

Allelic Frequency of Polymorphic DNA Sequences in Japanese
and Its Application to the Paternity Testing

T. Harumoto, K. Suzuki, K. Matsui, S. Ito, Y. Matsuo*,
T. Miyazaki and H. Matsumoto

Department of Legal Medicine,
*Department of Blood Transfusion,
Osaka Medical College, Takatsuki, Osaka, Japan

INTRODUCTION

The cellular oncogene Ha- ras-1 (HRAS-1) is localized to the short arm of chromosome 11. DNA sequence data suggest that this region consists of a variable tandem repetition (VTR) of a 28-base-pair consensus sequence at the 3' flanking region of the gene (Capon et al. 1983). Certain restriction enzymes cut sites closely outside of this tandem repetition resulting in restriction fragment polymorphisms (Goldfarb et al. 1982). The allele frequency and its application to the paternity determination, has been reported (Baird et al. 1986), and the frequencies were much different among American blacks, Caucasian and Hispanics. Here, we represent the allele frequency of HRAS-1 in the Japanese population, its pedigree analysis and application to paternity testing.

MATERIALS AND METHODS

High molecular DNA was isolated from leukocytes which had been separated by Hypaque-Ficoll method (Ferrante and Thong 1980). Approximately 10 μg of DNA was digested with *Hinf*I and the digests were fractionated by 0.8 % Agarose gel electrophoresis. After denaturation and neutralization, DNA was transferred to a nylon membrane and hybridized with labeled DNA probe. As a probe, a 2.5 kb *Bam*HI/*Sac*I fragment containing VTR was nick-translated with ^{32}P -dCTP (specific activity : $> 1 \times 10^8$ cpm / μg). Bands were visualized by autoradiography.

RESULTS AND DISCUSSION

Figure 1 shows the four observed alleles (a1, a2, a3 and a4) which were, when digested with other enzymes, corresponded to the common alleles previously reported (Krontiris et al. 1985; Thein et al. 1986; Heighway et al. 1986; Baird et al. 1986).

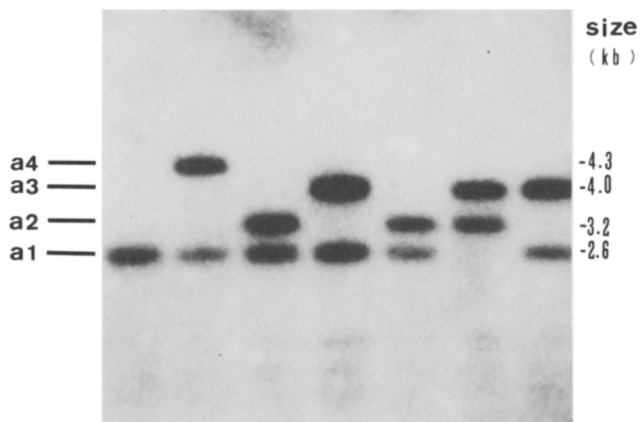


Fig. 1. Four HRAS-1 alleles (a1, a2, a3 and a4) observed in the Japanese population. Each lane shows HinfI - digested DNA from different individuals.

Table 1. Allele Frequencies of HRAS-1 in the Japanese Population and Other Ethnic Groups

Alleles	Size (kb)	Number of alleles(%)	Caucasian (Krontiris et al. 1985)	Unknown but not Japanese (Thein et al. 1986)	(Heighway et al. 1986)
a1	2.6	542 (84.6 %)	69 %	68 %	60 %
a2	3.2	1 (0.2 %)	12 %	6 %	16 %
a3	4.0	61 (9.5 %)	10 %	9 %	13 %
a4	4.3	35 (5.5 %)	6 %	13 %	7 %
other alleles		1 (0.2 %)	3 %	4 %	4 %
Total		640 (100.0 %)	100 %	100 %	100 %

The two right hand columns show frequencies reported from the United Kingdom. Baird et al. (1986) reported that the frequency of three alleles (a1, a2 and a4) was, in total, 82.4 % in Caucasians, 68.6 % in American blacks and 76.2 % in Hispanics. Previously reported allele sizes are assigned to those detected in HinfI - digested DNA.

As shown in Table 1, the frequency of a1 allele was higher in the Japanese population than in other ethnic groups, and the frequencies of a3 and a4 were similar. However, the significant difference was observed in the a2 allele, where only one allele was found among 320 Japanese (0.2 %). Besides these four alleles, rare alleles were observed which were measured as 2.5 kb and 3.8 kb in size. We include 2.5 kb allele to a1 (2.6 kb) allele because technical limitation was impossible to distinguish 2.5/2.6 from 2.6/2.6. The other allele (3.8 kb) was designated a5.

Table 2. Parents - offsprings Combinations in HRAS-1 RFLP

parental types	No. of families	offspring types									
		<u>a1</u> a1	<u>a1</u> a3	<u>a1</u> a4	<u>a3</u> a3	<u>a3</u> a4	<u>a4</u> a4	<u>a1</u> a2	<u>a2</u> a3	<u>a2</u> a4	<u>a2</u> a2
<u>a1</u> a1 × <u>a1</u> a1	7	16 (16)	-	-	-	-	-	-	-	-	-
<u>a1</u> a1 × <u>a1</u> a3	4	4 (3.5)	3 (3.5)	-	-	-	-	-	-	-	-
<u>a1</u> a1 × <u>a1</u> a4	3	5 (4)	-	3 (4)	-	-	-	-	-	-	-
<u>a1</u> a3 × <u>a1</u> a3	1	1 (0.5)	0 (1)	-	1 (0.5)	-	-	-	-	-	-
<u>a1</u> a4 × <u>a1</u> a4	1	0 (0.75)	-	1 (1.5)	-	-	2 (0.75)	-	-	-	-
<u>a1</u> a3 × <u>a1</u> a4	2	1 (1)	1 (1)	1 (1)	-	1 (1)	-	-	-	-	-
<u>a1</u> a2 × <u>a1</u> a3	1	0 (0.5)	0 (0.5)	-	-	-	-	1 (0.5)	1 (0.5)	-	-

√a/ Expected numbers are given in parentheses.

Gene frequencies of these alleles were : a1 = 0.8469, a2 = 0.0016, a3 = 0.0953, a4 = 0.0546, a5 = 0.0016, $X^2 = 2.564$
 0.30 < P < 0.50, 3 d.f. The observed phenotype distribution is in good accordance with Hardy-Weinberg equilibrium.

Pedigree Analysis of HRAS-1 Alleles

Table 2 shows Parents - offsprings phenotype combinations. The four alleles were consistent with their inheritance as Mendelian alleles.

Application of HRAS-1 Alleles to the Paternity Testing

Figure 2 shows the practical cases of paternity testing. The probability of paternity exclusion of HRAS-1 was calculated as 12.7 %, which is not very high, however, when HRAS-1 RFLP is used in addition to the ordinary methods, such as blood types and protein polymorphisms, it would be one of useful markers. The probability of paternity and of paternity exclusion by HRAS-1 is possible to be combined with the probabilities from the ordinary methods, which will result in more accurate determination of paternity.

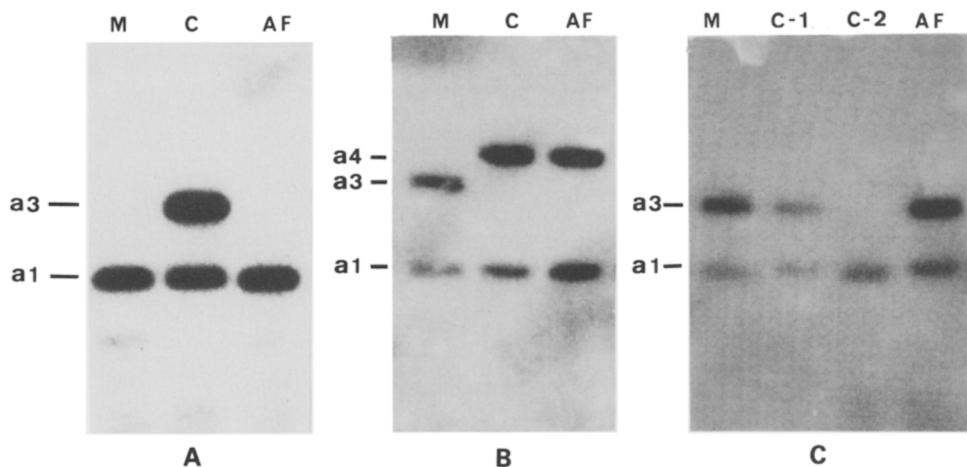


Fig. 2. Examples of paternity cases analyzed for HRAS-1 RFLP. M : mother; C : child; AF : alleged father. Panel A : a case of exclusion. Panels B and C : cases of inclusion. The probabilities of paternity were 0.9014 in Panel B, 0.5148 for child-1 and 0.3712 for child-2 in Panel C (C-1 : child-1, C-2 : child-2).

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
- Capon DJ, Chen EY, Levinson AD, Seeburg PH, and Goeddel DV (1983) Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature* 302:33-37
- Ferrante A, Thong, YH (1980) Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human blood by the Hypaque-Ficoll method. *J Immunol Methods* 36:109-117
- Goldfarb M, Shimizu K, Perucho M, Wigler M (1982) Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. *Nature* 296:404-409
- Heighway J, Thatcher N, Cerny T, Hasleton PS, (1986) Genetic predisposition to human lung cancer. *Br J Cancer* 53:453-457.
- Krontiris TG, DiMartino NA, Colb M, Parkinson DR, (1985) Unique allelic restriction fragments of the human Ha-ras locus in leukocyte and tumor DNAs of cancer patients. *Nature* 313:369-374
- Thein SL, Oscier DG, Flint J, Wainscoat JS, (1986) Ha-ras hypervariable alleles in myelodysplasia. *Nature* 321:84-85