CASE REPORT

Masao Ota,[1] *B.S.; Isamu Yonemura,*[1] *Ph.D.;*
Hirofumi Fukushima,[1] *M.D.; Hayato Hasekura,*[1] *M.D.;*
Goichi Ishimoto,[2] *Ph.D.; Youko Mizutani,*[3] *B.S.; and*
Takamichi Yamada,[3] *M.D.*

A Case of Paternity Testing Influenced by the Silent Allele of Rh Erythrocyte Groups

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ABSTRACT: A paternity test is presented in which a father and his two children possessed an extremely rare amorphic gene $R^{-29}(\overline{\overline{r}},---)$. One of the children was determined to be illegitimate at the first trial as her Rh phenotype was R2R2(ccDEE) and the father's phenotype was R1R1(CCDee). At the Court of Appeal, however, the rare Rh gene $\overline{\overline{r}}(---)$ was shown to be inherited from the father to the appellant child through extended tests including her brother whose phenotype was also R2R2(ccDEE). She was acknowledged to be legitimate.

KEYWORDS: pathology and biology, paternity, genetic typing, forensic immunogenetics, legitimacy, Rh blood groups, amorphic gene $\overline{\overline{r}}(---)$

In paternity testing, a false exclusion might result from atypical inheritance caused by the presence of suppressor genes, amorphic genes, or mutant genes. This report describes a rare case of paternity testing in a legitimacy denial suit, where the plaintiff father was excluded of his paternity to the defendant child at the first trial, but the High Court judged against him on the basis that a rare Rh amorphic gene was found to be transmitted.

History and Results

A man denied the legitimacy of his daughter. In the first trial, the girl was determined to be illegitimate as her Rh phenotype was R2R2(ccDEE) and her father's phenotype was R1R1(CCDee). These results apparently excluded paternity, although the expert suggested a possibility of her legitimacy in case she and her father had an extremely rare amorphic

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[1]Instructor, lecturer, associate professor, and professor, respectively, Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, Japan.

[2]Associate professor, Department of Legal Medicine, Mie University School of Medicine, Tsu, Japan.

[3]Instructor and professor, respectively, Department of Legal Medicine, Aichi Medical University, Nagakute, Japan.

TABLE 1—*Agglutination scores of erythrocytes with Rh antisera in a disputed paternity case.*[a]

Antisera Methods	Rh1(Rho,D)		Rh2(rh′,C)		Rh5(hr″,e)		Rh3(rh″,E)		Rh4(hr′,c)	
	Saline	Bromelin	Saline	Bromelin	Saline	Bromelin	Saline	Bromelin	Saline	Bromelin
Father	15	71	19	53	31	38	0	0	0	0
Mother	32	88	0	0	0	0	48	66	27	81
Child-1	22	71	0	0	0	0	36	49	20	66
Child-2	13	55	23	52	28	40	28	37	17	64
Child-3	22	71	0	0	0	0	33	55	22	69
R1R1, CDe/CDe	23.0	65.7	33.7	62.0	30.7	64.0	0	0	0	0
R1R2, CDe/cDE	16.5	52.5	23.0	49.5	19.7	37.0	31.5	45.0	15.5	62.5
R2R2, cDE/cDE	27.5	74.5	0	0	0	0	48.0	72.0	27.0	85.5

[a]Underlined figures show average scores of genotype.
R1R1, R1R2, and R2R2 erythrocytes were used from healthy donors.

gene. On the appeal of the mother and the daughter, the High Court ordered the expert to extend the test on her brother and sister whose legitimacy was not questioned. The test revealed the brother to have the same Rh phenotype R2R2(ccDEE) as the annulled daughter.

As the involvement of rare Rh genes, $\overline{\overline{R}}^{o}$(—D—) or $\overline{r}$(---), was suggested, strength of red cell agglutination of the family members of this case was observed using five Rh blood grouping sera anti-Rh1(Rho,D), anti-Rh2(rh′,C), anti-Rh3(rh″,E), anti-Rh4(hr′,c), and anti-Rh5(hr″,e). The results are shown in Table 1. Agglutinations were scored and summed according to Marsh [*1*]. Suspected heterozygotes of an amorphic gene in this disputed family did not disclose an increase in agglutinability to anti-Rh1 serum, excluding the possible participation of the $R_{1,-17}(\overline{\overline{R}}^{o}$, —D—) gene.

The agglutination scores of those heterozygous cells against anti-Rh2, anti-Rh3, anti-Rh4, and anti-Rh5 corresponded to those of R1R2(CcDEe) heterozygous cells and $R^{-29}(\overline{r}$,---) heterozygous cells as shown in the previous paper [*2*]. From these results, the putative father, the brother (Child-1), and the annulled daughter (Child-3) were judged to be heterozygotes of the amorphic gene of $R^{-29}(\overline{r}$,---). The Rh blood group genotypes of this family are shown in Fig. 1.

The test results of many erythrocyte groups, serum protein groups, erythrocyte isoenzyme groups, and leukocyte HLA groups did not show any exclusion of paternity (Table 2). The total probability of paternity exclusion was 99.87 in random Japanese males. The total probability of paternity likelihood of the putative father, reserving the Rh erythrocyte groups, was 99.97%. Assuming the $R^{-29}(\overline{r}$,---) gene frequency to be 0.008, as the $\overline{r}h$(---/---) propositus was found as the 16 000th random sample, although his parents were first cousins and the actual frequency should be much rarer, the total probability of paternity likelihood was more than 99.99%. These values corresponded to Hummel's criteria [*3*] of "practically proved" in estimating likelihood of paternity.

Dermatoglyphic tests including fingerprints and palmprints gave an impression that Child-3 was more similar to the disputed father than Child-1 or Child-2 was. Chromosomal tests showed that sizes of the C-bands on chromosomes 1, 9, and 16 of the three children corresponded to either of the chromosomes of the mother and the alleged father. This method, developed by us, was useful in supporting the immunohematological examination [*4*]. The High Court judged the alleged father to be the biological father of the once annulled daughter who was recognized to be legitimate.

Discussion

Many forensic geneticists agree that paternity exclusion should not be concluded by a single genetic system, but further tests are needed to find one or more inconsistent systems to

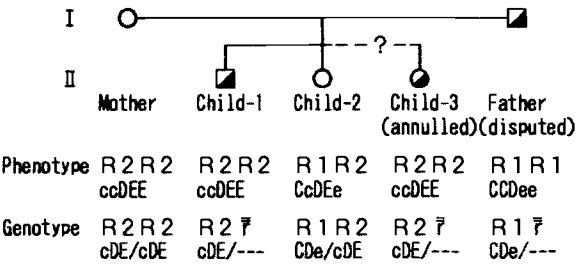

FIG. 1—*Pedigree of a disputed paternity case in which a* $R^{-29}(\overline{r}$,---) *gene was involved. Child-3, once excluded of legitimacy, was legitimatized through the additional tests including Child-1 who shared the --- gene.*

TABLE 2—*Blood grouping results of the parties.*[a]

	Mother	Child-1	Child-2	Appellant Child-3	Appellee Father	Paternity Exclusion in Random Males	Paternity Likelihood of the Appellee
			ERYTHROCYTE GROUPS				
ABO	O	O	O	A	A	0.514	0.6807
MNSs	MSs	Ms	Ms	Ms	Ms	0.265	0.6734
P	P1	P2	P2	P2	P2	0	0.5543
Rh	R2R2	R2$\bar{r}$	R1R2	R2$\bar{r}$	R1$\bar{\bar{r}}$	0.509	0.9843
Le	a−b+	a−b+	a−b+	a−b+	a−b+	0	0.5033
Fy	a+b+	a+b−	a+b+	a+b+	a+b−	0	0.5000
Jk	a+b−	a+b−	a+b−	a+b−	a+b−	0.275	0.6789
Di	a−b+	a−b+	a−b+	a−b+	a−b+	0.002	0.5110
Xg	a+	a+	a−	a+	a−	0	0.4510
			SECRETOR				
Se	Se	Se	Se	Se	Se	0	0.5151
			SERUM PROTEIN GROUPS				
Hp	2−2	2−2	2−2	2−2	2−2	0.076	0.5596
Gc	1F1S	1F1F	1F1F	1F1S	1F1F	0.079	0.5817
Tf	C1C2	C1C1	C1C2	C1C1	C1C1	0.063	0.5714
Pi	M1M1	M1M1	M1M1	M1M1	M1M1	0.068	0.5747
C6	AB	AB	AB	B	AB	0.247	0.4985
AHS	1−1	1−1	1−1	1−1	1−1	0.070	0.5760
F13B	3	3	3	1−3	1−3	0.496	0.6281
PLG	1−1	1−1′	1−1′	1−1	1−1′	0.002	0.3434
			ISOENZYME GROUPS				
PGM_1	1AB	1A	1A	1A	1A	0.092	0.5896
AcP	B	B	B	B	B	0.045	0.5596
EsD	1	1	1	1	2−1	0.123	0.4348
sGPT	2−1	2−1	1	2−1	1	0	0.5000
PGD	A	A	A	A	A	0.008	0.5227
			LEUKOCYTE GROUPS				
HLA-A	2,26	2,24	24,26	2,24	24,26	0.415	0.5842
HLA-B	w48,w61	w48,w52	w52,w61	w48,w52	w52,7	0.795	0.8218
HLA-C	—,w3	—,—	—,w3	—,—	—,w7	0.140	0.5073
HLA-DR	2,w8	2,2	2,w8	2,2	2,1	0.256	0.7143
Cumulative probability						0.998	0.9999

[a]Haplotype frequency considering recombination rates between HLA-A and B loci was used for cumulative probability of paternity likelihood. $\bar{\bar{r}}$(− − −) frequency in Japan was assumed to be 0.008.

meet the possibility of exceptional inheritance, including amorphic genes, modifying genes, or mutations. Henningsen [5] postulated the existence of a silent Rh gene − − − transmitted from a mother to a child. Prokop and Schneider [6] also presented two cases of paternity testing in which both mother and her child should possess − − − gene. Ishimori and Hasekura [7] proved the existence of this amorphic Rh gene $\bar{\bar{r}}$(− − −) by the discovery of the $\bar{\bar{r}}\bar{\bar{h}}$(− − −/− − −) homozygote. Difficulty in the present case was shown to be caused by the R−29($\bar{\bar{r}}$,− − −) gene.

In Japan over 30 Rh:−17(Rho,—D—) homozygotes[4] and 1 Rh:−29($\bar{\bar{r}}\bar{\bar{h}}$,− − −) homozy-

[4]List of rare blood donors in Japan (unpublished).

gote [7] are reported, indicating the presence of a considerable number of heterozygotes in the population. In case the paternity is excluded only by the result of R1R1(CCDee) and R2R2(ccDEE) combination, the expert should confirm if the —D— or --- gene might be involved.

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Address requests for reprints or additional information to
Masao Ota
Department of Legal Medicine
Shinshu University School of Medicine
Matsumoto, 390, Japan