calculation

The fragments detected by lambda DNA probe were used as internal markers in each lane to calculate the size of the fragments detected by the probes YNH24 and MS31 with the use of a computer assisted digitizing system.

Standard paternity testing methods

Routine paternity testing methods were taken from standard protocols and consisted of the serological analysis of red cell antigen systems (ABO, MNS, P, Rh, Kell, Duffy, Kidd, Lutheran and Wright), serum proteins (Gc, Hp, Gm and Km), red cell enzymes (AcP and PGM1) and leucocyte antigens (HLA-A,B,C).

Fragment size frequencies and calculation of the paternity index

The frequencies of the fragments detected by the RFLPs as described here were determined by testing unrelated Caucasian individuals. The ratio of the total range in which the fragments were detected and the square root of the total number of detected fragments has been used as class width.
The paternity index or likelihood ratio (I) is calculated according to Gjertson et al.'s (1988) method of calculation of probability of paternity (w). If equal a priori probabilities are assumed than
$W = \frac{P1}{P1+P0}$ and $I = \frac{W}{1-W}$ in which P1 and P0 are the respective probabilities for paternity.

RESULTS AND DISCUSSION

One hundred fifteen individuals were analyzed for the Hae III RFLP detected by probe YNH24. The fragments found ranged from 0.96 kb to 6.21 kb. After sorting the fragments in class widths of $(6.21 - 0.96)/\sqrt{(2x115)}$ = 0.35 kb, the fragment size frequency was calculated.
Ninety-nine unrelated individuals were analyzed for the Hinf I RFLP detected by probe MS31. The fragment size frequency was calculated after sorting of the fragments in ranges of 0.50 kb.
The paternity indices of 24 paternity cases were calculated after analysis by conventional systems with and without HLA-A,B,C, and the Hae III/YNH24 and Hinf I/MS31 RFLPs (Table 1).
The paternity indices calculated by testing RFLPs is based on a error of measurement of 1% (data not shown). After classification of the results the data were summarized (Table 2).

Table 1. Paternity indices of 24 cases obtained with 15 conventional systems, HLA-A,B,C and RFLPs

case	conv.systems - HLA	HLA	conv.systems + HLA	RFLP Hae III YNH24	RFLP Hinf I MS31	conv. systems - HLA + RFLP
1	5.1	5.4	27.5	7.5	3.2	122.4
2	10.3	86.0	885.8	12.2	30.6	3845.2
3	132.0	5.4	712.8	8.6	2.9	3292.1
4	757.0	9.3	7040.1	5.6	12.3	52142.2
5	-	-	-	1.3	0.0	-
6	87.0	8.6	748.2	2.3	32.5	6503.3
7	59.0	539.0	31801.0	52.3	257.2	793642.0
8	-	-	-	0.0	0.0	-
9	512.0	21.0	10752.0	16.1	46.4	382484.5
10	26.0	283.0	7358.0	2.3	8.5	508.3
11	19.1	156.3	2985.3	4.6	42.9	3769.2
12	16.4	539.0	8839.6	10.2	36.4	6088.9
13	74.9	14.9	1116.0	7.3	17.0	9295.1
14	73.3	14.7	1077.5	24.8	10.8	19632.7
15	212.5	13.4	2847.5	5.9	6.4	8496.0
16	50.0	1000.0	50000.0	4.9	3.9	955.5
17	-	-	-	0.0	0.0	-
18	502.0	21.0	10542.0	2.9	7.0	10187.6
19	-	-	-	0.0	0.0	-
20	86.2	156.6	13498.9	3.4	271.7	79629.8
21	29.0	31.0	899.0	4.3	16.9	2107.4
22	-	-	-	0.0	0.0	-
23	1428.0	258.0	368424.0	3.5	2.9	14344.3
24	-	-	-	0.0	0.0	-

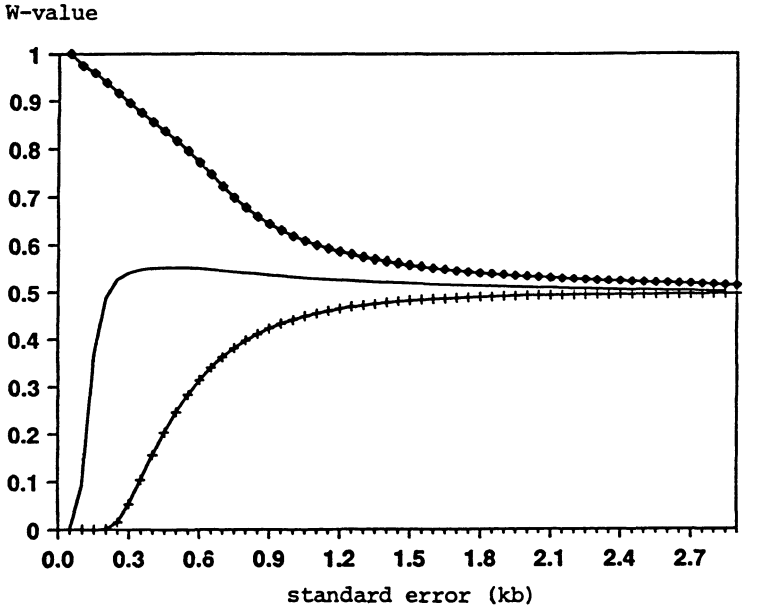
Table 2. Analysis of 24 Caucasian paternity cases with 15 conventional systems, HLA-A,B,C and RFLPs.

Category:	excluded	doubtful	probable	very probable
PI value:	0	0-19	20-99	$\geq$ 100
Conventional systems - HLA	6	4	8	6
Conventional systems + HLA	6	0	1	17
RFLPs:				
Hae III/YNH24 and Hinf I/MS31	6*	1	9	8
Conventional systems + RFLPs	6*	0	0	18

* In one case there was only an exclusion with Hinf I/MS31.

Using the standard tests the putative father was excluded in 6 cases. In 17 cases the probability was greater than 99% (I > 99) when the conventional systems including HLA were tested. Analysis for HLA showed a probability greater than 99% in 7 cases. In 7 other cases the results were doubtful ($0 < I \leq 19$).
When the 24 paternity cases were analysed for the two RFLPs the putative father was excluded for both in 5 cases ($I = 0.0$). In two cases the results were doubtful and in 8 cases the paternity index was greater than 99. Combining the results of the conventional systems without HLA and the two RFLPs, one case was doubtful and in 18 cases the paternity index was greater than 99.
The method for the calculation of the paternity index is a function of the size measurement error. If for example in case 9, the measurement error is 0 kb the standard Essen-Möller method is used for the calculation of the W-value is 1.0. If the measurement error is increased the W-value approaches 0.5 which results in a loss of information (fig. 1).

fig. 1 W-value as a function of the error of measurement



—— case 5, Hae III/YNH24: Child (2.0,1.6)
Mother (2.0,2.8)
Father (2.6,1.8): doubtful

+++ case 8, Hinf I/MS31 : Child (6.3,5.9)
Mother (6.3,5.1)
Father (8.3,4.9): exclusion

●●● case 9, Hinf I/MS31 : Child (6.8,4.6)
Mother (9.0,6.8)
Father (7.1,4.6): indication

In case 8, with a measurement error of 1% the putative father is excluded the pattern of the plot was identical to the other excluded cases: a curve starting with W = 0.0 (standard measurement error is 0 kb) to W = 0.5 (standard error 3kb). Raising the error rate to 2% the 5 non fathers remained excluded, The putative father of case nr 5 was excluded by conventional systems, HLA and by the Hinf I RFLP detected by probe MS31, (W = 0.0), but the result of the Hae III RFLP with probe YNH24 was doubtful (I = 1.32). When in this case the measurement error increases, the W-value increases from 0.0 to 0.55 and in the end the W-value approaches 0.5 from below.
By plotting the W-value as a function of the measurement error seems to be a good method to avoid wrong conclusions and to discover cases in which further testing of other genetic systems is necessary.
In spite of the small number of paternity cases tested in this study the results suggest that the Hae III RFLP detected by probe YNH24 together with the Hinf I RFLP detected by probe MS31 can be an powerful tool for the analysis of paternity testing, comparable with HLA-A,B,C-testing.

REFERENCES

Baird M, Balazs I, Giusti A, Miyazaki L, Nicholas L, Wexler K, Kanter E, Glassberg J, Allen F, Rubinstein P, Sussman L (1986) Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. Am J Hum Genet 39:489-501

Kanter E, Baird M, Shaler R, Balazs I (1986) Analysis of restriction fragment length polymorphism in DNA recovered from dried blood stains. J Forensic Sci 31:403-408

Balazs I, Baird M, Clyne M, Meade E (1989) Human population genetic studies of five hypervariable DNA loci. Am J Hum Genet 44:182-190

Essen-Möller E (1938) Die Beweiskraft der Ahnlichkeit in Vaterschaftsnachweis: Theoretische Grundlagen. Mitt Antropol Ges (Wien) 68:9-53

Gjertson DW, Mickey MR, Hopfield J, Takenouchi T, Terasaki PI (1988) Calculation of probability of paternity using DNA sequences. Am J Hum Genet 43:860-869

Maniatis T, Fritsch EF, Sambrook J (eds) (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503-517

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