

# Peripherin/*RDS* Gene Mutation (Pro210Leu) and Polymorphisms in Japanese Patients with Retinal Dystrophies

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**Purpose:** To determine the frequency of peripherin/*RDS* (retinal degeneration slow) gene mutations in Japanese patients with retinal dystrophies.

**Methods:** We analyzed the peripherin/*RDS* gene in 54 unrelated Japanese patients with retinal dystrophies. Genomic DNA was amplified by polymerase chain reaction (PCR) and the PCR products were sequenced. We also examined 100 healthy subjects, seeking mutations or variations of the peripherin/*RDS* gene.

**Results:** Of the 54 Japanese patients, one with retinitis pigmentosa had a heterozygous C to T change at the second nucleotide at codon 210 of exon 2 (CCT to CTT/Pro210Leu) of the peripherin/*RDS* gene. None of the 100 individuals with normal fundi had the Pro210Leu mutation of the peripherin/*RDS* gene. Three variants of the peripherin/*RDS* gene (GTC to GTT/Val106Val, Glu304Gln, and Gly338Asp) were also found. The first variation (GTC to GTT/Val106Val) was silent. Two concurrent missense variations (Glu304Gln and Gly338Asp) were seen in 25.9% of the affected patients and in 29% of the healthy individuals.

**Conclusion:** A novel mutation (Pro210Leu) of the peripherin/*RDS* gene has been found in one Japanese patient with retinitis pigmentosa. The alterations of Val106Val, Glu304Gln, and Gly338Asp may be polymorphic variants in the Japanese population. **Jpn J Ophthalmol 2001;45:355–358** © 2001 Japanese Ophthalmological Society

**Key Words:** Codon 210, Japanese, mutation, peripherin/*RDS* gene, retinitis pigmentosa.

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## Introduction

Several mutations and polymorphisms of the peripherin/*RDS* (retinal degeneration slow) gene have been found in patients with retinal dystrophies.<sup>1–14</sup> Fujiki et al<sup>13</sup> have suggested that peripherin/*RDS* gene mutations might be rare in Japanese patients. In the present study, we examined Japanese patients with retinal dystrophies to detect mutations in the peripherin/*RDS* gene.

## Materials and Methods

### Patients

We analyzed the peripherin/*RDS* gene in 54 unrelated Japanese patients with retinal dystrophies, including 9 with autosomal dominant retinitis pigmentosa, 22 with autosomal recessive retinitis pigmentosa, 15 with sporadic retinitis pigmentosa, and 8 with inherited macular dystrophies. We also analyzed the peripherin/*RDS* gene in 100 unrelated healthy individuals.

### DNA Sequencing

Informed consent was obtained from all subjects. Genomic DNAs were extracted from peripheral blood leukocytes of each subject. Three exons of the peripherin/*RDS* gene were amplified by polymerase

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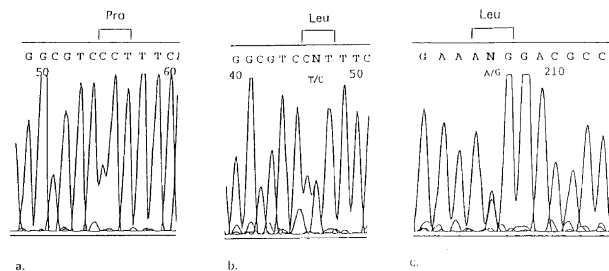
chain reaction (PCR) using four pairs of primers, as reported by Kohl et al.<sup>11</sup> The amplification of the peripherin/*RDS* gene was performed using 100 ng of genomic DNA in a 20- $\mu$ L mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M of each dNTP, 1.25 U of *Taq* polymerase (Perkin-Elmer, Norwalk, CT, USA) and 10 pmol of each primer. The PCR was performed by incubation at 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing) and 72°C for 1 minute (extension). All the PCR products were directly sequenced with an automatic DNA sequencer (ABI 310, Perkin-Elmer).

To determine the frequencies of the Pro210Leu mutation and two variants (Glu304Gln and Gly338Asp) found in 100 healthy individuals, exons 2 and 3 of the peripherin/*RDS* gene were amplified and directly sequenced.

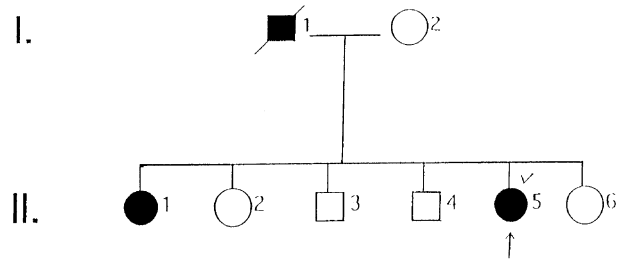
## Results

Of the 54 Japanese patients with retinal dystrophies, one (the proband) had a heterozygous substitution of T for C at the second nucleotide of codon 210 (CCT to CTT/ Pro210Leu) of the peripherin/*RDS* gene (Figure 1). None of the 100 individuals with healthy fundi had the Pro210Leu mutation of the peripherin/*RDS* gene.

The proband (Patient II-5, in Figure 2) was a 49-year-old woman who complained of night blindness and visual field constriction in both eyes. Her visual acuities were 1.5 with correction (sph + 2.5 diopters and cyl -1.25 diopters, axis 10°) OD, and 1.5 with correction (sph + 1.5 diopters) OS. The corneas, anterior chambers, and lenses appeared normal in both eyes. The vitreous bodies were liquefied. White-gray discoloration of the peripheral retina associated with



**Figure 1.** Nucleotide sequences around codon 210 of peripherin/*RDS* gene. (a) Sense DNA sequence of healthy control; (b) sense; and (c) antisense DNA sequences of proband (Patient II-5). Substitution of thymine for cytosine at second nucleotide position of codon 210 results in change of amino acid proline (CCT, Pro) to leucine (CTT, Leu).



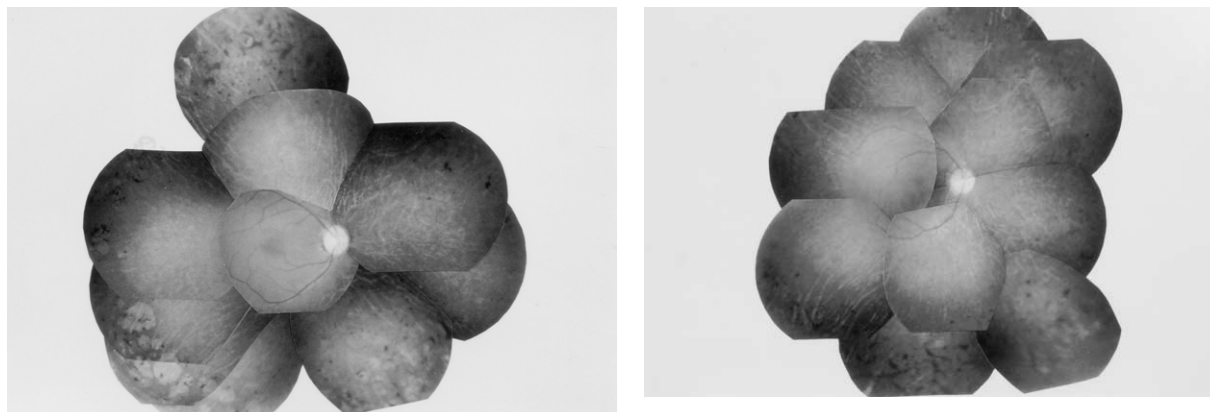
**Figure 2.** Family tree of proband. □: male with normal fundi, ○: female with normal fundi, ■: affected male, ●: affected female, ↑: proband, /: deceased, : examined in this study.

bone-spicule-like pigmentation was observed in both fundi (Figure 3). No macular lesions were seen in either fundus by ophthalmoscopy or fluorescein angiography. Goldmann visual field testing showed irregular ring scotomas in both eyes. The single bright-flash electroretinogram revealed nonrecordable responses in both eyes (Figure 4). The 30-Hz flicker electroretinogram revealed a diminished pattern in the right eye and no recordable pattern in the left eye. The proband's father (Patient I-1) was deceased, but reportedly had had retinitis pigmentosa from young adulthood. The proband's 62-year-old sister (Patient II-1) was reported to have poor vision (0.5), night blindness, mottled retina with bone-spicule-like pigmentation, ring scotomas, and nonrecordable electroretinographic responses in both eyes.

Three substitutions in the peripherin/*RDS* gene were observed in several subjects (Table 1.) The alteration (GTC to GTT/Val106Val) in exon 1 was found in 75.9% of affected patients. The concurrent variants (Glu304Gln and Gly338Asp) in exon 3 were seen in 25.9% of the affected patients and in 29% of the healthy individuals.

## Discussion

We performed a mutational analysis of the peripherin/*RDS* gene in 54 Japanese patients with retinal dystrophies, and found a novel mutation (Pro210Leu) in 1 patient, and Val106Val, Glu304Gln, and Gly338Asp variations in several patients. The Pro210Leu mutation was not found in the 100 individuals with healthy fundi. The Pro210Leu mutation of the peripherin/*RDS* gene has not been reported in the OMIM (<http://www.ncbi.nlm.nih.gov/Omim/>) or the HGMD (<http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>) databases. Although the exact pedigree analysis of this patient with the Pro210Leu mutation was not possible, an autosomal dominant trait was suspected. Sev-



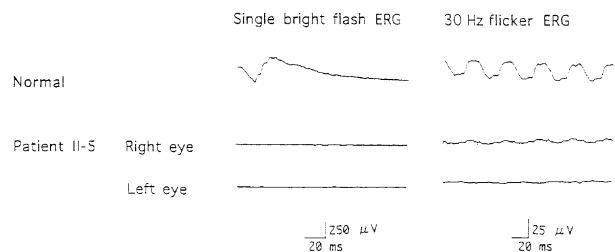
**Figure 3.** Fundus photographs of proband (II-5). White-gray discoloration of peripheral retina associated with bone-spicule-like pigmentation is observed in right and left fundi.

eral mutations at codon 210 of the *peripherin/RDS* gene have been previously reported.<sup>3-5,8</sup> Jackson et al<sup>3</sup> reported the Pro210Arg mutation in a family with butterfly pattern macular dystrophy. Feist et al<sup>4</sup> described the Pro210Arg mutation in a patient with adult foveomacular dystrophy. Kemp et al<sup>5</sup> reported the Pro210Ser mutation in a patient with autosomal dominant retinitis pigmentosa. Gorin et al<sup>8</sup> demonstrated that the Pro210Arg mutation was associated with macular and peripheral retinal degeneration. In the present study, the Pro210Leu mutation was associated with (probably autosomal dominant) retinitis pigmentosa. Different *peripherin/RDS* gene mutations can lead to different phenotypes: the mutations of codon 210 caused foveomacular dystrophy<sup>4</sup> or retinitis pigmentosa<sup>5</sup>; the alterations of codon 212 produced macular dystrophy<sup>7</sup> or retinitis pigmentosa<sup>2</sup>; and the mutations of codon 244 induced cone-rod dystrophy<sup>6</sup> or retinitis pigmentosa associated with Bull's-eye maculopathy.<sup>7</sup> Our findings describe an additional case in which the mutation of codon 210 led to retinitis pigmentosa. Although the exact mechanisms remain unclear, a variety of phenotypes have

thus been observed to result from the substitution of different nucleotides in the same codon of the *peripherin/RDS* gene.

The rarity of *peripherin/RDS* gene mutations in Japanese patients has been suggested.<sup>13</sup> In the present study of 54 Japanese patients, only 1 patient had this mutation. It is possible that mutations in the *peripherin/RDS* gene are uncommon in Japanese patients. Kohl et al<sup>11</sup> have proposed that the *peripherin/RDS* gene mutations are frequent causes of central retinal dystrophies. In the present study, the mutation was associated with peripheral retinal dystrophy.

The Pro210Leu mutation has occurred in the conserved regions of *peripherin/RDS* genes in humans, mice, cattle, and rats.<sup>12</sup> The mutations at codons 210 (Pro210Ser), 211 (Phe211Leu), 212 (Ser212Gly), 214 (Cys214Ser), 216 (Pro216Ser), and 219 (Pro219del,



**Figure 4.** Electoretinogram (ERGs) of proband (II-5). Single flash ERG shows nonrecordable responses in both eyes. The 30-Hz flicker ERG reveals diminished pattern in right eye and no recordable pattern in left eye.

**Table 1.** Frequencies of Val106Val, Glu304Gln, and Gly338Asp in Patients and Normal Controls\*

| Codon | Genotype | Frequency <sup>†</sup> |                           |
|-------|----------|------------------------|---------------------------|
|       |          | Patients (n = 54)      | Normal Controls (n = 100) |
| 106   | C/C      | 13 (24.1)              |                           |
|       | C/T      | 27 (50.0)              | ND <sup>‡</sup>           |
|       | T/T      | 14 (25.9)              |                           |
| 304   | G/G      | 40 (74.1)              | 71 (71.0)                 |
|       | G/C      | 12 (22.2)              | 27 (27.0)                 |
|       | C/C      | 2 (3.7)                | 2 (2.0)                   |
| 338   | G/G      | 40 (74.1)              | 71 (71.0)                 |
|       | G/A      | 12 (22.2)              | 27 (27.0)                 |
|       | A/A      | 2 (3.7)                | 2 (2.0)                   |

\*Val: valine, Glu: glutamic acid, Gln: glutamin, Gly: glycine, Asp: aspartic acid; n: number of examined subjects.

<sup>†</sup>Values in parentheses are percentages.

<sup>‡</sup>ND: not done.

3bp) in the intradiscal D2 loop domain of the peripherin/*RDS* gene have been reported to be pathogenic.<sup>9,12,14</sup> Travis et al<sup>15</sup> have proposed that the peripherin/*RDS* protein may act as an adhesion molecule to stabilize the outer segment disc through hemophilic interaction of glycan across the disc space of rod and cone photoreceptor cells. Therefore, the mutations in the D2 loop domain of the peripherin/*RDS* gene may have injurious effects on the formation and stability of the disc membrane protein.

The three variants (Val106Val, Glu304Gln, and Gly338Asp) in the present study may not be associated with retinal dystrophies. The DNA alteration (GTC to GTT) at codon 106 produces no amino acid change (Val106Val). The Glu304Gln and Gly338Asp substitutions in exon 3 of the peripherin/*RDS* gene were also found in healthy individuals. Fujiki et al<sup>13</sup> reported the frequency of Glu304Gln and Gly338Asp alterations in patients and normal controls. The frequency of the alterations in our present study were similar to those reported by Fujiki et al.<sup>13</sup> These alterations may be polymorphic variants in the Japanese population.

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