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RFLP Report

A NOVEL *Ava*I POLYMORPHISM WITHIN EXON 5 OF THE RHODOPSIN GENE

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Summary We identified a novel *Ava*I polymorphism within 3′ noncoding region within exon 5 of the human rhodopsin gene and determined the allele frequency in a Japanese population. The polymorphism was found to be due to A/G transversion at nucleotide 5510 of the gene.

Key Words rhodopsin gene, polymorphism, Japanese population, Oguchi disease

Gene mutations in the rhodopsin have been identified in hereditary retinal photoreceptor disorders including retinitis pigmentosa and congenital stationary night blindness, and on the other hand, a number of polymorphic DNA changes in the rhodopsin gene are also described which are innocent and not related to any retinal disease. During assessment of the rhodopsin gene in Oguchi disease, an autosomal recessive disease characterized by congenital night blindness associated with unique ocular fundus changes, we did not identify any disease-related mutation but found an innocent A/G transversion within the 3′ noncoding region of the exon 5. This paper reports a novel polymorphism with nucleotide alteration A5510G in the rhodopsin gene in a Japanese population.

PCR primers. Sense: 5′-TTCCGGAACTGCATGCTCACCACC-3′ and antisense: 5′-CGGCAGGACCAGGGATCTGGGATT-3′ that spans the exon 5 of the human rhodopsin gene from np 5168 to np 5829, as described by Hotta *et al.* (1992).

Allele frequency. Allele frequencies of A5510G were determined by *Ava*I digestion in 116 chromosomes from normal, unrelated individuals and 124 chro-

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mosomes from unrelated patients with retinitis pigmentosa, all Japanese in Kagoshima prefecture, southwestern Japan. Determination of the gene frequencies revealed: in normal subjects, allele A, 0.52; allele G, 0.48; the heterozygosity ratio (A/G), 51.7%; and in retinitis pigmentosa patients, allele A, 0.44; allele G, 0.56; the heterozygosity ratio, 56.5%. The gene frequencies did not differ between the two groups of subjects, and data from combined 240 chromosomes were: allele A, 0.48; allele G, 0.52; and the heterozygosity ratio, 54.2%.

Mendelian inheritance. Figure 1 shows the segregation profiles of A5510G in two independent families of hereditary retinal diseases, which are consistent with Mendelian inheritance. Each proband did not show any other pathological mutation in the rhodopsin gene.

Conditions for PCR and AvaI digestion. DNA was eluted from peripheral blood, and a 662-bp fragment encompassing exon 5 was amplified. Polymerase chain reaction (PCR) was performed for 30 cycles (denaturation at 95°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 2 min), followed by final extension at 72°C for 10 min. PCR product was reacted with *Ava*I at 40°C overnight, and separated by 1.8% agarose gel. A5510G creates an *Ava*I recognition site, which restricts the PCR product into 340- and 322-bp fragments.

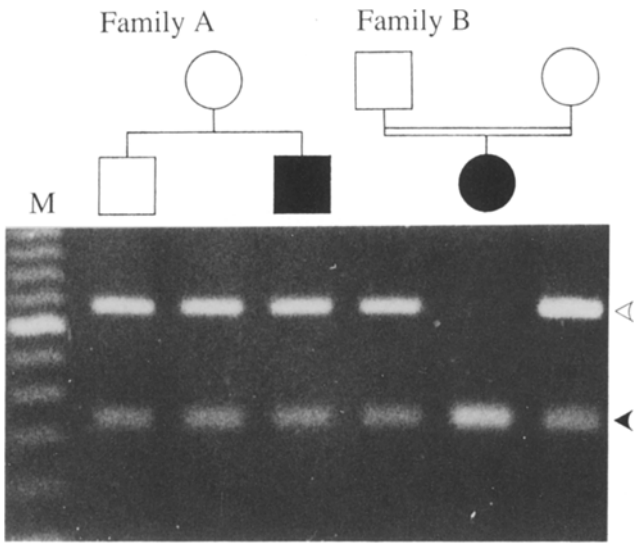

Fig. 1. PCR-restriction digestion for a nucleotide alteration at np 5510 (A to G) within the rhodopsin gene. The nucleotide alteration creates an *Ava*I recognition site, which restricts the 662-bp PCR product into 340- and 322-bp fragments. M, molecular weight markers (100 bp ladder); Family A, a family of Oguchi disease; Family B, a female patient with sporadic retinitis pigmentosa and her consanguineous parent; open arrowhead, undigested fragments (allele A); closed arrowhead, doublets of digested fragments (allele G).

Chromosomal location. The polymorphism is localized to 3q21-q24, within the rhodopsin gene.

Comments. Previously reported innocent polymorphisms within the human rhodopsin gene are A269G, C2557A, C3982T, G4142A, G5145A and C5321A which are identified in Caucasians (Dryja *et al.*, 1990; Sung *et al.*, 1991), and A269G, G5145A and C5321A which are observed in Japanese (Fujiki *et al.*, 1995). The above described A5510G indicates an additional polymorphism in the rhodopsin gene. There is yet no information available for its frequencies with reference to races or ethnic groups. The polymorphism A5510G was identified in a population of the southwestern area of Japan, and its survey is requested for different races or countries, as well as A269G, G5145A, C5321A, to speculate the anthropological characteristics of the rhodopsin gene.

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