A Novel Gly35Ser Mutation in the RDH5 Gene in a Japanese Family With Fundus Albipunctatus Associated With Cone Dystrophy

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Objective: To assess the clinical and genetic characteristics of a Japanese family with fundus albipunctatus with progressive cone dystrophy associated with a mutation in the RDH5 gene.

Design: Case report with clinical findings and results of fluorescein angiography, electroretinograms, kinetic visual field testing, dark adaptometry, and DNA analysis.

Setting: University medical center.

Patients: We studied the ocular findings in 6 members of a Japanese family with fundus albipunctatus with cone dystrophy and a guanine-to-adenine transversion at the first nucleotide in codon 35 of the RDH5 gene. The mutation resulted in a substitution of serine for glycine in amino acid 35 (Gly35Ser) of the RDH5 gene.

Results: Characteristic features included poor night vision, white dots in the retina, cone dystrophy, and a mottled appearance of the retinal pigment epithelium. Electroretinograms showed greater impairment of the rod-mediated responses than the cone-mediated responses. After 3 hours of dark adaptation, the a and b waves and scotopic b waves recovered.

Conclusions: Although the mutation of the RDH5 gene has been known as a causative gene of fundus albipunctatus, the Gly35Ser mutation in the RDH5 gene may be related to the pathogenesis of progressive retinal degeneration. This phenomenon may provide evidence of gene phenotype caused by a mutation in the RDH5 gene.

Clinical Relevance: The Gly35Ser mutation causes fundus albipunctatus with cone dystrophy. This finding provides evidence that some kinds of mutations in the RDH5 gene are related, in part at least, to the pathogenesis of progressive retinal degeneration.


UNDUS ALBIPUNCTATUS is a rare form of congenital stationary night blindness that is characterized by scattered white dots in the fundus and extremely slow dark adaptation of the rod photoreceptors. This disease is inherited as an autosomal recessive trait, and most patients with fundus albipunctatus show poor night vision.

In 1999, the RDH5 gene, which encodes 11-cis-retinol dehydrogenase, was reported to be a causative gene for fundus albipunctatus. It is well known that 11-cis-retinol dehydrogenase catalyzes the 11-cis-retinol to 11-cis-retinal reaction. To date, 2 kinds of mutation, designated as Gly238Trp and Ser73Phe mutations, have been reported. Earlier, we identified a common Leu310 (1-base pair [bp] deletion [del]; +4-bp insertion [ins]) (CTT to GAAGTT) mutation in the RDH5 gene in 4 unrelated Japanese families with fundus albipunctatus. Patients with this mutation in the RDH5 gene showed scattered white dots in the retina and poor night vision. Fluorescein angiography disclosed irregular hyperfluorescent areas mainly in the posterior portion in all patients. These results demonstrated that the RDH5 gene mutation caused the impairment of the retinal pigment epithelium and suggested the possibility that mutations in the RDH5 gene were related not only to fundus albipunctatus but also to progressive retinal degeneration. In addition, fundus albipunctatus associated with macular degeneration has been reported.

Thereafter, we screened 200 unrelated patients with autosomal recessive retinitis pigmentosa and 1 patient with fundus albipunctatus associated with cone dystrophy to search for mutations in the RDH5 gene. In this report, we describe the ocular findings associated with a newly identified RDH5 mutation in a Japanese family with fundus albipunctatus associated with cone dystrophy. The aim of this study was...
PATIENTS AND METHODS

We screened 200 genomic DNA samples isolated from unrelated patients with autosomal recessive retinitis pigmentosa and 1 patient with fundus albipunctatus associated with cone dystrophy to search for mutations in the RHDS gene. Informed consent was obtained from all patients before their entry into this study. Mutation screening was performed by the polymerase chain reaction followed by nonradioisotopic single-strand conformation polymorphism as previously reported. The amplified DNA fragment then underwent electrophoresis in 8% nondenaturing polyacrylamide gel containing 10% glycerol at 20 W for 8 hours at room temperature. The DNA bands were visualized by silver staining. Subsequently, amplified DNA fragments that showed abnormal band shift on a single-strand conformation polymorphism gel were directly sequenced (ABI sequencer; Applied Biosystems, PerkinElmer, Inc, Boston, Mass).

One pedigree with fundus albipunctatus associated with cone dystrophy was included in this study (Figure 1). A homozygous Gly35Ser mutation in the RHDS gene was identified in 1 affected member, and the parents were found to be heterozygous for the Gly35Ser mutation. In addition, we screened the peripherin/RDS, ROM1, rhodopsin, and RLBP1 genes to search for other mutations in the families.

The phenotypic features of the patient who showed the Gly35Ser mutation were studied to characterize the mutation. The ophthalmic examination included best-corrected visual acuity, kinetic visual field examination, slitlamp biomicroscopy, fundus examination, fluorescein angiography, and electroretinograms (ERGs). Kinetic visual field examination was performed by Goldmann perimetry with V-4-e and I-4-e isopters. The ERG recordings were performed under controlled conditions as reported previously and conformed to the International Society for Clinical Electrophysiology of Vision standards. Briefly, ERGs were obtained with a single flash or a 30-Hz flicker stimulus of red light under light-adapted conditions for cone-isolated responses, a dim blue flash in the dark-adapted condition (30 minutes) for rod-isolated responses, and a white standard flash (intensity of 1.69 candela/m² per second) in the dark-adapted condition for eliciting maximal responses of rods and cones. Ganzfeld dark-adapted thresholds were recorded with the use of a Goldmann-Weekers dark adaptometer (Haag-Streit AG, Liebefeld, Switzerland). Color vision was tested with the D-15 panel.

to assess the phenotypic manifestations associated with this molecular alteration. We shall show that the mutation gave rise to a guanine-to-adenine transversion in the first nucleotide at codon 35, resulting in substitution of a serine residue for a glycine residue (Gly35Ser). The clinical features associated with this mutation disclosed a degenerative macula in the right eye and chorioretinal atrophy in the left eye with scattered white dots in the retina. Eight years of observation disclosed a progressive decrease in visual acuity, an enlargement of the macular degeneration, and attenuation of the retinal vessels. These observations make up the natural course of the phenotype associated with the Gly35Ser mutation in the RHDS gene.

RESULTS

DNA ANALYSIS

One affected member (Figure 1) showed an abnormal band shift on a single-strand conformation polymorphism gel, and the abnormal band shift cosegregated with the disease. The obligate carriers (I:1 and I:2) showed both abnormal and normal bands. The abnormal nucleotide sequence was a homozygous guanine-to-adenine transversion at the first nucleotide of codon 35. This alteration caused a serine substitution for a glycine residue in codon 35 of the RHDS gene and was designated as the Gly35Ser mutation (Figure 2). The carriers were heterozygous for this mutation, and the nonaffected members did not show this mutation. We further screened our patients to search for mutations in the peripherin/RDS, ROM1, rhodopsin, and RLBP1 genes, and none was
found. We confirmed that the Gly35Ser mutation cosegregated with the disease and was not detected in the other 200 patients with autosomal recessive retinitis pigmentosa or 100 normal control subjects.

REPORT OF A CASE

A 51-year-old man had initially noticed poor night vision during his early teens and was diagnosed as having fundus albipunctatus by a local ophthalmologist. His parents were first cousins. The patient experienced a gradual progression of visual impairment, including constriction of the visual field, night blindness, and photophobia. In 1992, at 41 years of age, he visited our clinic to have a detailed assessment of his eyes. The visual acuity was corrected to 20/50 OD with a +0.25 −0.5 × 40 refraction and 20/20 OS with a refraction of −0.75 × 80 cyl.

Slitlamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous in both eyes. Fundus examination showed atrophic macular lesions in the right eye and 3 sharply demarcated macular lesions in the left eye with scattered white dots in the retina. The retinal vessels were attenuated (Figure 3). Fluorescein angiography demonstrated hypofluorescent areas, indicating chorioretinal atrophy in the macula of the left eye and granular hyperfluorescent areas mainly around the macula in the right eye. Irregular hyperfluorescent spots were seen in the central and midperipheral regions bilaterally (Figure 4). Kinetic visual field testing showed ring scotomas bilaterally (Figure 5).

After 30 minutes of dark adaptation, rod b waves and cone b waves were unrecordable, while the 30-Hz flicker responses were extremely reduced (42.6 µV in the right eye and 21.3 µV in the left eye) with delayed implicit times. The normal range in our laboratory is 83 to 175 µV. The standard flash ERG disclosed severely decreased a and b waves in both eyes. After 3 hours of dark adaptation, the standard flash ERG and scotopic ERG

Figure 3. Fundus photographs. A, Fundus photograph of patient II:1 at age 43 years. Scattered white dots and macular degeneration are observed in the right eye. B, Three sharply demarcated chorioretinal lesions are seen in the macula of the left eye. C, At age 51 years, the retinal degeneration and attenuation of retinal vessels have progressed. D, Enlargement of the chorioretinal atrophy, which is pigmented in the left eye.
showed recovery of the a and b waves and scotopic b waves, respectively, although they did not recover to the normal level. The cone b waves showed no significant change after 3 hours of dark adaptation. Blink artifacts were seen in the 3-hour dark-adapted scotopic and photopic ERGs (Figure 6). The patient could not discriminate colors. The dark-adaptation curve was 1.0 log unit above normal threshold after 1 hour of adaptation but attained normal threshold values after 3 hours in the dark.

The patient experienced accelerated deterioration of night vision and central visual acuity after his first visit in 1992. Visual acuity decreased to 20/200 OD and 20/250 OS at the age of 50 years. Fundus examination at this time disclosed bilateral atrophic macular degeneration, which appeared to have progressed since his first visit, and diffuse retinal mottling in the midperipheral areas. The retinal vessels appeared more attenuated (Figure 3). His parents had no visual symptoms, and slitlamp biomicroscopy of his parents showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous; fundus examination showed no retinal changes in both eyes; and the standard flash ERGs were normal.

It is well known that a deficiency of vitamin A causes impaired night vision and retinal degeneration. Thus, it has been thought that genes related to visual cycle or vitamin A metabolism were candidate genes for retinal degeneration or night blindness.

In 1999, it was reported that fundus albipunctatus was caused by mutations of the RDH5 gene, which is localized on chromosome 12q13-q14 and encodes 11-cis-retinol dehydrogenase. This enzyme is abundantly expressed in the retinal pigment epithelium and is a 32-kd membrane-bound enzyme with 318 amino acids. It catalyzes the conversion of 11-cis-retinol to 11-cis-retinal and plays an important role in the synthesis of functional visual pigment.

A previous study by our group showed that a Leu310 (1-bp del; +4-bp ins) (CTT to GAAGTT) mutation in the RDH5 gene was found as a frequent cause of fundus albipunctatus in the Japanese population. Moreover, we found slight variations in the clinical features of patients with this mutation. In particular, the presence of irregular hyperfluorescent spots on fluorescein angiography would appear to be a useful diagnostic tool.
raphy led us to suggest that the RDH5 gene might also be a candidate gene for progressive retinal degeneration. We therefore extended our study and identified a new Gly35Ser mutation in a previously unidentified patient with fundus albipunctatus with cone dystrophy.

As for the Gly35Ser mutation, Gly35 is the first sequence G-X-X-X-G-X-G, which is a highly conserved cofactor binding motif. Because Gly35 is considered to be at an important position, cases with a mutation at codon 35 may show more severe changes than those with other mutations in the RDH5 gene.

The clinical features produced by this mutation were decreased visual acuity, night blindness, white dots in the retina, progressive retinal degeneration, and attenuated retinal vessels. Furthermore, the ERGs showed that the recovery of rod function was abnormally delayed and cone responses were severely impaired. These features were different from those of patients associated with the Leu310 (1-bp del; +4-bp ins) (CTT to GAAGTT) mutation except for scattered white dots and night blindness. This mutation was not found in 200 patients with autosomal recessive retinitis pigmentosa.

Taken together with our previous results,2 the clinical expressions associated with the RDH5 mutation varied from fundus albipunctatus alone to fundus albipunctatus with cone dystrophy. It has been suggested that there may be a spectrum of gene phenotypes caused by mutations in the RDH5 gene.

Mutations in the rhodopsin and BPDE genes have been implicated not only in congenital stationary night blindness but also in retinitis pigmentosa.6-12 These findings can then support the earlier suggestion that mutations in the RDH5 gene are responsible not only for fundus albipunctatus but also for progressive retinal degeneration. However, they cannot explain the variable expressivity associated with the RDH5 gene mutation. As in the digenic inheritance pattern of retinitis pigmentosa caused by mutations in both the peripherin/RDS and ROM1 genes,13 there may be other mutations in the RDH5 Gly35Ser mutation that cause the cone dystrophy.

To examine this possibility, we also screened other candidate genes, such as rhodopsin, peripherin/RDS, ROM1, and RLBP1, to search for additional mutations. The results did not disclose any disease-causing mutation in these genes, and, therefore, mutations in at least these 4 genes are unlikely to be related to the pathogenesis of fundus albipunctatus with cone dystrophy in our patient. Further molecular genetic analysis is needed to eliminate this possibility.

In conclusion, we identified a homozygous Gly35Ser mutation in the RDH5 gene in a Japanese patient with fundus albipunctatus associated with cone dystrophy. This finding provides evidence that a mutation in the RDH5 gene is related, at least in part, to the pathogenesis of progressive retinal degeneration.

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REFERENCES


