Novel Mutations in the Cellular Retinaldehyde–Binding Protein Gene (RLBP1) Associated With Retinitis Punctata Albescens

Evidence of Interfamilial Genetic Heterogeneity and Fundus Changes in Heterozygotes

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Objective: To evaluate the molecular genetic defects associated with retinitis punctata albescens (RPA) in 5 patients from 3 families with this disease.

Methods: We examined 3 probands and 2 clinically affected relatives with RPA. Clinical examinations included best-corrected visual acuity, visual field testing, electroretinography, dilated fundus examination, and fundus photography. Leukocyte DNA was analyzed for mutations in the exons of the genes encoding cellular retinaldehyde–binding protein 1 (RLBP1), 11-cis-retinol dehydrogenase (RDH5), interphotoreceptor retinoid–binding protein (RBP3), and photoreceptor all-trans-retinol dehydrogenase (RDH8). Not all patients were evaluated for mutations in each gene. The exons were individually amplified and screened for mutations by single-stranded conformational polymorphism analysis or direct genomic sequencing.

Results: The 3 probands had similar clinical findings, including a history of poor night vision, the presence of punctate white deposits in the retina, and substantially reduced or absent rod responses on electroretinogram testing. One of the probands (patient 2:III:2) had 2 novel mutations in the RLBP1 gene (Arg151Trp and Gly31[2-base pair deletion], [GGA→G–]). Segregation analysis showed that the 2 mutations were allelic and that the patient was a compound heterozygote. Both parents of the proband manifested round white deposits in the retina. The other 2 probands had no detected pathogenic mutations in RLBP1 or in the other 3 genes evaluated.

Conclusions: The identification of novel RLBP1 mutations in 1 of our 3 probands, all with RPA, is further evidence of genetic (nonallelic) heterogeneity in this disease. The presence of round white deposits in the retina may be observed in those heterozygous for RLBP1.

Clinical Relevance: Patients with a clinical presentation of RPA can have genetically different mutations. Drusen-like lesions may be observed in heterozygotes in families with this disease and a mutation in RLBP1.

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tion by direct and indirect ophthalmoscopy, Goldmann VFs, and ERGs. Visual field examination was performed monocularly with a Goldmann perimeter using the II-2-e, II-4-e, and V-4-e test targets. The targets were moved from a nonseeing region to a seeing region. Not all of the patients were tested with all of these targets.

An ERG was obtained by either of 2 procedures previously described.16,17 The recording techniques adhered to the international standard for clinical electrophysiology established by the International Society for Clinical Electrophysiology of Vision.18 All recordings were obtained with fully dilated pupils and the use of a monocular Burian-Allen contact lens. Scotopic responses were obtained after 40 minutes of dark adaptation. Two patients were additionally tested after 17 hours of dark adaptation.

Three patients (1:III:11, 1:III:12, and 1:III:1) were from one Palestinian family (family 1, Figure 1 and Table), while the remaining 2 patients (2:III:2 and 3:III:3) were from separate African American and Eastern European families (families 2 and 3, respectively) (Figure 2, Figure 3, and Table). All 5 patients had night blindness in childhood, 1 (1:III:1) as early as 3 years of age and 2 (2:III:2 and 1:III:11) by 4 years of age. The best-corrected visual acuities ranged from 20/20 to 20/50 in all 5 patients. The spherical equivalent refractive errors of the subjects ranged from +3.75 to −1.00 diopters.

GENETIC STUDIES

Leukocyte DNA was analyzed for mutations in the 9 exons of RBP1 and the 5 exons of RDH5 by single-stranded conformational polymorphism analysis and direct genomic sequencing according to previously reported methods.7,13 We also searched for mutations in the genes encoding interphotoreceptor retinoid-binding protein (RBP3) and photoreceptor all-trans-retinol dehydrogenase (RDH8); oligonucleotide primers and polymerase chain reaction conditions for amplifying the exons of these genes are available on the Internet.19 Because our 3 families did not show an autosomal dominant mode of transmission, we did not evaluate our patients for mutations in the rhodopsin or peripherin/RDS genes.

RESULTS

At age 21 years, the proband in family 1 (1:III:11, Figure 1) showed round punctate white deposits in the midperipheral retina, fewer such lesions in the posterior pole, and normal optic disc, fovea, and retinal vessels. No pigmentary abnormalities were noted (Figure 4). Her sister (1:III:12, Figure 1), at age 9 years, showed multiple white spots temporal to the macula in each eye, with normal optic disc and retinal vessels (Figure 5A). In addition, a pepper-like mottling was observed, most apparent in the nasal midperipheral retina (Figure 5B). A cousin (1:III:1, Figure 1), age 7 years, showed numerous white spots throughout the retina, with a normal optic disc and attenuated retinal vessels. Moderate pigment granularity, particularly in the inferior retina, was also noted (Figure 6). This patient’s 47-year-old father and 32-year-old mother were each examined and found to have vision correctable to 20/20 in each eye. Neither showed the presence of white spots in the retina of either eye. The father showed normal ERG cone and rod a- and b-wave amplitudes.

The fundus examination of the proband in family 2 (2:III:2, Figure 2), age 7 years, showed white deposits in the posterior pole and midperipheral retina (Figure 7). Retinal pigmentary abnormalities, including hypopigmen-

| Figure 1 | Pedigree of family 1, with 3 affected individuals (proband 1:III:11 [arrow] and patients 1:III:12 and 1:III:1). The affected individuals are the offspring of consanguineous unions (double horizontal lines). X indicates individuals clinically examined by us; circle, female; and square, male. |
| Figure 2 | Pediculus to the macula in each eye, with normal optic disc, fovea, and retinal vessels. No pigmentary abnormalities were noted (Figure 4). |
| Figure 3 | Figure 11 | Pediculus to the macula in each eye, with normal optic disc, fovea, and retinal vessels. No pigmentary abnormalities were noted (Figure 4). |

tation, pigment mottling, and clumping, were also noted in the midperipheral retina. The retinal arterioles were not attenuated. The patient’s 35-year-old mother and 36-year-old father were each examined and found to have white spots in the posterior fundus, including nasal to the optic disc. Both parents had vision correctable to 20/20 or better in each eye, and neither complained of difficulty with night vision. The number of white spots was more numerous in the mother (Figure 8), who harbored a frameshift mutation, than in the father, who showed more isolated lesions and had a missense mutation. Cone and rod ERG amplitudes were normal in each parent.

The fundus examination of the proband in family 3 (3:III:3, Figure 3), age 49 years, showed a blunted foveal reflex, with mild retinal arteriole attenuation and mild waxy pallor of the optic disc. White spots were evident predominantly at and anterior to the vascular arcades (Figure 9A). Sparse pigmentary clumping, mainly in the inferior and midperipheral retina, was observed (Figure 9B). Isolated nummular lesions of chorioretinal atrophy, which were approximately three quarters of a disc diameter in size, were found in the inferior retina of each eye.

VISUAL FIELDS

Visual field testing with a Goldmann perimeter showed full VFs or only mild restriction to the II-4-e stimulus in all 5 patients. Two of the patients (1:III:1 and 3:III:3) showed a restriction to the II-2-e target. One patient (3:III:3) had central scotomas bilaterally.

ELECTRORETINOGRAPHY

The dark-adapted isolated rod ERG response to a low-intensity blue stimulus was nondetectable in 4 of the 5 patients (1:III:1, 1:III:12, 2:III:2, and 3:III:3; Figure 10). In patient 2:III:2, this was observed even after 17 hours of overnight patching. The patient’s parents each showed normal ERG cone and rod amplitudes. One patient (1:III:11) showed a 30% increase in the isolated rod b-wave amplitude after 17 hours of dark adaptation compared with the amplitude after 40 minutes of dark adaptation (Figure 11). The dark-adapted maximal ERG b-wave amplitude after 40 minutes of dark adaptation was within 60 µV of the light-adapted high-intensity b-wave amplitude in 4 of the 5 patients, reflecting a marked impairment of rod function (Table).
The proband of family 2 (2:III:2) carried 2 mutations in RLBP1. In exon 4, there was a frameshift mutation (Gly31[2-base pair deletion], [GGA→G–], complementary DNA bases 92-93delGA), and in exon 6, there was a missense mutation (Arg151Trp, CGG→TGG, complementary DNA sequence 451C→T) (Figure 12A). Segregation analysis in the patient’s family showed that the 2 mutations were allelic and that the patient was a compound heterozygote (Figure 12B). The other 2 probands had no likely pathogenic mutations in RLBP1 detected by single-stranded conformational polymorphism analysis or direct sequencing. Proband 3:III:3 was homozygous for a change in intron 7 (IVS7+20C→T) that was interpreted as likely not to be pathogenic, because it does not appear to create or destroy a splice site, based on splice-site prediction software. The probands without identified RLBP1 mutations (1:III:11 and 3:III:3) were screened for mutations in the RDH5, RDH8, and RBP3 genes. Patient 1:III:11 was homozygous for an isocoding polymorphism (Ile141Ile, ATC→ATA, 423C→A) in exon 3 of the RDH5 gene; no RDH5 sequence changes were found in patient 3:III:3. For RDH8, patient 1:III:11 was heterozygous at both of 2 bases in codon 202 (complementary DNA bases 116-117); depending on the phase of these changes, codon 202 had the allelic sequences ATG (Met) and ACA (Thr), ATA (Ile), or ACG (Thr). The alleles ATG, ACA, and ACG have been observed in additional patients we have evaluated in other investigations of this gene, but the ATA allele has never been

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encountered (data not shown). Patient 3:III:3 was heterozygous at 3 sites: Leu267Leu (CTC → CTT, 743C → T), Tyr277His (TAT → CAT, 829T → C), and Met202Thr (ATG → ACG, 116T → C). Each of these 3 variations is a non-pathogenic polymorphism in the RDH8 gene that we have found in other non-RPA individuals (data not shown). No changes in the RBP3 gene were found in patient 1:III:11 or in patient 3:III:3.

**COMMENT**

Maw et al.\(^2\) were the first to describe patients with recessive mutations in the RLBP1 gene in a retinal degeneration associated with diffuse “small white dots” throughout the fundus and the absence of bone spicule-like pigmentation. Subsequently, Burstedt et al.\(^3\) and Morimura et al.\(^4\) described features of RPA in patients with mutations in RLBP1. Other patients with RPA from Saudi Arabia\(^1\) and Newfoundland\(^5\) have been described with mutations in RLBP1. In addition to the clinical findings typically found in patients with RPA, those homozygous for the RLBP1 mutation Arg234Trp from northern Sweden have a predilection to exhibit an atrophic-appearing macular lesion, particularly in those older than 30 years.\(^6\) This form of RPA has been referred to as *Bothnia dystrophy*.\(^5,6\) A geographic atrophic macular lesion was not a feature observed in our patients with RPA; however, circular areas of geographic atrophy in the peripheral retina, a common finding among adult patients with *Bothnia dystrophy*,\(^5,6\) were observed in our oldest proband (3:III:3). The lesions were similar to those in a 52-year-old patient with RPA and an RLBP1 mutation described by Morimura et al.\(^7\)

Although certain phenotypic similarities exist between patients with RPA and fundus albipunctatus, as a group, these patients differ in certain important clinical and genetic features. Although younger patients with
either of these disorders complain of nyctalopia and typically show multiple round white deposits within the retina, most patients with fundus albipunctatus have a nonprogressive disease, ie, no deterioration in photoreceptor cell function, no attenuation of retinal vessels, and no pigment clumping in the retina. However, other patients with fundus albipunctatus and mutations in \textit{RDH5} have been described as having a cone dystrophy, leading to loss of central visual acuity and reduced cone ERG amplitudes; these findings were most often observed in those older than 30 years.22 Particularly in younger patients with characteristic features of fundus albipunctatus, cone and rod function can revert to normal after a prolonged period of dark adaptation.23 In comparison, those with RPA have a progressive loss of photoreceptor cell function and, not infrequently, develop attenuated retinal vessels and pigmentary clumping. Furthermore, mutations in different genes have been identified in these 2 diseases. Of interest, one of our patients with RPA showed a small partial recovery of rod function after prolonged dark adaptation. A partial recovery in dark adaptation has also been observed in younger patients with Bothnia dystrophy. In contrast, in our patient with an \textit{RLBP1} mutation, we did not observe an improvement in dark-adapted ERG responses even after 17 hours of dark adaptation.

Our findings suggest that the presence of a variable number of small white spots in the fundus may be a diagnostic feature observed in certain heterozygotes of this disease. These lesions may have a phenotypic appearance similar to drusen of the Bruch membrane. Their rather haphazard distribution, absence of a glistening (calcified) appearance, and associated pigmentary changes, in an individual younger than 40 years, are distinguishing features. Whether this finding is specific to RPA families with an \textit{RLBP1} gene mutation is yet to be verified by investigating a larger number of RPA families with or without a demonstrable \textit{RLBP1} mutation.

The novel mutations in \textit{RLBP1} observed in one of our patients, the absence of a demonstrable mutation in this gene in patients from 2 other families, and reports...
on genetic studies in RPA patients from the existing literature further underpin the genetic heterogeneity of RPA. Genetic screening may be necessary to discriminate between some patients with RPA and those with fundus albipunctatus, especially in those with early stages of RPA. Genetic heterogeneity exists in both of these disorders.

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