Mutations in the 11-cis Retinol Dehydrogenase Gene in Japanese Patients with Fundus Albipunctatus

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PURPOSE. To detect mutations in the RDH5 gene encoding 11-cis retinol dehydrogenase in patients from Japan with fundus albipunctatus.

METHODS. Polymerase chain reaction and direct genomic sequencing techniques were used to detect mutations of the RDH5 coding exons (exons 2–5) in two unrelated patients with fundus albipunctatus. Selected alleles that altered the coding region or intron splice sites were evaluated further through segregation analysis in the families of the index cases.

RESULTS. Two novel RDH5 mutations were identified. One of these was a missense mutation Val264Gly in exon 5, and the other was an in-frame insertion of 3 bp in exon 5.

CONCLUSIONS. The data indicate that mutations in RDH5 are the primary cause of fundus albipunctatus. (Invest Ophthal Vis Sci. 2000;41:3933–3935)

Fundus albipunctatus is a rare autosomal recessive form of stationary night blindness characterized by numerous small, subretinal, white or pale-yellow spots in the perimacula and the periphery of the retina. This disease was first described by Lauber in 1910 who distinguished it from an ophthalmoscopically similar disorder, retinitis punctata albescens.1 Although young patients with retinitis punctata albescens and fundus albipunctatus can have similar symptoms, electroretinograms (ERGs), and fundus appearances, patients with retinitis punctata albescens ultimately experience visual field deficits and attenuated retinal vessels, whereas those with fundus albipunctatus have a stationary condition. Mutations in the genes encoding rhodopsin, peripherin/RDS, and cellular retinaldehyde binding protein (CRALBP) have been reported in patients with retinitis punctata albescens.2–6 The only reported gene to cause fundus albipunctatus is that encoding 11-cis retinal dehydrogenase.7–9 The all-trans retinol produced is then transported to the RPE where it is converted back to 11-cis retinal.10 11-cis Retinol dehydrogenase is abundant in the RPE, where it converts 11-cis retinol to 11-cis retinal.11 The enzymatic activities of two of the reported mutant enzymes were strikingly reduced.7

METHODS

This study conformed to the tenants of the Declaration of Helsinki. We evaluated two patients from two families with fundus albipunctatus and 50 normal control subjects. The patients had symptoms of night blindness and multiple, subretinal, small white spots. There were no visual field deficits. The ERGs were examined using white flash (cone–rod response) or a 30-Hz red-flicker stimulus (cone-isolated response) under adequate dark-adapted conditions (15 minutes or 120 minutes; portable ERG PE-300; Tomey, Waltham, MA). Fifty persons with no known blood relative with hereditary retinal degeneration and no symptoms of retinal malfunction served as control subjects. All individuals resided in Japan. After informed consent was obtained, 10 to 20 ml venous blood was collected from each patient or control subject and DNA extracted.

Exons 2 through 5 (the coding exons) of RDH5 were individually amplified by polymerase chain reaction (PCR) using primer pairs based on the published genomic sequence.7,12 Each reaction used 50 to 100 ng DNA in 20 μl of a solution containing 20 mM Tris-HCl (pH 8.4); 0.25 to 1.5 mM MgCl2; 50 mM KCl; 0.02 mM each of dTTP, dCTP, dGTP, and dATP; 0.1 mg/ml bovine serum albumin (BSA); 0% or 10% dimethyl sulfoxide; and 0.25 U Tag polymerase. The pH, Mg2+ concentration, and presence or absence of 10% dimethyl sulfoxide were optimized for each primer pair. After initial denaturation (94°C for 5 minutes), 35 cycles of PCR were performed. Each cycle consisted of denaturation (94°C for 30 seconds), primer annealing (56°C–60°C for 30 seconds; exons 2A, 2B, 3A, 3B, 5A, and 5B at 60°C and exons 2C and 4 at 56°C), and extension (71°C for 30 seconds). All exons in all patients were evaluated by sequencing of PCR-amplified DNA segments by means of a
commercial sequencing protocol (Big Dye; Perkin–Elmer/Applied Biosystems, Foster City, CA). Each sequence variant expected to affect the protein sequence was further evaluated by segregation analysis. For this purpose, DNA samples from relatives were analyzed by direct genomic sequencing.

RESULTS

Sequence analysis of RDH5 revealed homozygous mutations in two index patients. One patient (P703) was homozygous for the missense change Val264Gly (GTG→GGG; Fig. 1A). This change was present homozygously in all three affected siblings of the index patient (Fig. 2A). The daughter of an affected sibling was a heterozygote. The second index patient (P724) was homozygous for an in-frame mutation in codon 310 in exon 5 (Fig. 1B). The mutation replaces codon 310, which normally specifies Leu, with two codons specifying Glu and Val (Leu310GluVal; CTT→GAAGTT). Only one unaffected sibling (homozygous wild-type) was available for segregation analysis (Fig. 2B). Neither of these mutations was found among a set of 50 Japanese control subjects.

Both index patients had normal visual acuity of 20/20 and full visual fields. The fundus of P703 at age 45 and P724 at age 50 showed small white deposits at the level of the RPE (Fig. 3). There was no attenuation of the retinal vessels. The ERGs showed a reduction in the b-wave amplitude to white flash light after 15 minutes of dark adaptation, which recovered to normal within 2 hours in P703 (Fig. 4).

DISCUSSION

The RDH5 mutations found in this study are likely to be pathogenic. Both mutations were present homozygously in affected...
individuals, in agreement with the recessive inheritance pattern. In both families the mutations cosegregated with the disease. Finally, results showed that the mutation Val264Gly changes a nonpolar residue to a polar one, and the mutation Leu310GluVal converts a nonpolar residue (Leu) into two residues, one negatively charged (Glu) and the other nonpolar (Val).

Two previously reported RDH5 mutations, Ser73Phe and Gly238Trp, were found to have 90% lower enzymatic activity than the wild-type enzyme in transfected COS-1 cells. Although we did not perform a similar analysis of the mutations, it is likely that they also result in reduced enzyme activity. 11-cis Retinol dehydrogenase has two potential membrane-anchoring domains. The position of a highly conserved cofactor-binding motif is located at residues 35 to 41, and a presumed catalytic domain is located at residues 175 to 179. Although residue Val264 is not within these domains, it is highly conserved in the superfAMILY of short-chain reductase dehydrogenases. The second mutation we found affects residue Leu310, which is located in a potential membrane-anchoring domain. Because this change alters two residues from nonpolar to negative charged and polar, this mutant protein may not be able to anchor to the membrane. Therefore, because the intracellular localization or membrane topology of this protein may be altered, the enzyme activity of the protein may decrease. However, because we did not perform biochemical analysis of these mutant proteins, further studies are needed to determine how the mutations affect the function of RDH5.

All the patients described in this study had similar clinical findings: small white deposits at the level of the RPE, normal visual acuity, full visual fields, and very slow dark adaptation. Because the patients were aged 45 and 55 years, it is unlikely that their disease was progressive. These clinical findings are similar to those previously reported in which mutations in RDH5 were found. Although the mutations found in RDH5 cause fundus albipunctatus in both the present and previous reports, recently a white family with fundus albipunctatus was reported to show no mutations in RDH5. Therefore, fundus albipunctatus may be genetically heterogeneous.

Acknowledgments

The authors thank Kaorn Nakano and Sachiko Takiuchi for technical assistance and Thaddeus P. Dryja and Terri L. McGee for helpful discussions.

References