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Case Report

MISSENSE MUTATION OF RHODOPSIN GENE CODON 15 FOUND IN JAPANESE AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA

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Summary Heterozygous missense mutation in codon 15 of the rhodopsin gene was detected in a patient with autosomal dominant retinitis pigmentosa (ADRP), where a transition of adenine to guanine at the second nucleotide in codon 15 (AAT→AGT), corresponding to a substitution of serine residue for asparagine residue (Asn-15-Ser) was detected. None of the remaining unrelated 42 ADRP, 24 autosomal recessive RP (ARRP) and 34 normal individuals had this alteration. Her funduscopic findings were sectorial in type similar to that of the patients with the same mutation found in an Australian pedigree (Sullivan *et al.*, 1993). This study shows phenotypic similarities in patients with the same mutation of a different ancestry.

Key Words autosomal dominant retinitis pigmentosa (ADRP), missense mutation, rhodopsin gene, *N*-glycosylation site

INTRODUCTION

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of eye disorders, characterized by night blindness, an eventual loss of visual field, a diminished response on electroretinogram (ERG), and pigmentary retinal degeneration. Mutations in rhodopsin gene (Dryja *et al.*, 1991; Sung *et al.*, 1991a; Inglehearn *et al.*, 1992; Macke *et al.*, 1993), peripherin/*RDS* gene (Farrar *et al.*, 1991; Kajiwara *et al.*, 1991) and other candidate genes have been found in patients

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with RP. In Japan, three families with Thr-17-Met (Fujiki *et al.*, 1992), Glu-181-Lys (Saga *et al.*, 1994), or Pro-347-Leu (Fujiki *et al.*, 1992) mutation in the rhodopsin gene, and two families with Cys-214-Ser (Saga *et al.*, 1993) or Asn-244-Lys mutation (Kikawa *et al.*, 1994) in the peripherin/*RDS* gene have been reported as autosomal dominant RP (ADRP).

We report here a mutation in codon 15 within the rhodopsin gene found in a Japanese patient with ADRP.

MATERIALS AND METHODS

Genomic DNAs were extracted from leukocytes of peripheral blood collected from unrelated 43 autosomal dominant RP (ADRP), 24 autosomal recessive RP (ARRP) and 34 normal individuals. DNA fragments, nucleotide (nt) position 74–403, encompassing codon 15 in exon 1 of the rhodopsin gene from each individual were amplified by polymerase chain reaction (PCR) using a pair of primer, 5′-TAGGCCCTCAGTTTCTGCAG-3′ and 5′-ACTGCCATGGCTCAGCCAGG-3′. The PCR was carried out in a volume of 100 μl in a DNA Thermal Cycler (Perkin-Elmer Corporation, USA) at 94°C for 1 min, at 60°C for 30 sec and at 72°C for 1 min, for a total of 30 cycles. The PCR products were digested with restriction enzyme *Bsr*I (New England Biolabs, USA) and the size was detected on 2% agarose gel containing ethidium bromide. A nucleotide substitution in codon 15 (AAT→AGT) creates a new recognition sequence for *Bsr*I.

The PCR products containing the mutation in codon 15 and normal controls cut out from 1.4% low melting agarose gels were purified and directly sequenced to confirm a substitution of the nucleotide using Taq Dye Deoxy™ Terminator Cycle Sequencing Kit by fluorescence-based DNA autosequencer, Model 373A (Applied Biosystems, USA).

RESULTS

A heterozygous mutation in codon 15 of the rhodopsin gene was detected in a patient with ADRP by restriction enzyme *Bsr*I (Fig. 1), where transition of adenine to guanine at the second nucleotide (AAT→AGT), corresponding to a substitution of serine for asparagine (Asn-15-Ser), was found by sequencing analysis (Fig. 2). None of the remaining unrelated 42 ADRP and 24 ARRP patients, and 34 normal individuals had the mutation in codon 15.

The patient with codon 15 mutation in rhodopsin gene is a 52-year-old female, whose fundus examinations showed a sectorial form of pigmentary retinal degeneration in the right lower hemiretina as well as along the left inferior vascular arcade (Fig. 3). She has an affected sister, brother, father, and cousin (Fig. 4). Other extended family members are still under investigation. Her sister (67-year-old, III-2 in Fig. 4) also has a similar fundus finding although no DNA analysis has been done.

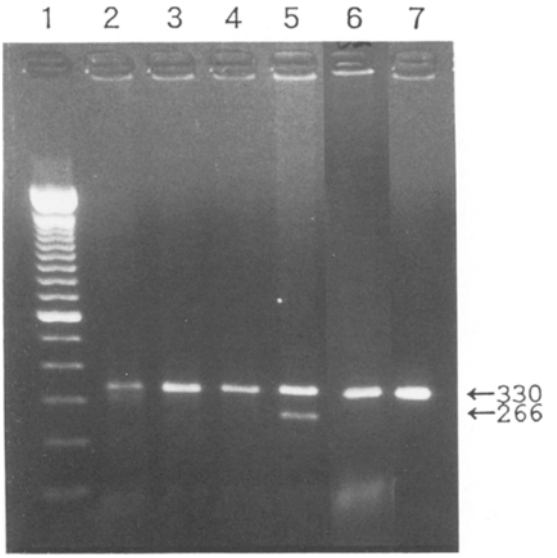

Fig. 1. Electrophoresis patterns by *Bsr*I digestion for PCR products encompassing codon 15 in the rhodopsin gene. Lane 1, 100 bp DNA ladder; lane 5, the patient with heterozygous mutation in codon 15 of the rhodopsin gene (330 and 266 base pairs (bp) bands); lane 6, normal cousin of the patient (III-11 in Fig. 4); other lanes are unrelated RP patients without alteration in codon 15.

DISCUSSION

Although there is no report of Asn-15-Ser mutation of the rhodopsin gene in the USA and the UK, this particular gene mutation in an Australian pedigree was found and shown to be associated with clinical features of a sectorial phenotype observed in four generations of a family which had been positively diagnosed as having RP with impairment of the inferior half of the fundus in all affected members and with no or only mild pigmentary changes occurring along the vascular arcades (Kranich *et al.*, 1993; Sullivan *et al.*, 1993).

Asn-15 is a *N*-glycosylation site near the amino terminus in the rhodopsin molecule. Therefore, replacement of Asn-15 would prevent normal glycosylation occurring at this position. Glycosylation of asparagine (Asn-15) requires the amino acid sequence Asn-Xaa-(Ser or Thr), where Xaa is any amino acid except proline. Sung *et al.* (1991b) supported this assignment. In transfection of cloned cDNA into tissue culture cells, Thr-17-Met mutation produces only the 31-kDa and 35-kDa protein which appeared to be degraded with no higher molecular mass species observed. We have reported a Japanese patient with Thr-17-Met mutation previously (Fujiki *et al.*, 1992) which showed also a pigmentary degeneration mainly

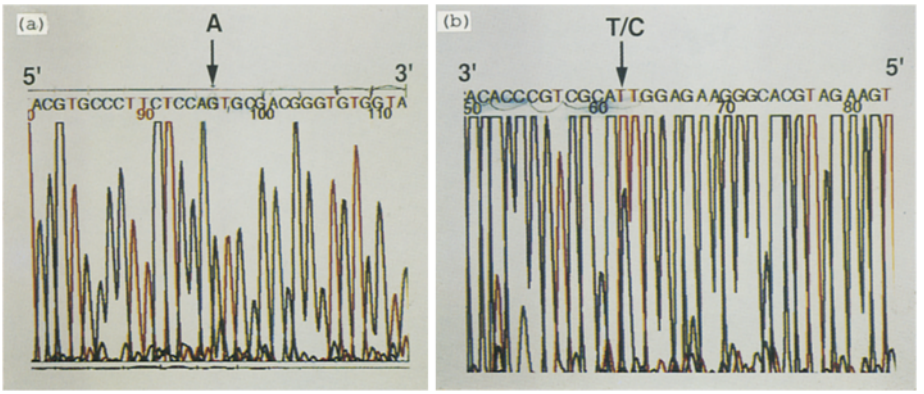

Fig. 2. Direct sequencing around codon 15 in the rhodopsin gene of a patient with autosomal dominant retinitis pigmentosa. (a), a heterozygous A to G transition in codon 15 has been detected from sense strand; (b), the heterozygous mutation has been detected from antisense strand, resulting in two-peaks in the position.

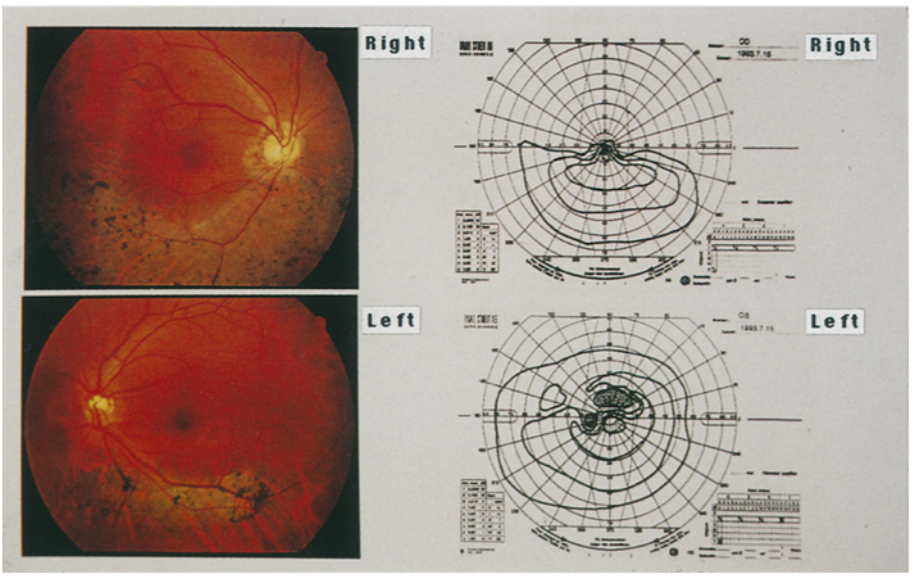

Fig. 3. Fundus photographs and visual field tests with Goldmann perimetry of the right and left eyes of the patient with Asn-15-Ser mutation.

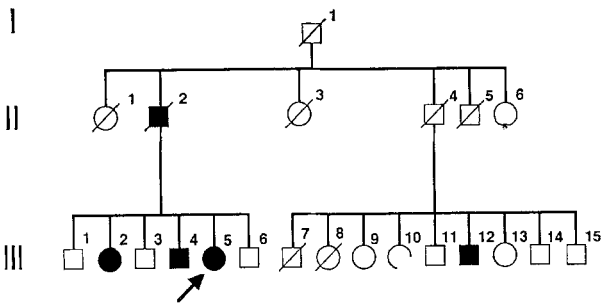

Fig. 4. Pedigree chart of the patient with Asn-15-Ser mutation within the rhodopsin gene.

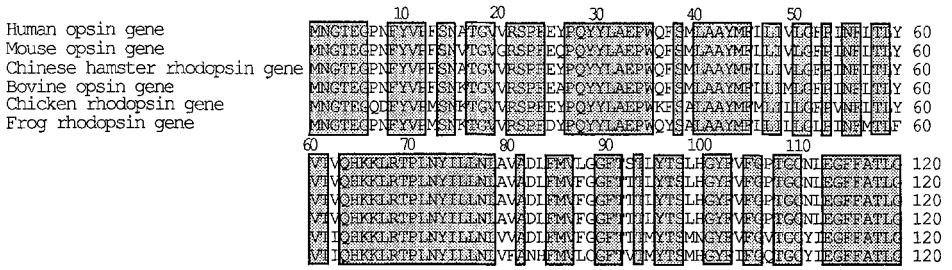

Fig. 5. Sequence similarity of rhodopsin from human and other vertebrates.

in the inferior part of the fundus, with a corresponding loss of the visual field in the superior part (Hayakawa *et al.*, 1993). There are similar cases of different racial backgrounds (Jacobson *et al.*, 1991; Fishman *et al.*, 1992a; Li *et al.*, 1994). The present case of Asn-15-Ser mutation has almost the same sectorial-like type. Therefore, similar findings of patients with codon 15 and 17 mutations would be the result of unglycosylation at Asn-15 position. Codons 15 and 17 are highly conservative in vertebrates. This is seen in Fig. 5 showing the sequence similarity of amino acid residues of the rhodopsin gene in human and other vertebrates. These positions are found to be important for photo-transductions. However, clinical features of sectorial or sectorial-like RP with relatively less severe overall functional impairment have been reported previously not only in codon 17, but also in codon 23 (Heckenlively *et al.*, 1991; Berson *et al.*, 1991a; Stone *et al.*, 1991), 58 (Fishman *et al.*, 1991; Richards *et al.*, 1991), 106 (Fishman *et al.*, 1992b), 190 and 267 (Fishman *et al.*, 1992c). All of these mutations are located either within the intradiscal domain of the photoreceptor or the membrane of the disc. They can have less severe clinical findings compared with the point mutations in codon 347 located closely at rhodopsin's carboxyl terminus at cytoplasmic regions which could give rise to a severe phenotype (Berson *et al.*, 1991b; Berson, 1993). This has also been reported in Japanese patients (Shiono *et al.*, 1992). Further studies will

be required on the relationship between the clinical features and the position of the mutation.

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