Late-Onset Autosomal Dominant Macular Dystrophy with Choroidal Neovascularization and Nonexudative Maculopathy Associated with Mutation in the RDS Gene

Shabrokh C. Khani,1 Athanasios J. Karoukis,2 Joyce E. Young,1 Rajesh Ambasudhan,2 Tracy Burch,2 Richard Stockton,1 Richard Alan Lewis,3 Lori S. Sullivan,4 Stephen P. Daiger,4 Elias Reichel,5 and Radha Ayyagari1

PURPOSE. To examine the molecular genetic basis and phenotypic characteristics of an unusual late-onset autosomal dominant macular dystrophy with features of age-related macular degeneration (AMD) in a large family (SUNY901), by using linkage and mutation analyses.

METHODS. Blood samples were collected from 17 affected members, 17 clinically unaffected members, and 5 unrelated spouses. Clinical analyses included a review of medical history and standard ophthalmic examination with fundus photography, fluorescein angiography, and electroretinography. Linkage and haplotype analyses were performed with microsatellite markers. Mutation analysis was performed by amplification of exons followed by sequencing.

RESULTS. A wide spectrum of clinical phenotypes including exudative and nonexudative maculopathy was observed, with onset in the late fifth decade. Linkage analysis excluded most of the previously known maculopathy loci. Markers D6S1604 (Z\text{max} of 3.18 at \(\theta = 0\)), and D6S282 (Z\text{max} of 3.18 at \(\theta = 0\)) gave significant positive LOD scores and haplotype analysis localized the disease gene to a 9-centimorgan (cM) interval between markers D6S1610 and D6S1459. Mutation analysis excluded the GUCA1A and GUCA1B genes and revealed a missense mutation in the RDS/peripherin gene leading to a Tyr141Cys substitution. A phenotype and haplotype comparison between this and a separate family with the Tyr141Cys mutation suggested the presence of a common ancestral haplotype.

CONCLUSIONS. The RDS mutation in codon 141 is associated with an unusual AMD-like late-onset maculopathy. An apparent selective bias was noted favoring the transmission of the mutant allele. These observations broaden the spectrum of phenotypes associated with RDS gene mutations. (Invest Ophthalmol Vis Sci. 2003;44:3570–3577) DOI:10.1167/iovs.02-1287

Chorioretinal atrophy and choroidal neovascularization are sight-threatening complications that frequently result in permanent loss of central vision in patients with many macular disorders including age-related macular degeneration (AMD). The molecular pathogenesis of these severely disabling and poorly treatable conditions is not well understood. Recent evidence suggests that chorioretinal atrophy in patients with various retinal dystrophies can be traced to a wide array of mutations in photoreceptor-specific genes.1–4 Evidence tying choroidal neovascular disease to these same or other mutations in photoreceptor-specific genes has been weaker, although rare patients with familial age-related macular degeneration manifest both nonexudative atrophic and neovascular exudative phenotypes in association with sequence variants in the photoreceptor-specific gene ABCA4.5 Most other macular degenerative disorders studied to date with prominent neovascular exudative complications, including Sorsby dystrophy, Doyne honeycomb dystrophy, and Best vitelliform dystrophy, are caused by genes that are predominantly expressed in the retinal pigment epithelium and not in photoreceptors.6–9

Among dystrophies with neovascular features, Sorsby macular dystrophy,10,11 stands out as the only late-onset autosomal dominant dystrophy in which choroidal neovascularization and disciform maculopathy are key defining clinical features, although atrophic features are also encountered. Mutations in the gene for tissue inhibitor of metalloproteinase (TIMP)-3, which is expressed primarily in the retinal pigment epithelium, were reported to cause this exudative disease.5 Most families and patients described with late-onset exudative phenotype either have been linked to a TIMP3 locus or carry a mutation in the TIMP3 gene, except for a family that we described earlier.12–14

We recently identified another large family (SUNY901) whose members have an unusual late-onset maculopathy with exudative and atrophic features. After excluding most of the previously described macular disease loci including the loci for exudative fundus dystrophies and AMD,5,7,15–17 we mapped the disease locus to a 9-centimorgan (cM) interval on chromosome 6. Mutation analysis of the RDS/peripherin, GUCA1A, and GUCA1B genes contained within the interval revealed a missense mutation resulting in a Tyr141Cys substitution in the RDS/peripherin gene product. Comparison of haplotypes between SUNY901 and a separate family (BCM-AD033) with the identical Tyr141Cys mutation suggests a possible common ancestral origin.18 Identification of Tyr141Cys establishes an association between an unusual exudative and nonexudative autosomal dominant late-onset macular degeneration with mutation in the photoreceptor-specific RDS/peripherin gene. Me...
tations in this essential outer segment structural gene have been associated with a wide array of central and peripheral human retinal dystrophies, but not typically the exudative phenotypes.19,20

METHODS

Clinical Studies

Informed consent was obtained from all participants before enrolling them in the molecular genetic studies that had been approved by the Institutional Review Boards for Human Subject Research at the State University of New York at Buffalo and Baylor College of Medicine (Houston). The research described in this study adhered to the tenets of the Declaration of Helsinki. A total of 39 individuals were recruited from SUNY901, including 17 with the disease, 17 without the disease, and 5 normal unrelated spouses. Five affected individuals were recruited from BCM-AD033. Blood samples and medical history were obtained from all participants. The ophthalmic examination included Snellen visual acuity measurements and ophthalmoscopy and/or fundus photography in most patients. Further ancillary testing including fluorescein angiography, visual field testing, electroretinography (ERG), and electro-oculography (EOG) were performed in selected participants.21,22

Genetic Analysis

Genotyping. Genomic DNA was isolated from leukocytes by standard techniques. Microsatellite marker analysis was conducted as described.23 Description of the polymorphic markers and genetic distances were obtained from Genethon (www.genethon.fr; provided in the public domain by the French Association against Myopathies, Evry, France).

Linkage Analysis. Two-point linkage analysis was performed between the disease locus and each microsatellite marker with the MLINK program of the LINKAGE package24 (http://www.hgmp.mrc.ac.uk/; provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge, UK). Linkage was assessed under the conditions of autosomal dominant inheritance of the disease trait with a frequency of 0.0001 for the disease-causing allele with the age-dependent penetrance and variable penetrance models and affected-only analysis.25 To calculate LOD scores using an age-dependent penetrance model, individuals in the pedigree were grouped into age-classes and the penetrance for each liability class was calculated as previously described.26

Mutation Analysis. The sequences containing exons and exon-intron boundaries of GUCA1A, GUCA1B, RDS/peripherin, and ROM1 genes were amplified with primers located in the flanking intronic regions as described.6,27–29 Sequence analysis was performed with amplified PCR products as templates and a 33P cycle sequencing reaction kit (Amersham, Arlington Heights, IL).

RESULTS

We recruited 39 participants from three living generations of the SUNY901 family. Thirty-four members were related through common ancestry. The other five were spouses who had married into the family but were unrelated to the members. Based on a well-established genealogy, the members of this family could be traced to a single ancestor who emigrated from Germany to North America.

To establish the inheritance pattern in SUNY901 pedigree, we traced the disease through five generations of descendants. In the pedigree shown in Figure 1, the disease appeared to have been passed sequentially from one generation to the next through affected individuals or unaffected carriers. In genera-
Evidence for the presence of genetic carriers can be explained by variable expressivity, especially given the dominant monogenic trait. Varying phenotypic features observed in the apparent confinement of the disease to macula. Retinal function can be mildly affected in some cases despite the patient’s report of night vision difficulty. The implicit times and the waveforms were unaffected in each eye of the fifth decade and nearly all reported fading spots and relative scotoma and/or distortion near the center of vision, even early on. Most retained visual acuity of 20/50 or better and had limited mild atrophic changes, including subtle pigmentary disturbance and drusen-like deposits in the macula (Figs. 2A–E). Others with more severe involvement displayed choriocapillaris loss (Figs. 2F, 2G), geographic atrophy (Fig. 2H), or exudative changes (Figs. 2I–L). Among those with the most severe exudative disease, IV:4 and IV:27 had active choroidal neovascular disease and III:2, IV:21, and IV:22 had disciform scars representing involved neovascular membranes (Table 1). The review of full-field ERG for IV:25 (Table 1, Fig. 3) showed only a mild generalized reduction in the amplitudes, despite the patient’s report of night vision difficulty. The implicit times and the waveforms were unaffected in each eye of this subject. The ERG and EOG responses on IV:6 were entirely normal according to this subject’s record, despite a similar self-report of nyctalopia. Visual field testing of IV:6 revealed pericentral scotomas but no peripheral constriction (data not shown). These visual functional studies suggest that the overall retinal function can be mildly affected in some cases despite the apparent confinement of the disease to macula.

Contemplating Sorsby fundus dystrophy as a possible first diagnosis in view of the propensity toward disciform disease, we set out to determine whether the TIMP3 locus might cause this disorder. We excluded this possibility by linkage analysis and by mutation screening of all five exons of the TIMP3 gene that contains nearly all Sorsby-associated mutations known to date. To determine whether the other retinopathies or macular dystrophies (Table 1, Figs. 2, 3). The patients in this family had undergone clinical evaluation by other ophthalmologists and were assigned numerous diagnoses including AMD, retinitis pigmentosa inversa, adult foveomacular dystrophy, and Stargardt disease. Their diseases usually manifested by the middle of the fifth decade and nearly all reported fading spots and relative scotoma and/or distortion near the center of vision, even early on. Most retained visual acuity of 20/50 or better and had limited mild atrophic changes, including subtle pigmentary disturbance and drusen-like deposits in the macula (Figs. 2A–E). Others with more severe involvement displayed choriocapillaris loss (Figs. 2F, 2G), geographic atrophy (Fig. 2H), or exudative changes (Figs. 2I–L). Among those with the most severe exudative disease, IV:4 and IV:27 had active choroidal neovascular disease and III:2, IV:21, and IV:22 had disciform scars representing involved neovascular membranes (Table 1). The review of full-field ERG for IV:25 (Table 1, Fig. 3) showed only a mild generalized reduction in the amplitudes, despite the patient’s report of night vision difficulty. The implicit times and the waveforms were unaffected in each eye of this subject. The ERG and EOG responses on IV:6 were entirely normal according to this subject’s record, despite a similar self-report of nyctalopia. Visual field testing of IV:6 revealed pericentral scotomas but no peripheral constriction (data not shown). These visual functional studies suggest that the overall retinal function can be mildly affected in some cases despite the apparent confinement of the disease to macula.

Contemplating Sorsby fundus dystrophy as a possible first diagnosis in view of the propensity toward disciform disease, we set out to determine whether the TIMP3 locus might cause this disorder. We excluded this possibility by linkage analysis and by mutation screening of all five exons of the TIMP3 gene that contains nearly all Sorsby-associated mutations known to date. To determine whether the other retinopathies or macular dystrophies (Table 1, Figs. 2, 3). The patients in this family had undergone clinical evaluation by other ophthalmologists and were assigned numerous diagnoses including AMD, retinitis pigmentosa inversa, adult foveomacular dystrophy, and Stargardt disease. Their diseases usually manifested by the middle of the fifth decade and nearly all reported fading spots and relative scotoma and/or distortion near the center of vision, even early on. Most retained visual acuity of 20/50 or better and had limited mild atrophic changes, including subtle pigmentary disturbance and drusen-like deposits in the macula (Figs. 2A–E). Others with more severe involvement displayed choriocapillaris loss (Figs. 2F, 2G), geographic atrophy (Fig. 2H), or exudative changes (Figs. 2I–L). Among those with the most severe exudative disease, IV:4 and IV:27 had active choroidal neovascular disease and III:2, IV:21, and IV:22 had disciform scars representing involved neovascular membranes (Table 1). The review of full-field ERG for IV:25 (Table 1, Fig. 3) showed only a mild generalized reduction in the amplitudes, despite the patient’s report of night vision difficulty. The implicit times and the waveforms were unaffected in each eye of this subject. The ERG and EOG responses on IV:6 were entirely normal according to this subject’s record, despite a similar self-report of nyctalopia. Visual field testing of IV:6 revealed pericentral scotomas but no peripheral constriction (data not shown). These visual functional studies suggest that the overall retinal function can be mildly affected in some cases despite the apparent confinement of the disease to macula.

Contemplating Sorsby fundus dystrophy as a possible first diagnosis in view of the propensity toward disciform disease, we set out to determine whether the TIMP3 locus might cause this disorder. We excluded this possibility by linkage analysis and by mutation screening of all five exons of the TIMP3 gene that contains nearly all Sorsby-associated mutations known to date. To determine whether the other retinopathies or macular dystrophies (Table 1, Figs. 2, 3). The patients in this family had undergone clinical evaluation by other ophthalmologists and were assigned numerous diagnoses including AMD, retinitis pigmentosa inversa, adult foveomacular dystrophy, and Star...
Afflicted loci may be associated with the disease in SUNY901, we also tested Stargardt macular degeneration 1 (STGD1), STGD3, STGD4, age-related macular degeneration (ARMD1), Doyne honeycomb dystrophy (DHRD), North Carolina macular dystrophy (MCIR1), Best macular dystrophy (VMD2), cone-rod dystrophy 2 (CORD2), CORD5, CORD8, and the rhodopsin loci for possible linkage. Linkage analysis was performed on affected individuals only, to avoid inaccuracies introduced into the final results as a consequence of ambiguities in the assignment of phenotype in case of asymptomatic participants with normal examination results. LOD scores of −2.0 or less were obtained, with most markers linked to previously mapped macular disease loci excluding them from linkage.

In contrast, microsatellite marker D6S282 linked to RDS/peripherin and cone dystrophy 3 (COD3) gave a significant positive LOD score ($Z_{\text{max}} = 3.18$) at zero recombination fraction with an affected-only model. The pericentromeric region of chromosome 6 containing the marker D6S282 harbors at least five known retinal disease loci. Thus we analyzed additional markers linked to D6S282 to map precisely the disease locus in family SUNY901 in conjunction with the previously mapped disease loci in this region. Markers D6S1552, D6S1582, D6S271, and D6S1604 all had significantly positive LOD scores, thus localizing the disease gene to the short arm of chromosome 6, using only the affected individuals in linkage analysis (Table 2). LOD scores were also calculated by using age-dependent penetrance and variable penetrance models. The results obtained from different models did not vary significantly. Haplotype analysis localized the disease gene in SUNY901 family to a 9-cM interval between markers D6S1616 and D6S459 (Fig. 4A).

The critical interval for the disease gene in family SUNY901, defined by the linkage and haplotype analysis, included the retinal genes encoding peripherin/RDS, GUCA1A, and GUCA1B. Sequence analysis revealed no mutations in either the GUCA1A or GUCA1B genes. However, a heterozygous missense A-to-G mutation was found in codon 141 of the RDS/peripherin gene leading to the putative Tyr141Cys substitution in the encoded protein in all affected members of family SUNY901. This mutation was also present in the asymptomatic carriers as well, including IV:1 and 17-19 (Fig. 4A) but not in our control population of 50 normal individuals (100 chromosomes). This sequence variation was not detected in more than 1000 chromosomes surveyed in control populations in other reports in the literature.

The protein product of the ROM1 gene localized to chromosome 11 has been reported to interact with RDS/peripherin. Mutations in this unlinked photoreceptor-specific gene have been reported to cause digenic retinitis pigmentosa. To evaluate the contribution of ROM1 to the variable phenotype observed in the SUNY901 family, we screened the gene for sequence alterations in affected individuals and carriers of the Tyr141Cys-RDS gene mutation. No sequence alterations were found in the coding region of ROM1 in either affected members or carriers.

A separate four-member pedigree BCM-AD033 with retinal dystrophy is the only other known family with the Tyr141Cys mutation. The four affected individuals examined from this
family exhibited a late-onset dominant macular phenotype similar to the one found in SUNY901. One had disciform macular degeneration. A haplotype comparison between these two families showed that marker alleles were shared in common (Fig. 4B). Based on the population frequency of alleles, probability of occurrence of this particular haplotype by chance is estimated at 0.00001. Although the phase could not be determined conclusively for the construction of haplotype in family BCM-AD033, the presence of the SUNY901 disease-associated ancestral haplotype in the affected members of BCM-AD033 suggests that these two families probably share common ancestry.

**DISCUSSION**

In this report we describe an unusual dominantly inherited late-onset macular dystrophy and elucidate its molecular ge-

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933711/)

**FIGURE 3.** Full-field ERG in patient IV:25. Dark-adapted (scotopic) and light-adapted (photopic) ERGs were recorded with bipolar Burian Allen contact lens electrodes in accordance with the 1999 International Society for Clinical Electrophysiology of Vision (ISCEV) standards. Representative responses under maximum (A) scotopic and (B) photopic conditions are shown. The included tables list absolute peak wave amplitudes for the maximum scotopic stimulus (threshold +4 log); scotopic a wave (max. rod b) and maximum photopic responses. Percentages represent the fraction of the lower limit of normal (mean, −2.5 sec/d) for the listed stimulus condition.

**TABLE 2.** Two-Point LOD Scores of Markers on Chromosome 6 Versus Pedigree SUNY901

<table>
<thead>
<tr>
<th>Markers</th>
<th>Distance*</th>
<th>0</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>$Z_{max}$</th>
<th>θ</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S1616</td>
<td>14</td>
<td>−∞</td>
<td>0.27</td>
<td>0.37</td>
<td>0.33</td>
<td>0.18</td>
<td>0.06</td>
<td>0.37</td>
<td>0.1</td>
</tr>
<tr>
<td>D6S426</td>
<td>1</td>
<td>−∞</td>
<td>1.17</td>
<td>1.38</td>
<td>1.24</td>
<td>0.85</td>
<td>0.39</td>
<td>1.37</td>
<td>0.1</td>
</tr>
<tr>
<td>D6S1552</td>
<td>3</td>
<td>1.91</td>
<td>1.84</td>
<td>1.66</td>
<td>1.21</td>
<td>0.73</td>
<td>0.29</td>
<td>1.91</td>
<td>0</td>
</tr>
<tr>
<td>D6S1582</td>
<td>2</td>
<td>2.49</td>
<td>2.16</td>
<td>1.83</td>
<td>1.18</td>
<td>0.60</td>
<td>0.19</td>
<td>2.49</td>
<td>0</td>
</tr>
<tr>
<td>D6S2771</td>
<td>1</td>
<td>2.85</td>
<td>2.49</td>
<td>2.12</td>
<td>1.41</td>
<td>0.76</td>
<td>0.26</td>
<td>2.84</td>
<td>0</td>
</tr>
<tr>
<td>D6S282</td>
<td>0</td>
<td>5.18</td>
<td>2.79</td>
<td>2.40</td>
<td>1.60</td>
<td>0.85</td>
<td>0.25</td>
<td>3.18</td>
<td>0</td>
</tr>
<tr>
<td>D6S1604</td>
<td>2</td>
<td>3.18</td>
<td>2.78</td>
<td>2.38</td>
<td>1.59</td>
<td>0.86</td>
<td>0.30</td>
<td>3.18</td>
<td>0</td>
</tr>
<tr>
<td>D6S459</td>
<td>2</td>
<td>−∞</td>
<td>1.31</td>
<td>1.50</td>
<td>1.26</td>
<td>0.76</td>
<td>0.27</td>
<td>1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>D6S1651</td>
<td>3</td>
<td>1.21</td>
<td>1.13</td>
<td>1.02</td>
<td>0.72</td>
<td>0.41</td>
<td>0.14</td>
<td>1.21</td>
<td>0</td>
</tr>
<tr>
<td>D6S1689</td>
<td>0</td>
<td>−1.86</td>
<td>0.83</td>
<td>0.88</td>
<td>0.69</td>
<td>0.38</td>
<td>0.10</td>
<td>0.83</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S452</td>
<td>2</td>
<td>−0.03</td>
<td>2.60</td>
<td>2.33</td>
<td>1.65</td>
<td>0.88</td>
<td>0.25</td>
<td>2.6</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S1714</td>
<td>1</td>
<td>0.94</td>
<td>0.84</td>
<td>0.72</td>
<td>0.47</td>
<td>0.24</td>
<td>0.06</td>
<td>0.94</td>
<td>0</td>
</tr>
<tr>
<td>D6S1662</td>
<td>4</td>
<td>−0.30</td>
<td>2.25</td>
<td>2.10</td>
<td>1.48</td>
<td>0.78</td>
<td>0.20</td>
<td>2.25</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S295</td>
<td>1</td>
<td>−2.22</td>
<td>0.47</td>
<td>0.47</td>
<td>0.27</td>
<td>0.09</td>
<td>0.01</td>
<td>0.47</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S294</td>
<td>1</td>
<td>1.78</td>
<td>1.55</td>
<td>1.33</td>
<td>0.89</td>
<td>0.49</td>
<td>0.17</td>
<td>1.77</td>
<td>0</td>
</tr>
<tr>
<td>D6S428</td>
<td>12</td>
<td>0.90</td>
<td>0.73</td>
<td>0.57</td>
<td>0.32</td>
<td>0.14</td>
<td>0.04</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>D6S286</td>
<td>2</td>
<td>−0.53</td>
<td>−0.29</td>
<td>−0.09</td>
<td>−0.03</td>
<td>−0.03</td>
<td>−0.03</td>
<td>−0.03</td>
<td>0.4</td>
</tr>
<tr>
<td>D6S445</td>
<td>1</td>
<td>−0.86</td>
<td>1.29</td>
<td>1.29</td>
<td>0.98</td>
<td>0.56</td>
<td>0.20</td>
<td>1.29</td>
<td>0.05</td>
</tr>
<tr>
<td>D6S1609</td>
<td>7</td>
<td>−∞</td>
<td>−0.84</td>
<td>−0.38</td>
<td>−0.06</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>D6S1570</td>
<td>8</td>
<td>−∞</td>
<td>−1.08</td>
<td>−0.65</td>
<td>−0.32</td>
<td>−0.12</td>
<td>−0.02</td>
<td>−0.02</td>
<td>0.4</td>
</tr>
<tr>
<td>D6S1717</td>
<td>−0.44</td>
<td>0.20</td>
<td>0.31</td>
<td>0.29</td>
<td>0.19</td>
<td>0.08</td>
<td>0.3</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

* Distance to the next distal marker.
Figure 4. Edited version of pedigrees (A) SUNY901 and (B) BCMAD-033 with haplotypes constructed with eight markers on chromosome 6 flanking the RDS/peripherin locus. Individuals' numbers are same as noted in Figure 1 for SUNY901. Numbers shown at top right of each circle or square represent the age of the individual. Filled bars: disease haplotype. +/−, presence of RDS Tyr141Cys mutation in the heterozygous state; −/−, mutant alleles in the homozygous state. Questions marks in the haplotype denote PCR failure.
nentic basis in a large family, SUNY901. Through a series of linkage, haplotype, and mutation analyses, a rare Tyr141Cys mutation in the RDS gene was uncovered and found to cosegregate with the atrophic and exudative disease phenotype in this family. Haplotype analyses revealed comparable profiles among affected individuals from SUNY901 and an independent family BCM-AD033 with the Tyr141Cys mutation, further implying a possible common ancestral origin for this mutation. These findings support the pathogenic role of the RDS mutation in the AMD-like phenotype described.

The Tyr141Cys RDS/peripherin mutation is unique because it predisposes to exudative complications in addition to a range of maculopathies with primarily atrophic consequences. Approximately one in four patients (6/21 = 29% ± 20%, P = 0.05) in SUNY901 and BCM-AD033 carrying the mutation had choroidal neovascularization and exudative changes including hemorrhage and edema superimposed on a background of atrophic macular changes. The prevalence of this serious complication was quite high in this family, considering the rarity with which choroidal neovascularization has been reported previously in association with RDS or other photoreceptor-specific gene mutations. Only two isolated RDS-associated cases, one of a patient with a Pro210Arg mutation and adult foveomacular disease and another with a 4-bp insertion in codon 140 with a pattern dystrophy phenotype, have been reported to manifest choroidal neovascular maculopathy.28,39 Given the gravity of this complication for visual prognosis, as is evident in studies on age-related macular degeneration showing that 90% of all blindness due to this disease is caused by this complication, the above findings take on a special significance because the cause of choroidal neovascularization is currently unknown.

The RDS mutation described herein seems to be a rare mutation originating from a common ancestor in Europe. Although the RDS/peripherin gene has been screened extensively in populations worldwide,18,44 the only families known to carry this mutation are the two we have described. Both appear to have originated in Germany. The SUNY901 ancestors first immigrated to the east coast of North America in the 1700s with the early settlers, whereas the BCM-AD033 ancestors reportedly moved to the midwestern United States directly from Germany in the mid-1800s. No direct connections or overlaps were found between the detailed six-generation genealogy on SUNY901 and the limited genealogy available on BCM-AD033. However, haplotype analysis revealed the presence of shared markers among the affected individuals from each family, further supporting common lineage and possibly a founder haplotype.

A selective bias was noted in our families, suggesting apparent inheritance of mutant allele in favor of the wild-type copy from heterozygous parents. At least 18 of the 23 members in generation IV born to affected or obligate carrier parents also carried the mutant allele. In addition, at least four of the five children born to II:1 and three of the five children born to II:2 carried the mutation. In the major branches studied, the affected outnumbered the unaffected, even after we conservatively labeled the few with unknown phenotype or genotype as unaffected. The calculated odds of encountering the mutant versus the wild-type copy comes closer to 3:1 than to the 1:1 ratio expected based on simple unbiased meiotic sorting of the two alleles into two gametes (odds ratio: 1000:1, Z = −3). The source of this bias is unclear and may not be solely attributable to an ascertainment bias or selective sampling, especially because the calculated proportions were based on the ratio of the affected or carrier phenotype relative to entire progesnies of II:1, III:1, III:5, III:6, and III:7. Indeed, entire segments of the family were enrolled well before any linkage assignment or gene identification had been made, again reducing any intentional selection bias. Although persistent ascertainment bias remains a possibility, the markedly skewed ratio in favor of the mutant copies in SUNY901 may also suggest a selective advantage conferred by the mutant RDS allele or a linked gene. Such selective bias or meiotic drive has not been reported previously with any of the other RDS-associated alleles, although patients with X-linked retinitis pigmentosa and Usher syndrome have been known to manifest alterations in sperm morphology without known alterations in fertility.42-43

The variability of clinical phenotype among individuals carrying the Tyr141Cys mutation in the SUNY901 pedigree suggests potential involvement of other modifier genes or the possible contribution of environmental and other stochastic factors to the pathogenesis of the maculopathy. We investigated this possibility in our group by mutation analysis of the unlinked ROM1 gene.35 ROM1 is a structurally related protein partner of RDS which is thought to interact with the intradiscal D2 loop in the heterodimeric (i.e., compound tetrameric) complex of RDS-ROM1. An additive effect of mutations in RDS and ROM1 resulting in retinal dystrophies has been demonstrated previously.37-39 We did not find any sequence alterations in the ROM1 coding sequence among the affected individuals and carriers of the Tyr141Cys mutation. This negative finding suggests contributions from sequence alterations elsewhere, either in the ROM1 gene or other genes.

The Tyr141Cys mutation is likely to exert its pathogenic effect through disruption of the normal structure and metabolism of the RDS protein. The additional cysteine in the mutant protein could compete and interfere with the formation of the normal disulfide bond in the D2 loop. Disruption in the protein structure could then translate into a disorganization of the outer segments giving the crucial role of RDS in maintaining the stacked configuration of outer segment disks.41 In addition, metabolic byproducts generated from processing the abnormal RDS protein by the retinal pigment epithelium could accumulate and change the composition of the extracellular matrix that normally forms part of the barrier against choroidal neovascularization.44 Alterations in physiology of extracellular matrix caused by mutations in TIMP3 and EFEMP1 have been associated with choroidal neovascular disease in Sorsby fundus dystrophy and Malattia Leventinese.45,46 The presence on fluorescein angiography of a dark choroid in several of the family members in the current study supports the possibility of accumulation of an abnormal metabolite in the pigment epithelium and underlying Bruch’s membrane.

Insights gained from the molecular genetic study of this family and other families with inherited maculopathies could benefit our understanding of the molecular basis of age-related macular degeneration in some fraction of the general population.47 The features encountered in the patients in this family mimic those in AMD, including late age of onset, variations in phenotypic expression, severity of the disease, differential age of expression, and the presence of drusen, geographic atrophy, and choroidal neovascularization. We would not be surprised if a fraction of patients with age-related macular degeneration harbored the RDS mutation described herein, especially given the clear evidence that this mutation and its associated haplotype can be selectively transmitted from one generation to next.

Acknowledgments

The authors thank all the members of the two families SUNY901 and BCM-AD033, whose willing and continued cooperation was essential to the success of these studies; all physicians who kindly provided the clinical records and diagnostic studies for review; Paul Sieving for reviewing the clinical photographs and histories; Andrew Vine and Julia Richards for critical review of the manuscript; and Brydon Grant for assistance with statistical analysis.
References


10. Sorsby A, Mason ME, Gardener J. A fundus dystrophy with unusual phenotypic variation including retinitis pigmentosa, pattern dys-...