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POINT MUTATIONS OF RHODOPSIN GENE FOUND IN JAPANESE FAMILIES WITH AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA (ADRP)

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Summary The mutations of codon 17, 23, 58, and 347 of rhodopsin gene were investigated in 24 unrelated Japanese families including 33 patients with autosomal dominant retinitis pigmentosa (ADRP). A patient with codon 17 mutation (Thr-17-Met, ACG→ATG) and a family including 4 patients with codon 347 mutation (Pro-347-Leu, CCG→CTG) were detected among them. Their clinical findings were extremely different between the two mutations. The former showed type 2 and the latter showed type 1 ADRP. No mutation of codon 23 and 58 was detected in any families so far analyzed in the present study. Clinical findings associated with the mutation in codon 17 and 347 of the rhodopsin gene show an existence of allelic heterogeneity.

Key Words autosomal dominant retinitis pigmentosa (ADRP), rhodopsin gene, point mutation, allelic heterogeneity

INTRODUCTION

Retinitis pigmentosa (RP), characterized by visual field loss, night blindness and fundus changes including bone corpuscle lumps of pigment, is clinically and genetically heterogeneous eye diseases with the mode of inheritance of an autosomal dominant, autosomal recessive or X-linked type. Linkage to locus D3S47 on chromosome 3q has been found in a large Irish family with ADRP (McWilliam *et al.*, 1989). Dryja *et al.* (1990a, b) studied intensively rhodopsin gene on chro-

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mosome 3q and found the mutations of a single base change in an allele of codon 23, 58, or 347 of the rhodopsin gene in patients with ADRP. Moreover, Dryja *et al.* (1991) reported a total of 17 different single-base mutations correlated with the ADRP. Forty-three (29%) of the 150 patients carry one of these mutations and no patient has more than 1 mutation. Rhodopsin gene encoding human rhodopsin has been isolated and completely sequenced (Nathans and Hogness, 1984). The coding region of the human rhodopsin gene is interrupted by four introns. The amino acid sequence is 348 residues long. Rhodopsin serves as the molecular photoreceptor in rod cell of the retina.

We have reported a Pro-347-Leu mutation of rhodopsin gene in a Japanese family with ADRP (Fujiki *et al.*, 1991; Hotta *et al.*, 1992; Shiono *et al.*, 1992). Present study is the further analyses for codon 17, 23, 58, and 347 of rhodopsin gene in 24 unrelated Japanese families including 33 patients with ADRP. Because, it has been known that codon 347 is "hot spot" for mutations (Dryja *et al.*, 1991); ninety-one European ADRP pedigrees have had no mutation in codon 23 (Farrar *et al.*, 1990), nevertheless approximately 13% of 150 unrelated American ADRP patients have had the codon 23 mutation (Dryja *et al.*, 1991); and the patients with codon 17 or 58 mutations have a distinct phenotypic expression of the gene defect (Fishman *et al.*, 1991, 1992; Richards *et al.*, 1991).

MATERIALS AND METHODS

Sample preparation: Twenty-four Japanese families including 33 patients with ADRP from Department of Ophthalmology, Juntendo and Tohoku Universities were studied as follows. Patients have been clinically diagnosed to be typical retinitis pigmentosa. The genetic type of disease was determined through a family history. A sample of 10–20 ml of venous blood was collected from each patient. High molecular weight DNAs were extracted from leukocyte nuclei.

PCR amplification and digestion by restriction enzyme: Two pairs of oligonucleotide primers were synthesized to amplify DNA fragments encompassing codon 58 in exon 1 and codon 347 in exon 5 by using DNA Synthesizer (Applied Biosystems, USA). Thirty rounds of PCR (polymerase chain reaction) amplification were performed by using a Thermal Cycler (Perkin Elmer Cetus, USA). The PCR products for codon 58 and 347 were cut by restriction enzyme *Dde*I and *Nci*I, respectively. A mutation in codon 58 creates a new recognition sequence for *Dde*I (Dryja *et al.*, 1990b), and a mutation in codon 347 loses the recognition sequence for *Nci*I. Using these approaches, we checked the mutation in codon 58 and 347 of rhodopsin gene of 24 unrelated ADRP families including 33 patients.

Dot blot hybridization by allele-specific oligonucleotide: To detect the mutation in codon 17 and 23, wild and mutant oligonucleotides were synthesized as follows: Codon 17 (wild); 5′-TAC•CAC•ACC•CGT•CGC•ATT•GGA-3′, Codon 17 (mutCAT): 5′-TAC•CAC•ACC•CAT•CGC•ATT•GGA-3′, Codon 23 (wild): 5′-GTA•CTC•GAA•GGG•GCT•GCG•TAC-3′, Codon 23 (mutGAG): 5′-GTA•CTC•

GAA•GAG•GCT•GCG•TAC-3, and Codon 23 (mutGTG): 5′-GTA•CTC•GAA•GTG•GCT•GCG•TAC-3. DNA fragments encompassing codon 17 and 23 were amplified by PCR from leucocyte DNA of the ADRP patients and normal persons. Ten microliters of a PCR reaction mixture were denatured, deposited onto a nylon membrane (PALL, USA), and fixed by UV light. The blots were hybridized by 5′-end-labeled 21-mers of the wild and the mutant oligonucleotides shown as above, annealed, washed, and autoradiographed to check the wild or mutant sequence (Kawaguchi *et al.*, 1990, 1991). By this approach 19 unrelated ADRP families including 20 patients were checked on the mutation of Thr-17-Met (ACG→ATG), Pro-23-His (CCC→CAC) and Pro-23-Leu (CCC→CTC).

Sequence analysis: PCR product containing mutant allele in codon 17 was directly sequenced to detect a transition of nucleotide using Gene Scanner (Applied Biosystems, USA).

RESULTS

The codon 347 mutation was detected in only one family already reported (Hotta *et al.*, 1992; Shiono *et al.*, 1992) and was not found in the remaining 23 families. The mutant allele of codon 347 was transmitted from the affected proband's mother to her son (proband) and affected two grand-daughters who are the proband's daughters as shown in Fig. 1. The C to T transition of the second nucleo-

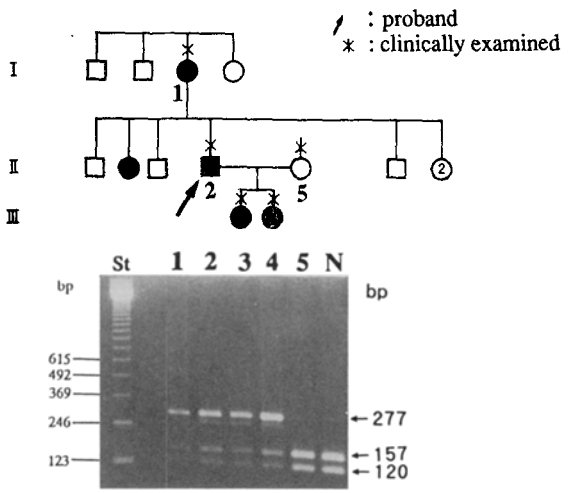

Fig. 1. Family pedigree and electrophoresis patterns by *Nci*I digestion for PCR products encompassing codon 347 in exon 5. St, 123 bp DNA ladder; 1, proband's mother (affected); 2, proband (affected); 3, 4, proband's daughters (affected); 5, proband's spouse who is mother of daughters (normal). 1, 2, 3 and 4 have lost the recognition site of *Nci*I by the muation of codon 347 in an allele of rhodopsin gene (277 bp band).

tide of codon 347, corresponding to a proline to leucine substitution was detected by sequencing (Hotta *et al.*, 1992). The 44 year-old proband had a night blindness since the age of 15 years. Fundus examination showed diffuse bone corpuscle pigmentation with concentric visual field loss and electroretinographic response was not recordable. Hewas diagnosed to be most likely type 1 ADRP of classification by Massof and Finkelstein (1981) and Fishman *et al.* (1985). Clinical findings of this family members have been detailed by Shiono *et al.* (1992).

Figure 2 shows the results of the dot blot hybridization by allele specific oligonucleotide probe for codon 17. Only one patient (No. 5) hybridized with the mutant probe carrying single nucleotide substitution (ACG→ATG). Other 19 patients (18 families) and a normal person did not react with the mutant sequences. In No. 5 patient, a C to T transition of the second nucleotide at the codon 17 of an allele of rhodopsin gene has been confirmed by sequencing analysis (Fig. 3), which results in a threonine to methionine substitution. In this family, although proband's mother, proband's sister and proband's niece were affected, only the proband was available for DNA analysis. He is a 40 year-old male, whose clinical findings showed extensive retinal degeneration with more bone spicule pigmentation in the inferior than superior retina and visual field showed loss of the superior field of both eyes. The amplitudes of b-wave in scotopic ERG and single flash ERG were reduced, but not extinguished. Ficker ERG showed moderately diminished amplitude. He was diagnosed to be type 2 ADRP described by Massof and Finkelstein (1981) and Fishman *et al.* (1985). No patients with Pro-23-His and Pro-23-Leu (Fig. 4) and Thr-58-Arg (data were not shown) were detected in present study.

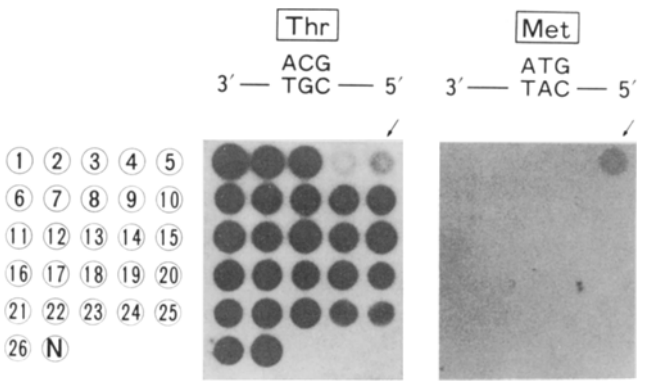

Fig. 2. Dot blot hybridization for the probe of normal (ACG) and mutant (ATG) sequences of codon 17. Amplified DNAs of a patient (No. 5) containing codon 17 hybridized to the probes of both normal and mutant sequences. Nos. 1–26: DNAs of ADRP patients (excluding 1, 2, 18, 21, 23, and 24 which are another retinal degenerative diseases), where Nos. 6 and 23 are sib. Therefore, 19 families with ADRP were available. N, normal person.

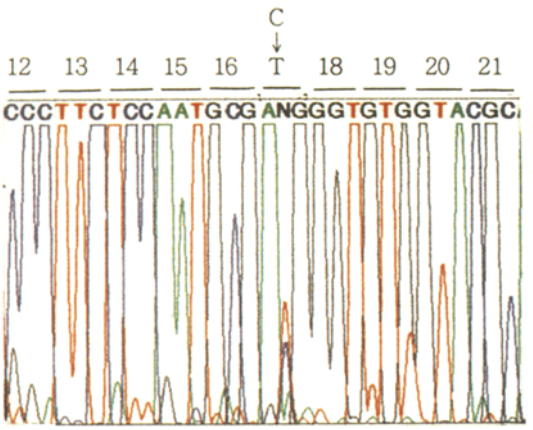

Fig. 3. Fluorescence-based direct sequence around codon 17 of No. 5 patient by Gene Scanner (Applied Biosystems, USA). A cytosine to thymine transition (ACG—ATG) in the second nucleotide of codon 17 has been detected, resulting in two peaks in the position.

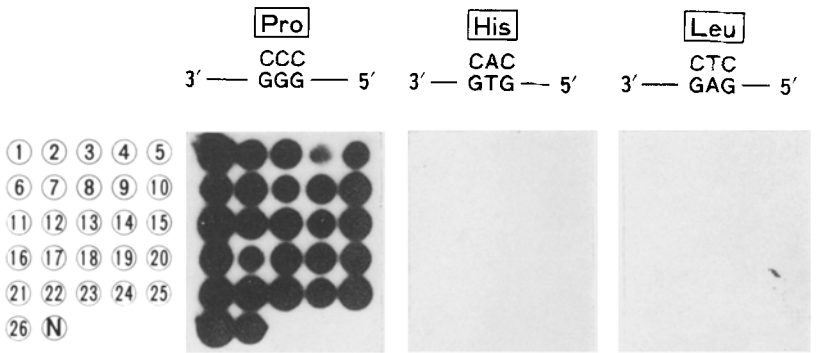

Fig. 4. Dot blot hybridization for the probe of normal (CCC) and mutants (CAC and CTC) sequences of codon 23. No body showed reaction with the mutant probes. Nos. 1–26: DNAs of ADRP patients (excluding 1, 2, 18, 21, 23, and 24 which are another retinal degenerative diseases), where Nos. 6 and 23 are sib. Therefore, 19 families with ADRP were available. N, normal person.

DISCUSSION

Two types of mutations of the rhodopsin gene in the two families among the 24 unrelated Japanese ADRP families were detected (19 families were available for codon 17 and 23). One was the codon 17 mutation (Thr-17-Met, ACG→ATG) in exon 1 and the other was the codon 347 mutation (Pro-347-Leu, CCG→CTG) in exon 5. No mutation of codon 23 and 58 in any families was detected so far analyzed in the present study.

As to codon 23 in exon 1, the mutation of Pro-23-His (CCC→CAC) has been observed in approximately 13% of 150 patients from different families residing in the United States and Canada (Dryja *et al.*, 1991). Nevertheless, this mutation has not been found in 91 European ADRP pedigrees including 21 Irish, 25 British, and 6 Swiss families (Farrar *et al.*, 1990), as well as 19 Japanese families in the present study. As to the fact that the mutation of codon 23 has been observed in an American population largely of British origin, it is interesting that it is not present in British and Irish ADRP families. Possibly the absence of the codon 23 mutation in the rhodopsin gene in British and Irish may be explained in terms of variation in the frequency of the mutation. Alternatively, the presence of the codon 23 mutation in the American population may be the result of a founder effect (Farrar *et al.*, 1990). As to codon 347, the Pro-347-Leu (CCG→CTG) mutation in 8 patients and Pro-347-Ser (CCG→TCG) mutation in one patient among 150 unrelated patients have been found in the United States (Dryja *et al.*, 1991). Pro-347-Arg (CCG→CGG) mutation was observed in a Swiss family (Gal *et al.*, 1991). As to codon 17 in exon 1, the Thr-17-Met (ACG→ATG) mutation detected in our study has been observed in the United States (Dryja *et al.*, 1991; Sung *et al.*, 1991a; Richards *et al.*, 1991; Fishman *et al.*, 1992). The codon 58 mutation reported in the United States was absent among 24 families in the present study.

Clinical findings of the proband and his mother with Pro-347-Leu mutation showed significantly concentric visual field loss and non-recordable electroretinograms. Their clinical findings were similar to those of American patients with Pro-347-Leu mutation reported by Berson *et al.* (1991). On the other hand, the patient with the Thr-17-Met mutation showed a regional preference for pigmentary changes to occur in the inferior part of the retina as well as corresponding field impairment predominantly in the superior. His electroretinographic responses were more pronounced than usually encountered in other forms of retinitis pigmentosa. His ocular findings were different from those of the patients with Pro-347-Leu mutation and showed almost the same phenomena as those of the cases with Thr-17-Met mutation in the United States reported by Jacobson *et al.* (1991) and Fishman *et al.* (1992).

Our results obtained in Japanese families of different ancestory from American, Canadian, and European pedigrees strongly support that the mutation of rhodopsin gene is the cause of some forms of ADRP. Now, more than 26 different kinds of single-base mutations and a few cases of deletion of codon (s) within the coding region of rhodopsin gene have been identified in ADRP patients (Inglehearn *et al.*, 1991; Keens *et al.*, 1991; Sung *et al.*, 1991a; Dryja *et al.*, 1991, *etc.*). Moreover, it has been identified that at least two distinct biochemical defects have been associated with different rhodopsin mutants in ADRP (Sung *et al.*, 1991b). Therefore, these allelic heterogeneities suggest a clinical variability. Almost 30% in ADRP patients have one of these mutations in the United States (Dryja *et al.*, 1991). On the other hand, it was found that the mutations of peripherin-*RDS* (retinal degenera-

tion slow: 6p12) gene locus cosegregate with ADRP in separate families (Farrar *et al.*, 1991; Kajiwara *et al.*, 1991). These facts indicate that some cases of ADRP are due to not only the mutation at rhodopsin gene locus but also at peripherin-*RDS* gene locus. Therefore, the mutation of other candidate gene(s) may also be the cause of ADRP.

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