

A CASE OF MATERNITY TESTING: EXCLUSION BY POLYMORPHIC VNTR MARKERS OF DNA

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Summary The genetic markers of a Korean woman and her allegedly adopted child were compared to test her maternity. None of 21 conventional markers, including 8 red cell antigens, 6 red cell enzymes, and 7 serum proteins, excluded the maternity. These results indicate a maternal probability of 0.31 which was too low to conclude the true maternity. Human leukocyte antigen (HLA)-A, -B, -C and -DR haplotyping was also uninformative in this case. The maternity was consequently excluded by the observation of a difference in 2 of 5 variable number of tandem repeat (VNTR) markers.

Key Words maternity testing, DNA analysis, VNTR markers

INTRODUCTION

Parentage is usually determined using such conventional genetic markers as red blood cell antigens, red cell enzymes, serum proteins and human leukocyte antigens (HLA). In addition, the variations of DNA such as restriction fragment length polymorphisms (RFLPs) are analyzed for the purpose (Akane *et al.*, 1990). Many RFLP markers have been found on various regions of every chromosome, and, among them, hypervariable RFLPs or variable number of tandem repeat

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Abbreviations: PGM1, phosphoglucomutase 1; ACP1, acid phosphatase 1; ESD, esterase D; GPT, soluble glutamic-pyruvate transaminase; PGD, phosphogluconate dehydrogenase; GLO1, glyoxalase 1; HP, haptoglobin; GC, group-specific component; TF, transferrin; PI, α_1 -antitrypsin; AHS₂, α_2 HS-glycoprotein; Gm, H chain of γ -globulin; Km, L chain of γ -globulin.

(VNTR) markers, which were developed by Nakamura *et al.* (1987a), have proved to be the most informative for this purpose (Akane *et al.*, 1990; Yokoi *et al.*, 1990).

Because the blood relationship between a mother and her child is usually obvious owing to the fact of delivery, it is uncommon to have to determine the maternity. Here is a peculiar case of maternity testing, in which only the VNTR markers were useful for the exclusion.

CASE REPORTS

A Korean woman claimed that a child legally registered as her progeny was not actually her child and that the child was the progeny of her husband and another woman. Her husband had deceased before the analysis was performed. The genetic markers of the child and the alleged mother were analyzed to test the maternity.

ANALYSIS OF GENETIC MARKERS

Conventional markers. Twenty-one conventional markers and HLA types were analyzed by antisera or by electrophoresis as described elsewhere (Akane *et al.*, 1990).

RFLPs. All VNTR probes used (Nakamura *et al.*, 1987a–1988b) were kindly supplied from the Japanese Cancer Research Resources Bank. DNA samples were extracted from peripheral blood leukocytes, and RFLPs were analyzed by Southern blotting method using ³²P-labeled probes as reported before (Akane *et al.*, 1990).

RESULTS AND DISCUSSION

Blood groups are used in elucidating problems of doubtful parentage because of their appearance in an individual in accordance with Mendelian laws of inheritance. Results in analyses of 21 conventional genetic markers and HLA types are shown in Table 1. The maternity of the alleged mother was excluded by none of these markers. The probability of maternity calculated from 21 markers based on allele frequencies in Korean population by the formula of Essen-Möller was 0.31, which was too low to affirm the maternity. Since frequencies of HLA haplotypes in Korean are unknown, the probability from HLA could not be obtained.

Then, 5 VNTR probes were additionally utilized for analyses of RFLPs as shown in Table 2. When pYNH24 or pEFD75.1 was used, each allele detected in the child's DNA was different from either of two alleles found in the alleged mother's genotype (Fig. 1): The maternity was excluded by 2 of 5 VNTR markers.

When hypervariable RFLPs are analyzed, the possibility of mutation event

Table 1. Conventional genetic markers.

Marker systems	Alleged mother	Child	Marker systems	Alleged mother	Child
Red cell antigens			Serum proteins		
ABO	B	B	HP	2	2-1
MNS	MNs	Ms	GC	2-1S	2-1F
Rh	CcDEe	CcDEe	TF	C1	C1
Duffy	a+b-	a+b+	PI	M1	M1
Kidd	a-b+	a+b+	AHSG	2-1	2-1
P	P ₁	P ₂	Gm	axg	axg
Lewis	a-b+	a+b-	Km	1-b+	1+b+
Diego	a-b+	a-b+			
Red cell enzymes			HLA		
PGM1	1A2B	1A	A	A2,A11	A2,A24
ACPI	B	B	B	B35,Bw62	B35,B51
ESD	1	2-1	C	Cw3,Cw4	Cw4,-
GPT	2-1	2-1	DR	DR4,DR9	DR4,DR9
PGD	A	A			
GLO1	2-1	2			

Table 2. VNTR markers.

Probe	Locus	Restriction enzyme	Genotypes ^a	
			Mother	Child
pYNH24 ^b	D2S44	MspI	3.00, 1.57	2.40, 1.70
pTHH59	D17S4	PvuII	1.80, 1.50	1.78, 1.50
pEFD75.1 ^b	D10S25	TaqI	2.41, 2.38	2.17, 2.17
pEFD126.3	D9S7	TaqI	2.70, 2.70	2.80, 2.70
pYNZ2	D1S57	RsaI	2.70, 1.60	2.15, 1.60

^a Genotypes are represented by restriction fragment lengths (kb) of alleles. ^b Maternity was excluded.

to generate a new length allele should always be taken into consideration (Akane *et al.*, 1990). For example, the spontaneous mutation rate might be as high as 5% for the most unstable hypervariable minisatellite locus (Jeffreys *et al.*, 1988). The mutation rate in each VNTR locus is thought to be very low: Only one case had been reported (Wolff *et al.*, 1988). Nevertheless, parentage could not be excluded when only one VNTR marker shows mismatching between genotypes of the alleged parent and the child in question whereas other many markers do not. Since the probability

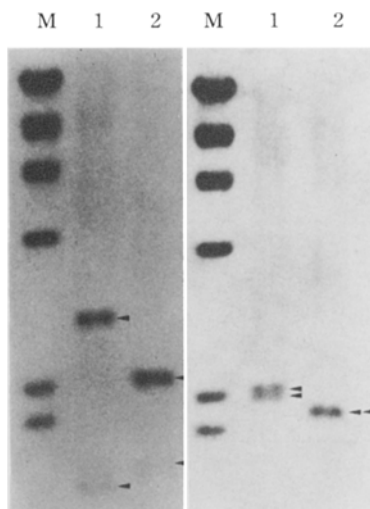


Fig. 1. The DNA alleles detected by pYNH24 (left) and pEFD75.1 (right) in the alleged mother (lane 1) and the child (lane 2). Lane M includes a molecular weight standard marker, λ -HindIII digest. Only by these probes could the maternity be excluded.

of mutations occurring on more than two VNTR loci at the same time is low (Wolff *et al.*, 1988), mismatching in more than two VNTR markers should be required for the exclusion of parentage, as in this case.

Parentage is usually determined by conventional genetic markers such as red cell antigens, red cell enzymes, serum proteins and human leukocyte antigens. Human genomic DNA includes numerous variations and conventional markers are reflections of only a small fraction of them. By DNA technology, it has become possible to detect DNA variations in various regions of individual human chromosomes. When RFLPs are analyzed in addition to the conventional genetic markers in parentage testing, the blood relationship can be determined more minutely and exactly.

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REFERENCES

- Akane, A., Matsubara, K., Shiono, H., Yuasa, I., Yokota, S., Yamada, M. and Nakagome, Y. 1990. Paternity testing: blood group systems and DNA analysis by VNTR markers. *J. Forensic Sci.* **35**: 1217-1225.
- Jeffreys, A.J., Royle, N.J., Wilson, V. and Wong, Z. 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature* **332**: 278-281.

- Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Kumkin, E. and White, R. 1987a. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* **235**: 1616-1622.
- Nakamura, Y., Gillilan, S., O'Connell, P., Leppert, M., Lathrop, G.M., Lalouel, J.-M. and White, R. 1987b. Isolation and mapping of a polymorphic DNA sequence pYNH24 on chromosome 2 (D2S44). *Nucleic Acids Res.* **15**: 10073.
- Nakamura, Y., Fujimoto, E., O'Connell, P., Leppert, M., Lathrop, G.M., Lalouel, J.-M. and White, R. 1987c. Isolation and mapping of a polymorphic DNA sequence (pEFD126.3) on chromosome 9q (D9S7). *Nucleic Acids Res.* **15**: 10607.
- Nakamura, Y., Holm, T., Gillilan, S., Leppert, M., O'Connell, P., Lathrop, G.M., Lalouel, J.-M. and White, R. 1988a. Isolation and mapping of a polymorphic sequence (pTHH59) on chromosome 17q (D17S4). *Nucleic Acids Res.* **16**: 3598.
- Nakamura, Y., Culver, M., Sergeant, L., Leppert, M., O'Connell, P., Lathrop, G.M., Lalouel, J.-M. and White, R. 1988b. Isolation and mapping of a polymorphic DNA sequence (pYNZ2) on chromosome 1p (D1S57). *Nucleic Acids Res.* **16**: 4747.
- Wolff, R.K., Nakamura, Y. and White, R. 1988. Molecular characterization of a spontaneously generated new allele at a VNTR locus: No exchange of flanking DNA sequences. *Genomics* **3**: 247-351.
- Yokoi, T., Nata, M., Odaira, T. and Sagisaka, K. 1990. Hypervariable polymorphic VNTR loci for parentage testing and individual identification. *Jpn. J. Human Genet.* **35**: 179-188.