Int J Leg Med (1991) 104:117–120

International Journal of
Legal Medicine

Application of single-locus hypervariable region DNA probes to deficiency cases in paternity testing

T. Yokoi, T. Odaira, M. Nata, Y. Aoki, and K. Sagisaka

Department of Forensic Medicine, Tohoku University School of Medicine, Aoba-ku, Sendai 980, Japan

Received March 16, 1990 / Received in revised form September 17, 1990

Summary. Seven DNA probes which recognize single-locus hypervariable region (HVR) were applied to a paternity test in which the putative father and his wife were deceased. Three legitimate children, an illegitimate child and her mother were available for analysis. The cumulative paternity index of the illegitimate child derived from 15 conventional blood group markers was 18.71 and from 7 DNA probes 92,572.08, that is, 4,948 times higher than the former. Thus the DNA analyses gave nearly conclusive evidence that the putative father was the biological father of the child. The application of highly discriminating polymorphisms of DNA which recognize single HVR loci is considered to be extremely informative in cases of disputed parentage.

Key words: DNA – Hypervariable polymorphism – HVR loci – Parentage testing

Zusammenfassung. In einem Vaterschaftsfall, in welchem der Putativvater und seine Frau verstorben waren, wurden 7 Arten von Single-Locus-Sonden, welche hypervariable Regionen erkennen, angewandt. Für den Test standen Blutproben dreier Vollgeschwister und eines Halbgeschwisters und dessen Mutter zur Verfügung, welche eine Freundin des Putativvaters gewesen war. Nach Untersuchung von 15 konventionellen Blutmarkern war der kumulative Paternitätsindex im Hinblick auf das illegitime Kind 18,71. Nach Analyse der 7 SLS-Polymorphismen war dieser 92.572, dies ist 4.948mal höher als der erste Wert. Auf diese Weise ergaben die DNA-Analysen einen nahezu eindeutigen Beweis dafür, daß der Putativvater der biologische Vater des Kindes war. Die Anwendung hochgradig diskreminierender Polymorphismen der DNA, welche Single-Locus HVR erkennen, wird als extrem informativ in Fällen strittiger Vaterschaft angesehen.

Schlüsselwörter: DNA – Hypervariable Polymorphismen (VNTR) – Vaterschaftstest

Offprint request to: T. Yokoi

Introduction

According to advances in hematogenetics, numerous genetic markers have been used in paternity tests. Recently, studies of DNA polymorphisms are in progress and many DNA restriction fragment length polymorphisms (RFLP) which recognize the presence or absence of particular restriction enzyme sites giving rise to two possible alleles at each locus [1], have been reported. Another class of polymorphisms is hypervariable polymorphic regions of DNA resulting from a variable number of tandem repeats (VNTR). A common feature of these probes is the presence of a short core sequence which is repeated in tandem along the chromosome and provides hypervariable polymorphism at the locus [2]. These are valuable genetic markers for human linkage maps [3], paternity testing [4–6] and individual identification [7–9].

In the present study, 7 hypervariable DNA probes were applied to paternity testing of the family in dispute and the statistical analyses were found to be much more informative for paternity testing.

Materials and methods

Case summary. This case was settled in a district court in April 1989 involving the putative father and his wife who were both deceased. The 3 legitimate children, one illegitimate child and her mother, who was the deceased's mistress, were available for analysis. The pedigree of the family is shown in Fig. 1. The parentage was not excluded with 15 standard blood group markers. The legitimate children, however, claimed a more affirmative evidence of paternity, and DNA analyses were ordered by the district court.

DNA hybridization. DNA samples of the family were prepared from peripheral blood using the standard technique [10]. Treatment with restriction enzymes, agarose gel electrophoresis, Southern blot, labelling of probes, pre-hybridization and hybridization were performed according to the method reported by Yokoi et al. [11].

DNA probes. The MR24/1, 3′globin, Ha-ras and Mucin were purchased from Amersham, UK and have been investigated in detail in a previous report [11]. The other 3 probes were kindly supplied by JCRB Gene Bank, Japan and have been reported in a previous paper [12].

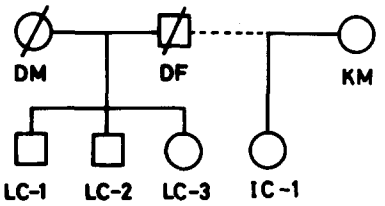

Fig. 1. Alleged pedigree of the family. DF = deceased father; DM = deceased mother; KM = kept mistress; LC-1, LC-2, LC-3 = Legitimate child 1, 2 and 3; IC-1 = illegitimate child; — = alleged relationship; --- = assumed relationship

Serological paternity testing methods. Paternity testing methods were taken from standard protocols including serological analyses of red cell antigens (ABO, Rh-Hr, MNSs, Duffy, Kidd and P_1), red cell enzymes (phosphoglucomutase-1 (PGM_1), esterase-D (EsD), acid phosphatase (AcP), phosphogluconate dehydrogenase (PGD)) and serum proteins (transferrin (Tf), haptoglobin (Hp), Gm allotypes (Gm), Km allotypes (Km), group-specific component (Gc), α_1-antitrypsin (Pi)).

Statistical methods. The polymorphic alleles of MR24/1, 3'globin, Ha-ras and Mucin were subdivided into 34, 37, 5 and 10 non-overlapping groups of fragments, respectively [11]. The polymorphic alleles of D2S44, D17S30 and D1S57 were also subdivided into 15, 28 and 5 nonoverlapping groups, respectively [12]. Therefore, the allele frequency values were used directly for calculation. The allele size was found to vary with increments which were smaller than the size measurement error [11, 12]. The size of the alleles was measured at 100-bp increments in D2S44 or 70-bp increments in D17S30. The database among the Japanese population used in this study is summarized in previous reports [11, 12]. The paternity exclusion was calculated using the formula described for RFLPs by Ito et al. [13].

Results

The results of conventional blood group markers for the family and the exclusion probability and paternity index are described in Table 1. The cumulative exclusion probability was 0.972 which was calculated using a gene frequency database from a Japanese population [11, 12]. The cumulative paternity index of the illegitimate child (IC-1) was 18.71 and the combined paternity probability 0.949.

DNA hybridization analyses were carried out using the combinations of probes and restriction enzymes as stated. Autoradiograms are shown in Fig. 2a–c. Allele sizes and paternity indices of the illegitimate child are summarized in Table 2. The 3.9 kb allele recognized in MR24/1-*Hin*fI system analysis, for example, could be inherited by the child (LC-2) from the alleged parent.

The cumulative exclusion probability of 7 probes was calculated as $1-(9.3 \times 10^{-5})$ using a gene frequency data from the Japanese population. The cumulative paternity index of IC-1 was 92,572.08 and the combined probability of paternity was 0.999989. This index was 4,948 times higher than the index from conventional blood group markers.

Discussion

Hypervariable DNA loci have been widely used especially for individual identification [7–9] and for paternity testing [4–6]. Most are multi-locus probes that recognize several loci under reduced stringency. The multi-locus system is very powerful, but statistical evaluation is still controversial [14, 15]. On the other hand, the single-locus probes are hybridized with high stringency, and are more sensitive and more reproduceable than the multi-locus probes [2, 11, 12]. In the present study, 7 hypervariable single-locus independent DNA probes were used which were except D1S57 locus on 1p and Mucin locus on 1q21. They might be assumed to be independent because allelic frequencies and confidence intervals among unrelated

Table 1. Summary of blood group markers (phenotypes) of the family in dispute and paternity indices of the illegitimate child (IC-1)

	Exclusion probability	LC-1	LC-2	LC-3	IC-1	KM	PI of IC-1
ABO	0.19	A	A	A	A	O	1.91
MNSs	0.24	MNs	MNs	Ns	Ns	MNs	1.45
Rh-Hr	0.24	CCDee	CCDee	CCDee	CCDee	CCDee	1.44
Duffy	0.08	a+b+	a+b+	a+b−	a+b−	a+b−	0.83
Kidd	0.18	a+b−	a+b−	a+b+	a+b+	a−b+	1.39
P_1	0.08	+	−	+	+	+	1.08
Hp	0.16	2-2	2-2	2-2	2-2	2-1	1.23
Tf	0.16	2-1	2-1	2-1	2-1	2-1	1.01
Gc	0.38	1F-1S	1F	1F-1S	1F-1S	1F-1S	1.35
Pi	0.22	M1M2	M1M2	M1M2	M1M2	M1M2	0.52
Gm	0.41	axg	axg	ab^3st	ab^3st	ab^3st	1.92
Km	0.17	lb	b	b	b	b	1.01
AcP	0.14	B	B	B	B	B	1.23
PGM	0.26	1−1+	1−1+	1−2+	2+2+	1+2+	1.70
EsD	0.18	2-1	1-1	2-1	1-1	1-1	1.07

Abbreviations: KM = kept mistress; LC = legitimate child; IC = illegitimate child

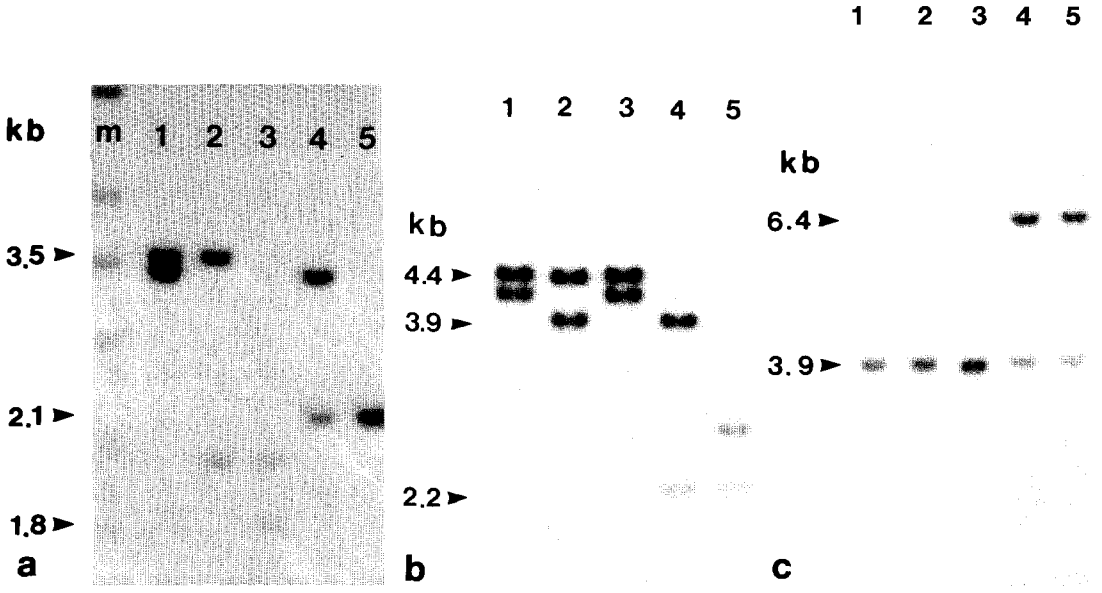

Fig. 2.a–c. Autoradiograms of southern blots of the disputed family are shown. The combinations of restriction enzyme and probe are as follows: **a** *Msp*I-D2S44; **b** *Hin*fI-MR24/1; **c** *Pvu*II-Mucin. *1:* LC-1, *2:* LC-2, *3:* LC-3, *4:* IC-1 and *5:* EW. Allele sizes in kb are indicated in Table 2. The arrow indicate the approximate size of the bands. The size was determined according to the method indicated in the text

Table 2. Allele size observed with HVR DNA probes and paternity indices (PI) of the illegitimate child (IC-1)

Probe enzyme	LC-1	LC-2	LC-3	IC-1	KM	PI of IC-1
D2S44	3.50	3.50	1.80	3.35	2.10	7.10
*Msp*I	3.35	2.80	1.50	2.10		
D17S30	0.93	0.93	0.93	0.93	1.14	3.23
*Msp*I	0.86	0.86	0.86	0.86	0.93	
D1S57	2.05	2.05	2.05	2.05	2.05	1.11
*Rsa*I	1.60		1.60	1.60	1.60	
MR24/1	4.4	4.4	4.4	3.9	2.7	7.54
*Hin*fI	4.2	3.9	4.2	2.2	2.2	
3′globin	2.6	2.6	2.4	2.6	2.6	13.05
*Pvu*II	2.4	2.4	2.1	2.4	2.1	
Ha-ras	2.7	2.7	2.7	2.7	2.7	1.29
*Pvu*II						
Mucin	3.9	3.9	3.9	6.4	6.4	28.65
*Pvu*II				3.9	3.9	

Individuals showing 1 band have 1 homozygotic allele and 2 bands have 2 heterozygotic alleles

Japanese individuals were previously reported and codominant segregation of the polymorphism was confirmed in family studies [11, 12].

We adopted the indication of fragment size in 100-bp increments for the probes MR24/1, 3′globin, D2S44 and D1S57 (partially 50-bp increment in D2S44) or 70-bp increments for D7S30. For example, the alleles detected by D2S44 have been grouped into 28 size classes among Japanese population, but these categories do not represent individual alleles. The recognition of a single repeat of the core sequence (30 base pair [2]) is beyond the capability of agarose gel electrophoresis. Theoretically D2S44 has over 110 different repeat units among Japanese population, but this method using 100-bp increments might be a conservative approach for standardization of the fragment size.

The 3′globin probe was used for paternity testing among populations in the United States [6] and the paternity index was calculated to be between 3 and 25 in 10 cases of 2-generation families. In the present study, the paternity index was calculated to be 13.05 with 3′globin. This means that the distribution of allelic sizes differs among races, as was reported with some probes in American populations [16]. In our study, 4 out of the 7 probes, D2S44, MR24/1, 3′globin and Mucin, gave a paternity index greater than 7. In this case the paternity index using these 4 probes, was 20,025.

No mutation has been observed in more than 200 cases examined with the 3′globin single-locus probe [6]. Wolff et al. [17] mentioned that the mutation rate of D17S30 single-locus probe was 0.2%. Thefore, investigation with single-locus hypervariable probes might be a more practical approach for paternity testing. In cases where parentage is negated with one hypervariable probe confirmation with other probes located on an other chromosome should be carried out.

The probes, MR24/1, 3′globin, Ha-ras and Mucin, are available from Amersham U.K., and D2S44, D17S30 and D1S57, from ATCC, U.S.A. or from JCRB Gene Bank, Japan. The combination of independent single-locus DNA polymorphisms is considered to be informative enough not only for paternity test but also for forensic, anthropological and other human genetic purposes.

Acknowledgments. This study was supported in part by the TAKEDA Life Science Foundation and the Ministry of Education, Science and Culture.

References

1. Cooper DN, Schmidtke J (1984) DNA restriction fragment length polymorphisms and heterozygosity in the human genome. Hum Genet 66:1–16
2. Nakamura Y, Leppert M, O'Connell P, Woff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1622
3. Nakamura Y, Lathrop M, O'Connell P, Leppert M, Lalouel JM, White R (1988) A mapped set of DNA markers for human chromosome 15. Genomics 3:342–346
4. Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable "minisatellite" regions in human DNA. Nature 314:67–73
5. Baird M, Balazs I, Giusti A, Miyazaki L, Nicholas L, Wexler K, Kanter E, Glassberg J, Allen F, Rubinstein P, Sussmann 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
6. Allen RW, Bliss B, Pearson A (1989) Characteristics of a DNA probe (pa3'HVR) when used for paternity testing. Transfusion 29:477–485
7. Jeffreys AJ, Brookfield JEY, Semenoff R (1985) Positive identification of an immigration test-case using human DNA fingerprints. Nature 317:818–819
8. Gill P, Jeffreys AJ, Werrett DJ (1985) Forensic application of DNA "fingerprints". Nature 318:577–579
9. Giusti A, Baird M, Pasquale S, Balazs I, Glassberg J (1986) Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm. J Forensic Sci 31:409–417
10. Maniatis T, Fritsch EF, Sambrook J (1981) Molecular cloning, a laboratory manual. Cold Spring Harbor, Cold Spring Harbor NY
11. Yokoi T, Nata M, Odaira T, Sagisaka K (1990) Hypervariable regions of DNA for parentage testing and individual identification. Z Rechtsmed 103:487–497
12. Yokoi T, Nata M, Odaira T, Sagisaka K (1990) Hypervariable polymorphic VNTR loci for parentage testing and individual identification. Jpn J Hum Genet 35:179–188
13. Ito H, Yasuda N, Matsumoto H (1985) The probability of parentage exclusion based on restriction fragment length polymorphisms. Jpn J Hum Genet 30:261–269
14. Evett IW, Werrett DJ, Buckleton JS (1990) Paternity calculations from DNA multilocus profiles. J Forensic Sci Soc 29: 249–254
15. Odelberg SJ, Demers DB, Westin EH, Hossaini AA (1988) Establishing paternity using minisatellite DNA probes when the putative father is unavailable for testing. J Forensic Sci 33: 921–928
16. Balazs I, Baird M, Clyne M, Maeda E (1989) Human population genetic studies of five hypervariable DNA loci. Am J Hum Genet 44:182–190
17. Wolff RK, Nakamura Y, White R (1988) Molecular characterization of a spontaneously generated new allele at a VNTR locus: no exchange of flanking DNA sequence. Genomics 3: 347–351