A Frequent 1085delC/insGAAG Mutation in the RDH5 Gene in Japanese Patients with Fundus Albipunctatus

Yuko Wada, Toshiaki Abe, Nobuo Fuse, and Makoto Tamai

PURPOSE. To identify the frequency of a mutation of the RDH5 gene in Japanese patients with hereditary retinal degeneration and to characterize clinical findings for the patients associated with a 1085delC/insGAAG mutation in the RDH5 gene.

METHODS. Mutation screening by single-strand conformation polymorphism was performed on 6 patients with fundus albipunctatus and 150 patients with autosomal recessive retinitis pigmentosa. The DNA fragment that showed abnormal mobility on SSCP was then sequenced. Clinical features were characterized by visual acuity, slit-lamp biomicroscopy, electroretinography, fluorescein angiography, kinetic visual field testing, and dark adaptometry.

RESULTS. A novel 1085delC/insGAAG mutation in the RDH5 gene was identified in all 6 patients, from 4 unrelated families with fundus albipunctatus. The ophthalmic findings of each affected member were very similar, which may provide the natural course of the phenotype produced by the 1085delC/insGAAG mutation.

CONCLUSIONS. A homozygous 1085delC/insGAAG mutation in the RDH5 gene produces fundus albipunctatus in Japanese patients. These findings suggest that this mutation was a founder effect in Japanese patients with fundus albipunctatus. (Invest Ophtalmol Vis Sci. 2000;41:1894–1897)

Fundus albipunctatus is one form of autosomal recessive, congenital, stationary night blindness in which all other visual functions (including visual acuity, color vision, and visual field) are usually normal. A characteristic finding of this disease is the presence of numerous white dots in the midperipheral retina, typically sparing the macula.

It is well known that a deficiency of vitamin A causes impaired night vision and retinal degeneration, and genes related to vitamin A metabolism have been candidate genes for retinal degeneration or night blindness. In 1997, it was reported that RPE65 and RLBP1 genes, which were expressed in retinal pigment epithelium (RPE) and played an important role in vitamin A metabolism, were causative genes of Leber’s congenital amaurosis and early onset retinitis pigmentosa.1–3

In 1999 it was reported that fundus albipunctatus was caused by mutations of the RDH5 gene, which is localized on chromosome 12q13-q14 and encoded 11-cis retinol dehydrogenase.4,5 11-cis retinol dehydrogenase, which is also abundantly expressed in the RPE, is a 32-kDa membrane-bound enzyme and is made of 318 amino acids. It catalyzed the conversion of the 11-cis retinol to 11-cis retinal with the cofactor NAD+ and played an important role in synthesis of visual pigment.

Although the RPE65 and RLBP1 genes were shown to be the causative genes of severe progressive retinal dystrophy, mutations of the RDH5 gene caused stationary night blindness.

In this study, we characterized the clinical features of patients associated with a newly identified 1085delC/insGAAG mutation in the RDH5 gene.

METHODS

Subject and Mutation Analysis

We screened genomic DNA samples isolated from 6 patients from 4 unrelated Japanese families with fundus albipunctatus and 150 unrelated patients with autosomal recessive retinitis pigmentosa (ARRP) for mutations in the RDH5 gene by means of nonradioisotopic single-strand conformation polymorphism (SSCP) with a modification previously described.6 We also screened 200 control chromosomes with this gene. Genomic DNA was isolated from leukocytes prepared from 10 to 15 ml of each patient’s blood using the protocol previously described.7

The sequence from exon 2 to exon 5 of the RDH5 gene was amplified by polymerase chain reaction (PCR). We used eight sets of oligonucleotide primer pairs to amplify the entire coding region of the RDH5 gene.5 The PCR products were analyzed by SSCP. After electrophoresis, DNA bands were visualized by silver staining. The mutation or polymorphism was detected by the presence of abnormal bands derived from a mutant allele. The DNA fragment that showed abnormal mobility on SSCP was then directly sequenced to identify the mutation in the RDH5 gene on an ABI 310 sequencer (Applied Biosystems; Perkin-Elmer).

The tenets of the Declaration of Helsinki were followed, and informed consent was obtained from all subjects who participated in this study.

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Clinical Examination

We examined 6 affected patients and 6 carriers from four pedigrees at Tohoku University Hospital, Sendai, Japan (Fig. 1). The ophthalmic examinations included best-corrected visual acuity, slit-lamp biomicroscopy, kinetic visual field examination, fundus examination, fluorescein angiography, color vision testing, and electroretinography (ERG). We performed all these tests in patients I-4, II-I, and IV-1, and some of them in the other affected members and obligate carriers. Kinetic visual field examination was performed by a Goldmann perimeter with V-4-e, II-4-e, and I-2-e isopters. The ERG recordings were obtained by a single flash or 30-Hz flicker stimulus of red light under light-adapted conditions for the cone-isolated responses. A dim blue flash after 30 minutes of dark-adaptation elicited the rod-isolated responses, and a bright white flash (20 J) in the dark-adapted state elicited the maximal responses of rods and cones. These conditions conformed to the protocol of the International Society of Clinical Electrophysiology Standards.

RESULTS

DNA Analysis

The results of nonradioisotopic SSCP analysis of exon 5 of the RDH5 gene for the 6 patients with fundus albipunctatus showed abnormal band shifts in all (Fig. 2). The subsequent nucleotide sequencing analysis disclosed an identical homozygous 1085delC/insGAAG mutation, viz., one base (C) of nucleotide 1085 deleted and four bases (GAAG) inserted instead. This alteration caused 1 extra amino acid of glutamate residue and 1 substitution of valine for leucine residue in codon 310 of the 11-cis retinol dehydrogenase (Fig. 2). The carriers were heterozygous for this mutation, and nonaffected members had this mutation. We further screened our patients to search for mutations in the RALBP1 gene, and none was found. We confirmed that the 1085delC/insGAAG mutation was cosegregated with the disease in all pedigrees with fundus albipunctatus and was not detected in the other 150 ARRP patients or 200 control chromosomes.

Ophthalmologic Examination

The clinical and molecular genetic findings of the 6 patients are summarized in Table 1. All patients had night blindness from childhood. The visual acuity of patients with 1085delC/insGAAG mutation ranged from 20/15 to 20/25. Results of visual field testing disclosed that these patients had normal to slightly restricted peripheral fields. Fundus examinations showed many white dots scattered in the retina, without pigmentation and attenuation of retinal vessels (Fig. 3). Color vision testing showed mildly impaired color sensation on panel D-15 testing. The fluorescein angiogram of patients IV-1 disclosed irregular hyperfluorescent areas mainly in the posterior portion, indicating the loss of pigment in the RPE. The white dots showed neither hyperfluorescence nor hypofluorescence (Fig. 3). Results of dark-adaptation tests exhibited a delayed diphasic pattern for three patients. The ERGs of patients with 1085delC/insGAAGmutation are shown in Figure 4. The scotopic...
responses were nonrecordable, and single-flash, standard ERG results showed reduced a- and b-waves in each patient after 30 minutes of dark-adaptation. Patients I-4 and II-1, who did not have decreased visual acuity, had slightly reduced amplitudes of 30-Hz flicker ERG (right/left: 68/64 and 3.3/74.6 μV, respectively). The normal range in our laboratory is 83 to 175 μV. The carriers from 4 families, who had heterozygous 1085delC/insGAAG mutation, had no visual complaints. Slit-lamp biomicroscopy disclosed normal-appearing cornea, anterior chamber, iris, lens, and vitreous in each eye. Ophthalmoscopic examination also showed no retinal change.

**DISCUSSION**

In 1999, Yamamoto et al. first reported that mutations of the RDH5 gene encoding 11-cis retinal dehydrogenase caused delayed dark-adaptation and fundus albipunctatus. However, no previous report of genotype–phenotype correlation has been made in Japanese patients with fundus albipunctatus. In the present study, we evaluated 6 Japanese patients with fundus albipunctatus and 150 Japanese patients with ARRP. Interestingly, molecular genetic analysis disclosed that all our patients with fundus albipunctatus had an identical 1085delC/insGAAG mutation in the RDH5 gene and that no mutation was detected in the patients with ARRP. Although genealogic studies have not been performed for three or four generations, the 1085delC/insGAAG mutation in the RDH5 gene is considered to be the common mutation for Japanese patients with fundus albipunctatus.

The previously reported mutations in the RDH5 gene were all missense mutations, such as the homozygous Gly238Trp mutation and the compound heterozygous Gly238Trp and Ser73Phe mutations. The mutation we detected, on the other hand, was a homozygous one base (C) deletion and four base insertions (GAAG), resulting in one amino acid insertion of glutamate residue and one amino acid substitution of valine for leucine residue of codon 310. This deletion/insertion mutation is very rare. To our knowledge this type of mutation has not been reported among patients with eye disease, such as retinal degeneration, corneal dystrophy, and stationary night blindness, although the 1094delGCTG/insTC mutation was reported in a patient with glycogen storage disease type 1b.

Human and bovine amino acid sequences of 11-cis RDH showed a high homology (91%), and both had 318 amino acids. The site of the 1085delC/insGAAG mutation occurred at the conserved site. We suggest that this mutation might generate a different protein coding for the 319 amino acids instead of the 318 amino acids, and it might alter the structure and function of 11-cis retinal dehydrogenase.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>1085 delC/insGAAG</th>
<th>Symptom</th>
<th>Visual Acuity</th>
<th>Visual Field</th>
<th>Dark Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-4</td>
<td>36</td>
<td>M</td>
<td>+</td>
<td>NB, PB</td>
<td>20/15</td>
<td>Slight constriction</td>
<td>Delayed diphasic</td>
</tr>
<tr>
<td>II-1</td>
<td>57</td>
<td>F</td>
<td>+</td>
<td>NB</td>
<td>20/20</td>
<td>Slight constriction</td>
<td>Delayed diphasic</td>
</tr>
<tr>
<td>II-2</td>
<td>55</td>
<td>F</td>
<td>+</td>
<td>NB</td>
<td>20/15</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II-3</td>
<td>48</td>
<td>F</td>
<td>+</td>
<td>NB</td>
<td>20/20</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II-4</td>
<td>45</td>
<td>F</td>
<td>+</td>
<td>NB</td>
<td>20/25</td>
<td>Slight constriction</td>
<td>ND</td>
</tr>
<tr>
<td>IV-1</td>
<td>40</td>
<td>M</td>
<td>+</td>
<td>NB</td>
<td>20/20</td>
<td>Slight constriction</td>
<td>Delayed diphasic</td>
</tr>
</tbody>
</table>

NB, night blindness; PB, photophobia; ND, not determined.
The affected members showed similar subjective symptoms, visual acuity, and fundus appearance. The fundus showed scattered white dots in the retina that were very similar to those of patients who have a homozygous Gln238Trp mutation or the compound heterozygous Gln238Trp and Ser73Phe mutations.

Mutations of the RLBP1 gene also cause white dots in the retina and lead to progressive retinal degeneration at the late stage, but the RDH5 gene mutation gives rise to stationary night blindness with white dots regardless of ethnic origins. 5

On fluorescein angiography, irregular hyperfluorescence was detected in our patients that did not correspond to white dots. This feature of fundus albipunctatus has been reported, and it has been suggested that the fluorescein angiographic abnormality resulted from diffuse and irregular abnormality of the RPE.10 Our results of fluorescein angiography are compatible with this suggestion. We considered that the mutation in the RDH5 gene caused not only functional but also morphologic abnormalities in the RPE.

We did not detect other mutations in the RDH5 gene, so we could not examine whether the defect of the RDH5 gene causes only fundus albipunctatus or not. Therefore, additional families with fundus albipunctatus, ARRP, and other retinal degenerations must be studied for mutations to ascertain the phenotype-genotype correlation in the RDH5 gene.

References