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Short Communication

FREQUENCIES OF POLYMORPHISMS IN THE RHODOPSIN GENE OF JAPANESE RETINITIS PIGMENTOSA AND NORMAL INDIVIDUALS

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Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of eye disorders in which defects in the retina are associated with night blindness, an eventual loss of visual field, a diminished response on the electroretinogram (ERG) and pigmentary retinal degeneration. Mutations in the genes of rhodopsin (Dryja *et al.*, 1991; Sung *et al.*, 1991; Inglehearn *et al.*, 1992), peripherin/*RDS* (Farrer *et al.*, 1991; Kajiwara *et al.*, 1991), and other candidate genes have been found in different patients with RP, where most of them have been found in the autosomal dominant RP (ADRP).

The purpose of this report is to estimate the frequencies of three kinds of DNA polymorphisms within the rhodopsin gene in the Japanese patients with RP and normal individuals, which are very different from that of the American population reported by Sung *et al.* (1991).

Materials and methods

Genomic DNAs were extracted from leucocytes of peripheral blood collected from three groups of unrelated 38 autosomal dominant RP (ADRP), 23 autosomal recessive RP (ARRP), and 66 normal individuals among which only two ADRP

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patients have the rhodopsin gene mutation cosegregated with the disease and no other patients have been yet found with any mutation of candidate genes. As it is impossible to determine the genetic types of RP by clinical phenomena, the patients were divided into ADRP or ARRP through their family history.

To detect A269→G (5′ non-coding region), G5145→A (4th intron), and C-5321→A (3′ non-coding region) substitutions within the rhodopsin gene, two pairs of primers were designed; sense and antisense, 5′-TAGGCCCTCAGTTTCTGCAG-3′, 5′-ACTGCCATGGCTCAGCCAGG-3′ for amplification of the fragment of nucleotide (nt) position 74–403, and 5′-CGTGAGGGGCAGAAGCAGGC-3′, 5′-GTGACTTCGTTCATTCTGCA-3′ for the fragment of nt5061–5389, respectively. The DNA fragments were amplified by polymerase chain reaction (PCR) using a DNA Thermal Cycler (Perkin-Elmer Cetus, USA). The PCR products purified were digested with restriction enzyme, *Sac*II, *Hin*fI, and *Fok*I, respectively, and size of the fragments was detected on 2% agarose gel containing ethidium bromide. The A269→G substitution creates a new recognition sequence for *Sac*II. The G5145→A and C5321→A substitution loses the recognition sequence for *Hin*fI and *Fok*I, respectively. Twenty or more DNA fragments from ARRP, ADRP, and normal individuals were purified by NACS PREPAC™ (GIBCO BRL, Life Technologies, USA) from low melting agarose gels and directly sequenced using a fluorescence-based DNA autosequencer, Model 373A (Applied Biosystems, USA) to confirm the results obtained by the restriction enzyme.

Results and discussion

Electrophoresis patterns of the digestion by each restriction enzyme to detect the A269→G, G5145→A, and C5321→A substitutions were shown in Fig. 1. The frequencies of each genotype of polymorphisms within the rhodopsin gene among the three groups of unrelated ADRP, ARRP, and normal individuals were shown in Table 1. Expected values were calculated from the frequency of substitution per an allele in each group. There was no significant deviation from the Hardy-Weinberg's law in each genotype in any group. And also there were no significant differences in the frequencies of A269→G, G5145→A, and C5321→A substitutions per an allele between ADRP and ARRP, or between each RP and normal group as well as bias to specific genotype. Therefore, the frequency of A269→G, G-5145→A, and C5321→A substitutions pooled three groups was 57.0, 36.1, and 5.1%, respectively. These values were different from that of the American (Sung *et al.*, 1991), in which the frequency of A269→G, G5145→A, and C5321→A was 14%, infrequent (no exact value has been shown) and 13%, respectively. The 57.0% (A269→C) and 36.1% (G5145→A) in our data are far higher than that in the American, and 5.1% (C5321→A) is lower than 13% in the American.

The difference between the American and the Japanese has also been seen in the frequency of mutations within the rhodopsin gene in ADRP patients. In three large population studies in the USA (Dryja *et al.*, 1991; Sung *et al.*, 1991) and the

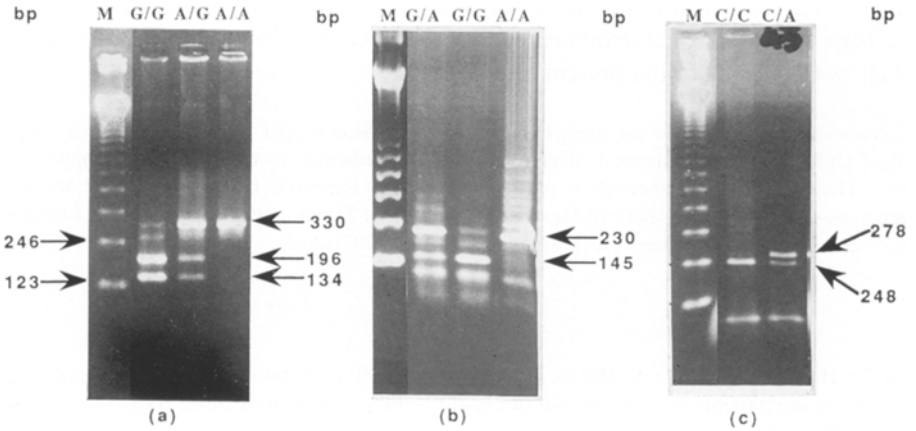

Fig. 1. Electrophoresis patterns digested by restriction enzymes. (a) A269→G, *Sac*II digestion detects A by 330 base pair (bp) band, and G by 134 and 196 bp bands, respectively. (b) G5145→A, *Hin*fI digestion detects G by 145 bp and A by 230 bp band. (c) C5321→A, *Fok*I digestion detects C by 248 bp and A by 278 bp band. M: 123 bp DNA ladder.

Table 1. Frequencies of DNA polymorphisms in the rhodopsin gene of ADRP, ARRP, and normal individuals.

Substitution	Geno-type	ADRP		ARRP		Normal		Total	
		Obs.v. (%)	Exp.v.	Obs.v. (%)	Exp.v.	Obs.v. (%)	Exp.v.	Obs.v. (%)	Exp.v.
A269→G	A/A	8 (20.5)	7.9	3 (13.0)	3.9	9 (15.3)	10.6	20 (16.5)	22.4
	A/G	19 (48.7)	19.3	13 (56.5)	11.2	32 (54.2)	28.8	64 (52.9)	59.3
	G/G	12 (30.8)	11.8	7 (30.4)	7.9	18 (30.5)	19.6	37 (30.6)	39.3
	Total	39 (100)	39.0	23 (100)	23.0	59 (100)	59.0	121 (100)	121.0
	G allele frequency	43/78 (55.1)		27/46 (58.7)		68/118 (57.6)		138/242 (57.0)	
G5145→A	G/G	18 (48.6)	18.3	9 (39.1)	9.8	24 (36.4)	23.6	51 (40.5)	51.4
	G/A	16 (43.2)	15.5	12 (52.2)	10.4	31 (47.0)	31.7	59 (46.8)	58.1
	A/A	3 (8.1)	3.3	2 (8.7)	2.8	11 (16.7)	10.6	16 (12.7)	16.4
	Total	37 (100)	37.1	23 (100)	23.0	66 (100)	65.9	126 (100)	125.9
	A allele frequency	22/74 (29.7)		16/46 (34.8)		53/132 (40.2)		91/252 (36.1)	
C5321→A	C/C	33 (94.3)	33.0	20 (90.9)	20.0	53 (86.9)	53.3	106 (89.8)	106.3
	C/A	2 (5.7)	1.9	2 (9.1)	1.9	8 (13.1)	7.5	12 (10.2)	11.4
	A/A	0 (0.0)	0.0	0 (0.0)	0.0	0 (0.0)	0.3	0 (0.0)	0.3
	Total	35 (100)	34.9	22 (100)	21.9	61 (100)	61.1	118 (100)	118.0
	A allele frequency	2/70 (2.9)		2/44 (4.5)		8/122 (6.6)		12/236 (5.1)	

U.K. (Inglehearn *et al.*, 1992), more than 50 mutations in the rhodopsin gene co-segregated with RP have been reported in which most of these mutations results in single amino acid substitution, and these mutations have been identified in 25–30% of unrelated ADRP patients in U.S.A.. However, in the Japanese ADRP patients, only four families with mutation of codon 15 (Fujiki *et al.*, 1994), 17 (Fujiki

et al., 1992), 181 (Saga *et al.*, 1994) or 347 (Fujiki *et al.*, 1992) in rhodopsin gene have been reported. These differences are considered to be due to racial differences as well as the results in the present study.

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