ABCA4 Gene Sequence Variations in Patients With Autosomal Recessive Cone-Rod Dystrophy

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Objective: To identify sequence variations in the ABCA4 gene in a cohort of patients with autosomal recessive cone-rod dystrophy.

Methods: The coding sequences of the ABCA4 gene were analyzed in 30 unrelated probands. In those patients with plausible disease-causing variations, correlations were made between genotype and fundus phenotype as well as with electrophysiological and visual field findings.

Results: Sixteen (53%) of 30 probands were found to harbor plausible disease-causing variations in the ABCA4 gene. Two distinctly different fundus phenotypes were observed in our cohort of patients. Twelve patients showed diffuse pigmentary degenerative changes, whereas 4 showed either no pigmentary changes or only a mild degree of peripheral pigment degeneration. An association between certain sequence variations and each of these 2 different phenotypes was observed.

Conclusions: Our findings confirm that a substantial percentage of patients with autosomal recessive cone-rod dystrophy are likely to harbor a mutation in the ABCA4 gene as the cause of their disease. The fundus phenotype observed in such patients is quite variable, and certain fundus phenotypes may be more associated with certain genotypes.

Clinical Relevance: Identification of the molecular genetic basis for various inherited human retinal dystrophies, such as cone-rod dystrophy, facilitates a potentially better understanding of the mechanisms by which photoreceptor cells degenerate. This in turn provides guidance as to how to better proceed in identifying the most optimal future therapeutic strategies.

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HE ABCA4 protein (previously denoted as ABCR) is a member of the adenosine triphosphate–binding cassette transporter superfamily of proteins that are involved in the energy-dependent transport of a wide variety of substrates across cellular membranes.1,2 The ABCA4 protein is localized exclusively within photoreceptor outer segments.3,4 This protein is currently thought to participate in the transmembrane transport of all-trans retinal (the aldehyde derivative of vitamin A), facilitating its reduction to all-trans retinol.5

Mutations in this gene have been reported in patients with autosomal recessive Stargardt retinal dystrophy,1,6,7 fundus flavimaculatus,7 autosomal recessive retinitis pigmentosa,8,9 and autosomal recessive cone-rod dystrophy.8,10,11 Mutations in this gene have also been suggested to cause some cases of age-related macular degeneration.12,13

Progressive cone-rod dystrophy is a clinically heterogeneous disorder in which patients characteristically complain of reduced visual function and impairment of color vision. Findings include central scotomas or various degrees of peripheral or midperipheral visual field loss and electroretinogram (ERG) recordings that show either a predominant loss in cone function compared with rod function or a similar reduction in ERG a and b wave amplitudes for the cones and rods.14,15 Funduscopic examination may show either minimal and nonspecific pigmentary changes within the fovea, an easily discernible atrophic appearing macular lesion (which may have a bull’s-eye–like appearance), or more diffuse pigmentary degenerative changes involving both the macula and more peripheral regions of the retina.16 Although some patients with a more advanced stage of cone-rod dystrophy may show a somewhat similar fundus phenotype and ERG amplitude reduction as observed in patients described as having retinitis pigmentosa, the initial history of impaired central vision and, not infrequently, impairment of color vision, as opposed to an initial history of poor night vision and impairment of peripheral vision, are useful in distinguishing between the 2 disorders. Additionally, although exceptions do occur, most patients with retinitis pigmentosa, when ERG amplitudes are still recordable, show a greater...
impairment of rod compared with cone function. Further, overall, the pigmentary changes observed in patients with some forms of cone-rod dystrophy have a greater predilection for the more posterior portion of the fundus than do the pigmentary changes observed in patients with retinitis pigmentosa.

In the current study, we evaluated 30 unrelated probands diagnosed with cone-rod dystrophy. The purpose of the study was to determine the prevalence of ABCA4 sequence variations in this cohort of patients and to ascertain any potential genotype-phenotype correlations.

METHODS

From a review of the medical records of patients seen by one of us (G.A.F.), 30 unrelated probands with the clinical diagnosis of cone-rod dystrophy were selected for inclusion. This cohort of patients was selected from a total group of 212 patients with cone-rod dystrophy based on their availability for participation and their having had a comprehensive ophthalmologic examination. Additionally, we selected patients who either fulfilled the genetic criteria for autosomal recessive transmission (n=17) or who likely had inherited an autosomal recessive trait (n=13), as there were no other known family members afflicted with a similar retinal disease.

An ophthalmic examination was performed by one of us (G.A.F.) on all patients. This included best-corrected visual acuity with a Snellen projection chart or, in individual instances, a Feinbloom Distance Test Chart for the Partially Sighted. Slit-lamp examination of the cornea, anterior chamber, lens, and vitreous was also performed. Ocular pressure was determined by applanation tonometry.

A dilated fundus examination with direct and indirect ophthalmoscopy was performed. An ERG was obtained by either of 2 procedures previously described. These recording techniques adhered to the international standard for clinical electroneuromiologic measurements. All results were compared with the 90th percentile limits or an appropriate range for a healthy population. A comparison of relative reductions in cone and rod b-wave amplitudes was obtained after normalizing the logs of the ratios between the single-flash light-adapted and rod-isolated dark-adapted responses from patients with the respective mean amplitudes from a normal control population.

Visual field examination was performed monocularly with a Goldmann perimeter using II-2-e, II-4-e, III-4-e, and V-4-e test targets. The targets were moved from nonseeing to seeing regions. All of the above targets were not necessarily used on each patient.

All patients had central vision impairment and visual field changes, including central scotomas and peripheral field loss, and most patients showed some degree of fundus pigmentary change. Cone and rod ERG amplitude reductions were observed in all patients. Light-adapted (cone) b-wave amplitudes were either more reduced than dark-adapted (maximal and rod-isolated) responses or both the light- and dark-adapted amplitude responses were similarly reduced.

After informed consent was obtained, blood samples were drawn and used to prepare genomic DNA. From each patient, 12.5 ng of DNA was used as a template in an 8.35-µL polymerase chain reaction containing 1.25 µL of 10X buffer (100 mM Tris hydrochloride, pH 8.3; 300 mM potassium chloride; and 13 mM magnesium chloride); 300 nmol each of deoxythymidine triphosphate, deoxyadenosine triphosphate, deoxyguanosine triphosphate, and deoxyctydine triphosphate; 1 pmol of each oligonucleotide primer; and 0.25 U of Taq polymerase (Boehringer Mannheim, Indianapolis, Ind). Samples were denatured for 5 minutes at 94°C and incubated for 35 cycles under the following conditions: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds in a DNA thermocycler (OmniGene; Hybaid, Teddington, England). After amplification, 5 µL of stop solution (95% formamide, 10 mM sodium hydroxide, 0.05% bromophenol blue, and 0.05% xylene cyanol) was added to each sample. Amplification products were analyzed by single-strand conformation polymorphism analysis (SSCP) as follows. Amplification products were denatured for 3 minutes at 94°C and electrophoresed on 6% polyacrylamide and 5% glycerol gels at 25 W for approximately 3 hours. After electrophoresis, the gels were stained with silver nitrate, as described by Bassam et al. When variant electrophoretic bands were observed by SSCP, polymerase chain reaction products were further analyzed by bidirectional sequencing using fluorescent dye-labeled primers on an automated sequencer (model 373; Applied Biosystems, Foster City, Calif). All 30 exons of the ABCA4 gene were amplified using 51 primer pairs, as previously described. Sequence changes were considered to be plausible disease-causing variations if they were expected to change the amino acid structure of the ABCA4 protein and if they were also present in less than 1% of the 192 control alleles. The clinical and genetic investigations were approved by the institutional review boards at both the University of Illinois at Chicago and the University of Iowa, Iowa City.

RESULTS

Of the 30 probands, 16 (53%) were found to have a plausible disease-causing variation on at least 1 allele. Nine
patients were white, 5 African American, 1 Hispanic, and 1 Palestinian. Ten of 16 patients had 1 or more affected siblings, but neither parent was known to have eye disease. Of the remaining 6 patients, none had a history of eye disease affecting either their parents or other family members.

These 16 patients each exhibited 1 of 2 ophthalmoscopically distinct phenotypes. Twelve patients showed diffuse pigmentary degenerative changes with both hypopigmentation and pigment clumping (type 1) (Figure 1), whereas 4 patients exhibited either normal or negligible and nonspecific mottling of pigment within the fovea (type 2) (Figure 2) and either no peripheral pigmentary changes (3 patients) or a mild degree of peripheral pigment clumping (1 patient).

All 16 patients showed either a central scotoma (n=6) or both a central scotoma and some degree of peripheral field loss (n=10). Additionally, in each patient, both cone and rod a- and b-wave ERG amplitudes were reduced, which is diagnostic for patients with cone-rod dystrophy (Figure 3). Amplitude reductions for cone and rod ERG responses were considered similar if the differences from their respective lower limits of the reference range were less than 20%. Of the 12 patients categorized as type 1, 10 showed a similar degree of cone and rod ERG b-wave amplitude reduction, whereas 2 had a comparatively greater reduction (35% and 36%) in cone compared with rod b-wave amplitude. In the type 2 group, 3 of 4 patients showed a similar reduction of cone and rod b-wave amplitudes, whereas 1 had a greater degree of cone b-wave amplitude reduction (78%) compared with the reduction in the rod b-wave amplitude. Of note, 2 of 4 patients categorized as type 2 showed nondetectable ERG amplitudes for both the cones and rods. The Table shows the age, sex, race, visual acuity, type of visual field loss, cone compared with rod ERG b-wave amplitude reductions, category of fundus phenotype, and ABCA4 sequence variations for each of the 16 patients with cone-rod dystrophy.

Of the 12 patients with diffuse pigmentary changes (type 1), 4 harbored an Ala1038Val change, which is the second most common ABCA4 variant we have observed in patients with Stargardt disease.19 Two of these 4 patients were African American. In 2 of 4 patients, this missense mutation was the only sequence variation identified, whereas in the other 2 cases, it was observed with a second missense mutation. In the additional 8 patients classified as type 1, 2 showed 2 different heterozygous missense mutations, 3 a single heterozygous missense mutation, and 3 a heterozygous splice site mutation within intron
Patients With Cone-Rod Dystrophy

<table>
<thead>
<tr>
<th>Patient No./ Age, y</th>
<th>Race/Ethnicity</th>
<th>Visual Acuity OD</th>
<th>Visual Acuity OS</th>
<th>Visual Field</th>
<th>Fundus Type*</th>
<th>Mutation</th>
<th>Cone vs Rod ERG Reduction</th>
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<tbody>
<tr>
<td>1/74/F</td>
<td>AA 20/60 – 2</td>
<td>5/600</td>
<td>Central and peripheral loss</td>
<td>1 Gly1448Arg</td>
<td>C = R</td>
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<td></td>
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<td>2/35/F</td>
<td>W 10/350</td>
<td>5/400</td>
<td>Central and peripheral loss</td>
<td>1 Ala1038Val</td>
<td>C = R</td>
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<td></td>
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<tr>
<td>3/42/F</td>
<td>H 20/400</td>
<td>20/400</td>
<td>Central and peripheral loss</td>
<td>1 Ala1038Val</td>
<td>C = R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/54/F</td>
<td>W 10/180</td>
<td>10/140</td>
<td>Central scotoma</td>
<td>1 Donor splice, 5bp3' g-a intron 40</td>
<td>C = R</td>
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<td>5/36/F</td>
<td>W 10/120</td>
<td>10/60 – 1</td>
<td>Central scotoma</td>
<td>1 Leu541Pro</td>
<td>C ↓ R</td>
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<td>W 5/160</td>
<td>5/180</td>
<td>Central and peripheral loss</td>
<td>1 Donor splice</td>
<td>C = R</td>
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<tr>
<td>7/49/F</td>
<td>W 10/350</td>
<td>4/350</td>
<td>Central and peripheral loss</td>
<td>1 Glu328Stop</td>
<td>C = R</td>
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<tr>
<td>10/39/M</td>
<td>W 10/225</td>
<td>20/400</td>
<td>Central and peripheral loss</td>
<td>1 Ala1038Val</td>
<td>C = R</td>
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<tr>
<td>9/36/M</td>
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<td>5/350</td>
<td>Central and peripheral loss</td>
<td>1 Gly550Arg</td>
<td>C = R</td>
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<td>11/26/F</td>
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<td>20/400</td>
<td>Central scotoma</td>
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<td>C = R</td>
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<td></td>
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<tr>
<td>12/36/M</td>
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<td>20/200</td>
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<td>1 Val2050Leu</td>
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<tr>
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<td>20/200</td>
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<td>20/50</td>
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<td>C = R (ND)</td>
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<tr>
<td>15/44/M</td>
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<td>20/400</td>
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<td>2 Leu2027Phe</td>
<td>C ↓ R</td>
<td></td>
<td></td>
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<tr>
<td>16/56/M</td>
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<td>20/40</td>
<td>Central scotoma</td>
<td>2 Leu2027Phe</td>
<td>C = R</td>
<td></td>
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</table>

Abbreviations: AA, African American; C ↓ R, cone responses more reduced than rod amplitudes; C = R, cone and rod amplitudes were similarly reduced; CF, counting fingers; ERG, electroretinogram; H, Hispanic; ND, nondetectable; P, Palestinian; W, white.

*Type 1 fundus phenotype indicates diffuse pigmentary degenerative changes; type 2, normal or negligible and nonspecific mottling of pigment.

As originally recognized by Maugeri et al,10 we observed that sequence variations in the ABCA4 gene are a common cause of autosomal recessive cone-rod dystrophy. We documented this finding in 53% of our probands compared with 13 (65%) of 20 observed by Maugeri et al. Among our 16 patients with plausible disease-causing variations, 4 harbored 2 different variations (presumably on different alleles), whereas among the 13 patients described by Maugeri et al, 6 showed a similar finding. Twelve patients exhibited a fundus phenotype consisting of diffuse pigmentary changes, and 4 of these harbored an Ala1038Val change, one of the most common ABCA4 variants observed in patients with Stargardt disease. Four patients exhibited minimal fundus pigmentary changes, and 2 of these patients harbored a Leu1201Arg variation. The most common ABCA4 change seen in Stargardt patients, Gly1961Glu, was not observed at all in this cohort of patients with cone-rod dystrophy. Maugeri et al10 commented that a complex allele consisting of Ala1038Val and Leu541Pro variations on the same allele was found exclusively in their German patients (6 of 14). In our series, 1 patient with this complex allele was identified. This individual was of German and Polish ancestry.

To date, 2 genetic loci20,21 in addition to the ABCA4 locus have been associated with autosomal recessive cone-rod dystrophy. Our findings confirm that a substantial number of patients with a cone-rod dystrophy phenotype will show a sequence variation in the ABCA4 gene. We observed 2 distinctly different fundus phenotypes among our cohort of patients with an ABCA4 change, and these phenotypes were not correlated with differences in ages between patients classified as type 1 (mean age, 43 years) vs those considered to have a type 2 phenotype (mean age, 44 years). There was a predilection for certain sequence variations to be associated with these 2 different phenotypes.

It will be possible to make more meaningful genotype-phenotype correlations once the mutations on both alleles of the ABCA4 gene can be consistently identified. Additionally, a more accurate prognosis regarding loss of visual function will likely emerge when a more complete genotype analysis is possible. Nevertheless, it is of interest that different mutations within the same ABCA4 gene can result in such different fundus phenotypes.

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