Analysis of Peripherin/RDS Gene for Japanese Retinal Dystrophies

Keiko Fujiki,* Yoshihiro Hotta,* Mutsuko Hayakawa,* Takuro Fujimaki,* Misako Takeda,* Yasushi Isashiki,† Norio Ohba‡ and Atsushi Kanai*

*Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan; †Division of Molecular Pathology and Genetic Epidemiology, Center for Chronic Viral Diseases and ‡Department of Ophthalmology, Kagoshima University Faculty of Medicine, Kagoshima City, Japan

Abstract: We studied 133 Japanese patients with retinal dystrophies to detect peripherin/RDS gene defects. The patients analyzed included 52 with autosomal dominant retinitis pigmentosa, 36 with autosomal recessive retinitis pigmentosa, 3 with simplex retinitis pigmentosa, 12 with cone-rod dystrophy, 5 with rod-cone dystrophy, 3 with vitelliform macular dystrophy (Best’s disease), 4 with macular dystrophy, 2 with cone dystrophy, 2 with fundus flavimaculatus, 2 with fundus albipunctatus, and 12 with retinitis pigmentosa with macular degeneration as well as 40 unrelated normal persons. Three exons of the peripherin/RDS gene cut into 150–200 base-pair fragments were amplified by polymerase chain reaction and screened by single-strand conformation polymorphism. The DNA fragments with any suspected variations were directly sequenced. Eight point mutations were detected. Among them, two missense mutations at codons 304 and 338 result in an amino acid substitution of glutamine for glutamic acid and aspartic acid for glycine, respectively. However, they were not cosegregated with the diseases, and these mutations were also commonly found in normal controls. For these controls, the proportion of transversion from G to C at codon 304 (GAG→CAG) and transition from G to A at codon 338 (GGC→GAC) were 0.192±0.045 and 0.173±0.053, respectively. Our results suggest that a peripherin/RDS gene mutation might be rare in Japanese patients.


Key Words: DNA polymorphism, macular dystrophy, peripherin/RDS, retinal dystrophy, retinitis pigmentosa.

Introduction

The peripherin/RDS (retinal degeneration slow) gene located on the short arm of chromosome 6 consists of 3 exons and 346 amino acid residues. It codes for the photoreceptor-specific glycoprotein expressed in the disc membranes of photoreceptor outer segments of both rods and cones. The glycoprotein is considered to be important to the structural stability of the rim of outer segments.1–3 Several mutations of one or a few base pairs resulting in deletion, insertion, or a missense mutation in the peripherin/RDS gene have been identified. These mutations give striking variations of retinal dystrophies such as autosomal dominant retinitis pigmentosa (ADRP), butterfly-shaped pigment dystrophy of the fovea, autosomal dominant retinitis punctata albscens, macular dystrophy, cone-rod dystrophy, and fundus flavimaculatus.4–18 In addition, digenic retinitis pigmentosa because of mutations of the unlinked peripherin/RDS and ROM1 genes has been found,19 in which only double heterozygotes develop retinitis pigmentosa.

The purpose of the present study is to identify peripherin/RDS gene defects in Japanese patients with ADRP and other retinal dystrophies.

Materials and Methods

We analyzed 133 unrelated Japanese patients with retinal dystrophies, including 52 with ADRP, 36 with autosomal recessive retinitis pigmentosa (ARRP), 3 with simplex retinitis pigmentosa (RP), 12 with cone-rod dystrophy, 5 with rod-cone dystrophy, 3
with vitelliform macular dystrophy (Best’s disease), 4 with macular dystrophy, 2 with cone dystrophy, 2 with fundus flavimaculatus, 2 with fundus albipunctatus, and 12 with RP with macular degeneration, as well as 40 unrelated normal persons.

Sample Preparations

Genomic DNAs were extracted from leukocytes of peripheral blood. Exons 1 and 3 of the peripherin/RDS gene were cut into four fragments, and exon 2 was cut into two fragments. These were amplified by PCR (polymerase chain reaction) with a pair of primers at each condition of amplification (Table 1).

Analyses by Single Strand Conformation Polymorphism

The DNA fragments were screened using two kinds of single strand conformation polymorphism (PCR-SSCP). For non-radioisotopic (RI)-SSCP, aliquots of PCR products (3–5 μL) were mixed with 2 vol. of formamide sample buffer (95% formamide, 20 mM ethylenediaminetetraacetic acid (EDTA), 0.05% xylene cyanol, 0.05% bromophenol blue, 10 mM NaOH), heated at 94°C for 15–20 minutes, and applied on a nondenaturing 6% acrylamide gel containing 10% glycerol (gel size: 33 × 30 × 0.04 cm, 48 lanes). Electrophoresis was done at 1300–1500 V, 50–64 mA for 2–3 hours using 1 × TBE with an aluminum plate attached to one side of the glass plates. The gel was dried on filter paper and then exposed to x-ray film at −80°C for 1–2 hours with an intensifying screen.

Table 1. Primer Sequences and Polymerase Chain Reaction Conditions

<table>
<thead>
<tr>
<th>Aimed Locus</th>
<th>Primer Sequence</th>
<th>Annealing Temperature (°C)</th>
<th>Length of Fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1–1 Sense: 5’-GGGAAGACAACCGGACGACTCA-3’ 5’-CAGAATATTATCATCACATCG-3’</td>
<td>50</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>Exon 1–3 Sense: 5’-TTCCTGAGATTTGAACTCCG-3’ 5’-TAGCCAGTACGGCTTCAGC-3’</td>
<td>52</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Exon 1–4 Sense: 5’-AGTACTACCGGGGACACAGAC-3’ 5’-TCTGACCCCAGGACTGGAG-3’</td>
<td>52</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Exon 2–1 Sense: 5’-AACCCCATTTCCTCAGCTTCT-3’ 5’-TTCTGAACTGTAGTGTGCTGA-3’</td>
<td>52</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>Exon 2–2 Sense: 5’-CCCTGCAATCCAGATCTACAG-3’ 5’-TTACCCCTTACCCCCAGCTG-3’</td>
<td>50</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Exon 3–1 Sense: 5’-TTAATACTCTCCTCCCACACTAC-3’ 5’-CGGCAGCTCCTCCTCGAACG-3’</td>
<td>50</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Exon 3–2 Sense: 5’-AGAGGCGGAGACGGGACTGG-3’ 5’-GCCCCTGCGGCTGGCCGCG-3’</td>
<td>52</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Exon 3–3 Sense: 5’-TGCGGGAAGACGGCAAGGC-3’ 5’-TGCGGGAAGACGGCAAGGC-3’</td>
<td>62</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Exon 3–4 Sense: 5’-CCAAGTGAAAGCGGGGGCG-3’ 5’-GGAGATTCAGACTTTCGGAG-3’</td>
<td>62</td>
<td>203</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mutations Identified in the Present Study

<table>
<thead>
<tr>
<th>Exon</th>
<th>Codon</th>
<th>Nucleotide Wild→Mut</th>
<th>Amino acid Wild→Mut</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98</td>
<td>C → T</td>
<td>Leu → Leu</td>
</tr>
<tr>
<td>106</td>
<td></td>
<td>GTC → GTT</td>
<td>Val → Val</td>
</tr>
<tr>
<td>189</td>
<td></td>
<td>TCC → TCT</td>
<td>Ser → Ser</td>
</tr>
<tr>
<td>266</td>
<td></td>
<td>GGT → GGA</td>
<td>Gly → Gly</td>
</tr>
<tr>
<td>303</td>
<td></td>
<td>AGC → AGT</td>
<td>Ser → Ser</td>
</tr>
<tr>
<td>304</td>
<td></td>
<td>GAG → CAG</td>
<td>Gln → Gln</td>
</tr>
<tr>
<td>338</td>
<td></td>
<td>GCC → GAC</td>
<td>Gln → Asp</td>
</tr>
</tbody>
</table>

3’ noncoding region 1294 | C → T

* Nucleotide position. Leu: leucine; Val: valine; Ser: serine; Gly: glycine; Gln: glutamic acid; Cys: glutamine; Asp: aspartic acid.
DNA Sequencing

The DNA fragments suspected of any mutations by PCR-SSCP were directly sequenced by Taq Dye Deoxy Terminator using Gene Scanner, Fluorescent Fragment Analyzer (Applied Biosystems, Foster City, CA, USA).

Analyses by Restriction Enzyme

To calculate the frequencies of codon 304 and 338 mutations among the patients and normal controls, DNA fragments containing these codons were digested by restriction enzyme, where codon 304 mutation (GAG→CAG) is a recognition site of Mva I and codon 338 mutation (GGC→GAC) is the site of Hga I.

Statistical treatments

The proportion, \( p \), of substitution of the nucleotide and standard deviation (SD) were calculated by the following formulas:

\[
p = \frac{W/M + 2 \times M/M}{N}
\]

and

\[
SD = \sqrt{p(1-p)/2N},
\]

where \( W \) refers to wild and \( M \) to mutant allele at the codon. \( W/M \) is the number of heterozygotes of mutant and wild allele. \( M/M \) is the number of homozygotes of the mutant allele. \( N \) is the total number of individuals investigated. Significance between patients and normal controls for substitution of nucleotide was analyzed by a test of proportions.

Results

Eight kinds of mutations were detected (Table 2) by sequencing of the fragments. However, they were not cosegregated with the disease, although amino acids were substituted.

Figure 1 shows the electrophoretic pattern of the 190 base-pair fragments of exon 1–3 of the peripherin/RDS gene by non-RI-SSCP. The mutant band coincided with a transition from C to T at codon 106 (GTC→GTT, Val106Val).

Figure 2 shows electrophoretic fragment patterns of exons 3–1 as analyzed by RI-SSCP, where mutant bands were detected. The mutant bands were coin-
cided with the transition from C to T of codon 303
\((\text{AGC} \rightarrow \text{AGT}, \text{Ser303Ser})\) and transversion from G to C of codon 304 \((\text{GAG} \rightarrow \text{CAG}, \text{Glu304Gln})\). Transition from G to A of codon 338 \((\text{GGC} \rightarrow \text{GAC}, \text{Gly338Asp})\) was also detected.

Figure 3 shows the sequencing around codons 304 and 338 and the agarose gel pattern of DNA fragments digested by restriction enzyme—Mva I for codon 304 and Hga I for codon 338. These two mutations were also found in the normal controls in spite of the substitution of amino acid.

Table 3 shows the frequencies of the transversion from G to C at codon 304 and transition from G to A at codon 338 among the patients and normal controls. There were no significant differences in the proportion of the substitution of nucleotide between patients and normal controls in both codons.

The conservation of the amino acid of codons 304 and 338 among human, bovine, mouse, rat, and feline species (cat) was compared (Figure 4). Although the amino acid of codon 304 was well conserved, codon 338 in humans was different from other mammals and interestingly showed a high frequency of the mutation to the amino acid, Asp(D), of other mammals.

**Discussion**

Several mutations in the peripherin/RDS gene cause retinal dystrophies. Various phenotypes are manifested by these mutations from macular dystrophies to RP. Now more than 25 distinct mutations in the peripherin/RDS gene have been found in patients with ADRP and other retinal dystrophies all over the world. Most cases are diagnosed as ADRP, but some mutations are found in patients with macular dystrophy, vitelliform macular dystrophy, fundus flavimaculatus, and so on. In the USA, approximately 5-6% of patients with nonrhodopsin ADRP have a defect in the peripherin/RDS gene. In Japan, five point mutations in five families have been reported: Cys214Ser mutation in ADRP, Asn244Lys mutation in ADRP with bull's eye, Asn244His mutation in cone-rod dystrophy, Arg172Trp mutation in macular dystrophy, and Tyr184Ser mutation in cone-rod dystrophy.

We have tried to check for other mutations in the...
peripherin/RDS gene causing various retinal dystrophies. Our results were inconclusively lower. Although transitions have been detected for codon 338 and transversions for codon 304, none of these cosegregated with the disease. There was the same frequency of mutation in the patients and controls, suggesting spontaneity of instability of these codons. These codons also appear not to be vital for the proper function of the gene.

Other candidate genes may also be implicated for these retinal dystrophies. These candidate genes include the rhodopsin gene, of which the mutations were associated with the disease in four families27–30 in Japanese ADRP patients in the past, and genes at chromosomes 7q31–q35,31,32 7p15.1–p13,33 8p11–q21,34 17p13,35 and 19q13.436 in ADRP; 1q31–q32.1,37 β-cGMP-PDE (phosphodiesterase) gene (4p16.3),38 α-cGMP-PDE (5q31.2–q34),39 and α-cGMP-gated channel40 in ARRP; and at chromosome 11q13 in Best’s disease.41 For these patients, therefore, another candidate gene must be analyzed to determine if mutations are responsible for these heterogeneous retinal dystrophies. In the present study, although we attached much importance to clinical diagnosis for patients with retinal dystrophies except ADRP and ARRP, the patients with affected relatives in three or more

<table>
<thead>
<tr>
<th>Codon</th>
<th>Genotype</th>
<th>Number (%) of Patients</th>
<th>Number (%) of Controls</th>
<th>Total Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>G/G</td>
<td>50 (62.5)</td>
<td>25 (64.1)</td>
<td>75 (63.0)</td>
</tr>
<tr>
<td></td>
<td>G/C</td>
<td>29 (36.3)</td>
<td>13 (33.3)</td>
<td>42 (35.3)</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>1 (1.3)</td>
<td>1 (2.6)</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>80 (100)</td>
<td>39 (100)</td>
<td>119 (100)</td>
</tr>
<tr>
<td></td>
<td>Proportion of transversion from G to C</td>
<td>0.194 ± 0.031</td>
<td>0.192 ± 0.045</td>
<td>0.193 ± 0.026</td>
</tr>
<tr>
<td>338</td>
<td>G/G</td>
<td>30 (63.8)</td>
<td>17 (65.4)</td>
<td>47 (64.4)</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>17 (36.2)</td>
<td>9 (34.6)</td>
<td>26 (35.6)</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>47 (100)</td>
<td>26 (100)</td>
<td>73 (100)</td>
</tr>
<tr>
<td></td>
<td>Proportion of transition from G to A</td>
<td>0.181 ± 0.040</td>
<td>0.173 ± 0.053</td>
<td>0.178 ± 0.032</td>
</tr>
</tbody>
</table>

ADR P: autosomal dominant retinitis pigmentosa; ARRP: autosomal recessive retinitis pigmentosa.

aADRP 38, ARRP 23, retinal dystrophies 19.
bThere is no significant difference in the proportion between patients and normal controls.
cADRP 18, ARRP 10, retinal dystrophies 19.

Figure 4. Conservation of amino acid of codons 304 and 338 of the peripherin/RDS gene in human, bovine, mouse, rat, and feline species. Although the amino acid of codon 304 was well conserved, codon 338 in humans was different from other mammals. Interestingly, Gly338Asp (G338D) mutation in humans showed the same amino acid as other mammals. Asterisks show homologous amino acid in the species compared.
consecutive generations, which show strongly autosomal dominant inheritance, might have been selected and analyzed separately.

On the DNA polymorphism in the peripherin/RDS gene, three sequence alterations giving rise to amino acid substitutions at codons 304, 310, and 338 have also been reported, where the proportion of the transition from G to C at codon 304 was 0.51 in normal controls and 0.58 in ADRP patients. The transition from A to G at codon 310 was 0.53 in normal controls and 0.45 in ADRP patients, and transition from G to A at codon 338 was 0.59 in the normals and 0.70 in ADRP patients. These values were higher than that of the present study. We did not find the mutation at codon 310 in the 30 patients randomly selected, although the substitutions at codons 304 and 338 were found in relatively high frequencies. Because three mutations are for both normal and affected ADRP patients, it appears that substitution in these codons could not be used to determine predisposition to retinal dystrophy. However, it might be useful in studying racial differences. For example, frequency of polymorphism in the rhodopsin gene varies for Japanese and Americans. The transition from A to G at nucleotide (nt) position 269 in the 5’ noncoding region, the transition from G to A at nt position 5145 in the 4th intron, and transversion from C to A at nt position 5321 in the 3’ noncoding region were 0.57, 0.36, and 0.05, respectively, in Japanese. In the Americans these values were 0.14, a few, and 0.13, respectively. These facts may therefore be useful for studying Japanese anthropological origin.

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References


