Autosomal Dominant Hemorrhagic Macular Dystrophy Not Associated With the TIMP3 Gene

Radha Ayyagari, PhD; Irina B. Griesinger, PhD; Eve Bingham; Kurt K. Lark, MD; Sayoko E. Moroi, MD, PhD; Paul A. Sieving, MD, PhD

Objective: To describe the ophthalmic and genetic findings of a large kindred (UM:H389) with autosomal dominant hemorrhagic macular dystrophy.

Methods: The disease state of family members was documented by dilated fundus examination, electroretinography, color vision tests, fluorescein angiography, measurement of visual fields, biomicroscopy, gonioscopy, and intraocular pressure measurement. Linkage and haplotype analyses were carried out with markers flanking the Sorsby fundus dystrophy TIMP3 (tissue inhibitor of metalloproteinase 3) gene locus, and mutation analysis was carried out by screening exon 5 of the TIMP3 gene.

Results: This 4-generation pedigree with autosomal dominant hemorrhagic macular degeneration has visual symptoms beginning in the sixth decade of life. Several family members developed choroidal neovascular membrane formation in the macula of both eyes. The phenotype overlaps that of Sorsby fundus dystrophy. Some of the affected members have unusual zonularlike radial striations on the anterior lens capsule surface, and glaucoma or ocular hypertension has developed in 2 of them. Involvement of the TIMP3 gene was excluded by linkage, haplotype, and mutation analyses.

Conclusions: The phenotype of this family with autosomal dominant macular dystrophy overlaps that of Sorsby fundus dystrophy. Exclusion of the TIMP3 gene in this family indicates genetic heterogeneity for hemorrhagic macular dystrophy. Anterior segment anomalies may occur with this condition, but cosegregation has not yet been established.

Clinical Relevance: This study broadens the spectrum of hemorrhagic macular dystrophy by identifying a family in which the TIMP3 gene is not involved. Once the gene is cloned, we are eager to learn whether this gene may be involved in age-related macular degeneration.


We have studied a family (UM:H389) with vision loss from an autosomal dominant form of macular dystrophy that includes recurrent choroidal neovascular membrane (CNVM) formation and hemorrhage. The phenotype corresponds well with that of Sorsby fundus dystrophy (SFD; OMIM 136900 [available at: http://www.ncbi.nlm.nih.gov/Omim/]), including the phenotype of a family with SFD that we described earlier with a Ser181Cys mutation in the gene encoding tissue inhibitor of metalloproteinase 3 (TIMP3). In SFD, affected individuals experience loss of central vision from choroidal and subretinal neovascularization and hemorrhage that occur in the fourth to sixth decades of life. The histopathological features of SFD include atrophy of the choriocapillary layer and retinal pigment epithelium (RPE) and an abnormal accumulation of confluent lipid-containing material in the inner portion of the Bruch membrane. Individuals with SFD exhibit a range of phenotypes, as noted in the early reports. Some patients experience nyctalopia before developing maculopathy, and most have a normal electroretinogram (ERG) before developing overt maculopathy. Nonretinal findings can include pigment dispersion glaucoma. The family studied in this article (UM:H389) shares this general phenotype.

Sorsby fundus dystrophy maps to chromosome 22q12-q13 and point mutations in the TIMP3 gene were demonstrated in SFD pedigrees. In spite of the phenotypic range, all SFD pedigrees thus far show mutations in the TIMP3 gene, and all these mutations involve exon 5 or the intron 4–exon 5 junction. We have excluded the TIMP3 gene by linkage, haplotype, and mutation analyses of exon 5, indicating a non–TIMP3 cause for the hemorrhagic macular dystrophy in this family.
PATIENTS AND METHODS

CLINICAL STUDIES

Standard clinical examination methods and ancillary tests were used to assess macular dystrophies, as described previously. Gonioscopy and biomicroscopy were conducted to evaluate anterior segment structures. Institutional review board approval was obtained for this study, and informed consent was obtained from all participating subjects.

GENETIC ANALYSIS

Genomic DNA was isolated from 26 family members according to standard protocols. Markers D22S273, D22S280, D22S281, and D22S283 linked to the SFD locus, and a 10-centimorgan interval encompassing the TIMP3 gene was analyzed via the Genome database (available at: http://gdb.wjei.edu.au/gdb/gdbtop.html). Microsatellite marker analysis was carried out as described previously. The sequence of the primers for the markers was obtained from the Genome database.

Linkage analysis was performed using a computer program (MLINK, version 5.1) of a computer package (LINKAGE) using the age-dependent penetrance model. Allele frequencies given in the Genome database were used. The phenotype in this family was analyzed as an autosomal dominant trait with a frequency of 0.0001 for the disease-causing allele. Haplotype analysis was performed to test for the presence of association between the haplotype and the affection status of the individuals in the pedigree studied.

The order of the markers in the region was reported to be 22qcen, D22S273, D22S280 - W17837 (TIMP3), D22S281, D22S283, 22qter (information available at: http://carbon.wi.mit.edu:8000/cgi-bin/gdb/wjei_genomeutr/contig/sis_info/642).

Because all mutations reported in the TIMP3 gene thus far occur in exon 5, we sequenced (cycle sequencing kit; Amersham, Arlington Heights, Ill) this exon for individuals IV-8, IV-29, and III-15 (Figure 1) using the primers described earlier.

RESULTS

CLINICAL FINDINGS

We studied a 4-generation autosomal dominant family having 6 living affected individuals (Figure 1). We have examined 9 affected or at-risk family members and obtained a fluorescein angiogram for 1 additional affected member. The clinical findings of these 10 family members are given in Table 1. In general, the phenotype is characterized by progressive maculopathy that becomes symptomatic in the sixth decade of life. The fundus does not show angiod streaks in any individual, and there is no family history of pseudoxanthoma elasticum.

Three affected sisters (IV-8, IV-11, and IV-13) (Figure 1) were followed up for nearly 10 years; each developed macular CNVM formation between the ages of 55 and 64 years. The earliest clinical findings in these women were seen in the temporal macula, with extensive RPE punctate atrophy that extended into the inferior midperipheral retina and nasal region. The punctate drusenlike and pigmentary changes extended across the macula, as reported in some SFD cases, including a family we described with SFD with a TIMP3 gene Ser181Cys mutation. With time, the RPE changes advanced toward the fovea, and multiple small, subretinal, macular hemorrhages occurred. Recurrent hemorrhages and progressive RPE and choriocapillary layer atrophy eventually reduced the acuity in both eyes to less than 20/200 (Table 1).

The fundus photographs (Figure 2, A-C) of individual IV-11 (Figure 1) show the early course of the disease. At the age of 58 years, she noted blurred central vision in her left eye, and widespread early atrophic RPE changes were seen in the temporal macula (Figure 2, A). Within months, she developed 2 regions of CNVM in the temporal macula (Figure 2, B). The CNVM responded readily to laser photocoagulation and disappeared within days after treatment. However, additional parfoveal neovascular lesions occurred over the subsequent months (Figure 2, C). Some of these lesions could be considered recurrences, with CNVM developing adjacent to treated areas. Other foci of CNVM, however, developed at independent sites. After multiple occurrences and recurrences, the CNVM reached the fovea and caused a permanent decrease in the acuity, at which point we ceased laser treatments. The CNVM thereafter had variable appearance with episodes of regression and hemorrhages, but the progressive RPE atrophy continued across the macula. Her right eye followed a similar course 18 months later. We delayed treatment in her second eye initially, thinking that the laser could possibly have contributed to recurrences at the edge of treated areas by ablating some biological factor critical for a barrier function, but this had no beneficial effect in slowing CNVM occurrence.

The fluorescein angiograms (Figure 2, D-F) of her sister, individual IV-8 (Figure 1), show a similar outcome. She developed CNVM in the temporal macula of the left eye at the age of 64 years. The angiogram (Figure 2, D) showed geographically extensive RPE changes in the temporal retina. The CNVM resolved completely with laser application (Figure 2, E), but a new subfoveal CNVM developed at the margin 3 months later (Figure 2, F).

The RPE response to treatment was clinically unusual in that the choriocapillary layer and RPE essentially disappeared within days after even quite light laser application (Figure 2, E). This reaction of the diseased RPE to additional insult was mirrored in the third affected sister, IV-13 (Figure 2, G-I), who was first seen at the age of 55 years with an asymptomatic hemorrhage in the temporal extramacular region of the left eye; we did not treat this condition because of the extramacular location. When examined only months afterwards, the RPE and choriocapillary layer surrounding the previous hemorrhage had involuted and were absent (Figure 2, G), without developing any gliotic disciform scar that more typically occurs after untreated CNVM. This eye showed additional foci of CNVM and hemorrhage 3 years later (Figure 2, H), and the macula was atrophic 7 years later (Figure 2, I).
later (Figure 2, I). The right eye also developed recurrent CNVM and subsequent RPE atrophy (Table 1). A fourth sister, IV-6, is clinically unaffected at the age of 73 years (Table 1). We did not examine individual III-15, but she was described as unaffected and could read without difficulty at the age of 96 years.

Three male cousins of similar age are also affected (IV-17, IV-20, and IV-29). The fluorescein angiogram of IV-17 showed extensive RPE loss across the macula and particularly at 20° to 30° from the fovea, which is the zone of greatest rod density (Figure 2, J). His brother, IV-29, showed multiple foci of RPE loss and a large area of roughened RPE in the temporal macula (Figure 2, K). From a family history that was compiled by the 3 sisters in conjunction with the elderly aunt, the affected aunts and uncles in the previous generation (III [now deceased]) had all lost their central vision and some subsequently had also experienced some peripheral visual field loss.

Color vision and ERG tests were performed (Table 1). Color discrimination in individual IV-8 was excellent on Farnsworth dichotomous D-15 color testing only 1 year before acuity loss, indicating that this is not a cone dystrophy. The ERG in the 3 affected sisters, IV-8, IV-11, and IV-13, showed normal waveform and amplitudes for rods and cones even at the stage of early maculopathy. However, rod dark-adapted psychophysical thresholds were elevated in the 3 sisters when tested outside of the macular regions ablated by laser or lost to CNVM and hemorrhage. The rod dark-adapted threshold was also severely affected for individual IV-29 and was uni-
formally elevated by 3 log units at all 6 points tested in each eye. His rod scotopic ERG amplitude was greatly reduced by 80% to 90% despite normal cone ERG amplitudes; this implies widespread preservation of the cone photoreceptors but functional impairment of the rods. Some individuals with SFD with TIMP3 gene mutations have impaired rod thresholds.2,19

One affected sister, IV-11, developed glaucoma, with intraocular pressures of 27 mm Hg OD and 22 mm Hg OS, and she had cup-disc ratios of 0.8 × 0.8 OD and 0.5 × 0.5 OS (vertical times horizontal). The anterior lens capsule showed an unusual radial “spokewheel” pattern of zonularlike structures that ended unusually near the center of the lens, leaving approximately a 3.5-mm central clear zone (Figure 2, L). The zonular clear zone normally is larger than the lens, leaving approximately a 3.5-mm central clear zone larlike structures that ended unusually near the center of the midperipheral iris.22 Furthermore, there was no pseudoxfoliation material found on the anterior lens surface or at the iris margin of the pupil.

Two other sisters with macular dystrophy, IV-8 and IV-13, also showed an anterior insertion of zonularlike fibrils on the lens capsule, with abnormally small central clear zones. One (IV-8) has developed an elevated intraocular pressure and is being treated for glaucoma. We have not been able to perform careful anterior segment examinations on the 3 affected male cousins (IV-17, IV-20, and IV-29). Three children of these sisters, V-20, V-39, and V-42, also show prominent zonularlike filaments on the anterior lens capsule and have only small central clear zones (Table 1). These individuals are young, and none has yet developed maculopathy, although the 39- and 45-year-old individuals (V-39 and V-20, respectively) show roughened RPE in the temporal macula. Consequently, statistical evidence of cosegregation of the maculopathy and the lens zonularlike abnormalities is lacking, but this association is suspected.

**GENETIC FINDINGS**

Because of the similarity of the phenotype of this family UM:H389 to the phenotype for SFD, we analyzed 4 markers on chromosome 22 that are linked to the SFD locus and that flank the TIMP3 gene for linkage.4,11 Markers D22S280, D22S281, and D22S283 gave lod scores of less than −2, which excludes linkage to the TIMP3 locus of SFD (Table 2).

Haplotypes were constructed using the genotype data from these 4 markers that flank the TIMP3 gene (Figure 3). The 4 daughters of III-3 (IV-6, IV-8, IV-11, and IV-13) showed 3 haplotypes (5,2,2,8; 3,2,2,2; and 3,4,6,10), but all 3 haplotypes can be eliminated as co-

### Table 1. Clinical Observations on Members of Family UM:H389*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visual Acuity</th>
<th>Refraction</th>
<th>ERG Results</th>
<th>Dark-Adapted Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-6</td>
<td>20/20 OU at 64 y and 20/25 OU at 73 y (clinically unaffected)</td>
<td>-0.75 Sphere OU</td>
<td>Scotopic B wave at 270 µV, photopic B wave at 75 µV, and 30-Hz flicker at 65 µV (34 ms)</td>
<td>Normal</td>
</tr>
<tr>
<td>IV-8</td>
<td>20/20 OU at 63 y and 20/300 OU at 64 y</td>
<td>+0.250.50 × 56 OD and plano + 0.75 × 29</td>
<td>Scotopic B wave at 310 µV, photopic B wave at 115 µV, 30-Hz flicker at 95 µV (32 ms), and bright flash electrongative</td>
<td>Normal except 0.9 log inferior OD</td>
</tr>
<tr>
<td>IV-11</td>
<td>20/30 OD and 20/15 OD at 56 y, 20/20 OD and 20/200 at 57 y, and CF OU at 59 y</td>
<td>Plano OU</td>
<td>Scotopic B wave at 360 µV, photopic B wave at 95 µV, 30-Hz flicker at 100 µV (32 ms), and bright flash electrongative</td>
<td>0.5 Log OU outside laser-treated areas</td>
</tr>
<tr>
<td>IV-13</td>
<td>20/20 OU at 55 y, 20/30 OD and 20/200 at 60 y, and 20/300 OU at 64 y</td>
<td>Plano OU</td>
<td>Scotopic B wave at 180 µV, photopic B wave at 105 µV, 30-Hz flicker at 100 µV (33 ms), and bright flash not available</td>
<td>0.7 Log OU at 55 y and 1.3 Log OU at 64 y</td>
</tr>
<tr>
<td>IV-17</td>
<td>72 y</td>
<td>. . .</td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
<tr>
<td>IV-29</td>
<td>20/25 OU at 61 y and 20/30 OD and 20/60 OD at 67 y</td>
<td>-2.00 + 0.50 × 132 OD and -1.50 + 0.25 × 78 OD</td>
<td>Scotopic B wave at 35 µV, photopic B wave at 120 µV, 30-Hz flicker at 85 µV (36 ms), and bright flash electrongative</td>
<td>3-Log loss at 6 points tested in each eye</td>
</tr>
<tr>
<td>V-20</td>
<td>20/15 OU at 45 y</td>
<td>-2.75 Sphere OU</td>
<td>Not performed</td>
<td>All points normal</td>
</tr>
<tr>
<td>V-31</td>
<td>20/15 OU at 33 y and 20/15 OD at 45 y</td>
<td>-3.75 + 0.25 × 70 OD and -4.00 + 0.25 × 110 OS</td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
<tr>
<td>V-39</td>
<td>20/15 OU at 39 y</td>
<td>Plano OU</td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
<tr>
<td>V-42</td>
<td>20/15 OU at 32 y</td>
<td>-2.25 Sphere OU</td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

*ERG indicates electroretinogram; OU, in both eyes; OD, in the right eye; OS, in the left eye; CNVM, choroidal neovascular membrane; Rx, treatments; C/D, cup-disc ratio; CF, counting fingers; RPE, retinal pigment epithelium; FA, fluorescein angiogram; HVF, Humphrey visual field; and ellipses, data not applicable.
mutations in the TIMP3 gene reported in white families

Sequences in the affected individuals of the family we studied (UM:H389) showed no mutations in exon 5 of the TIMP3 gene.

The family studied herein exhibits hemorrhagic maculopathy and has clinical symptoms and signs similar to those found in persons with SFD. Haplotype and mutation analyses demonstrate that the TIMP3 gene is not associated with the disease.

Similar to some previous reports of SFD, dark-adapted absolute thresholds are elevated in some of these affected family members (Table 1); rod and cone photoreceptor function, however, is not disturbed early in the course of the disease, as judged from the normal ERG waveforms and amplitudes. In SFD, this was proposed to result from the abnormal processing or trafficking of vitamin A by the RPE–Bruch membrane complex. We do not know whether this mechanism also applies to the present family.

Several issues warrant discussion. First, the maculopathy in this family has clinical features that overlap SFD. This eponym designates a clinical phenotype of hemorrhagic macular dystrophy beginning in the fourth to sixth decades of life and transmitted in an autosomal dominant manner. Sorsby fundus dystrophy genotype-phenotype studies show a range of clinical states even...
Figure 2. Findings in affected members of family UM:H389. A, B, and C, Individual IV-11. A, Geographically extensive retinal pigment epithelium (RPE) changes are seen in the temporal macula by the age of 58 years. B, By the age of 59 years, multiple foci (arrows) of subretinal neovascularization developed. C, Moderate laser application caused RPE ablation and resolution of the subretinal neovascularization, but 3 additional choroidal neovascular membrane (CNVM) foci (arrow) developed. These responded to laser treatment, but the subsequent occurrences were juxtafoveal and then subfoveal, resulting in acuity loss. D, E, and F, Individual IV-8. D, A fluorescein angiogram at the age of 64 years showed geographically extensive RPE changes in the temporal macula that extended across the inferior fundus just beyond the macular arcade vessels. E, The large neovascular membrane in the temporal macula was readily treated with moderate laser application that, surprisingly, also ablated the RPE and choriocapillary layer. F, Within 3 months, new CNVM had occurred at the inferior margin (arrows) and extended into the fovea, with loss of acuity. G, H, and I, Individual IV-13. G, At the age of 56 years, she developed a small subretinal hemorrhage in the temporal macula of the left eye (not shown); 6 months later, this left a large atrophic zone that was devoid of RPE and the choriocapillary layer. H, By the age of 59 years, this atrophic region had enlarged and perifoveal atrophic changes had progressed; 2 additional subretinal hemorrhages were evident (arrows). I, By the age of 64 years, the macula was extensively atrophic and her visual acuity was 20/300 OS. J, Individual IV-17. A fluorescein angiogram of the right eye at the age of 79 years showed geographically extensive RPE and choriocapillary layer loss across the macula and extending into the midperiphery of both eyes (right eye shown). K, Individual IV-29. A fluorescein angiogram at the age of 67 years showed atrophic RPE changes in the temporal macula of both eyes (right eye shown) with punctate pigmentary changes. L, Individual IV-11. On retroillumination, the anterior lens capsule shows long, radial, "zonularike" structures that leave only a small central clear zone. Iris transillumination defects and loss of the pupillary ruff are evident at the pupillary margin between the arrows.
within individual families, and not all patients with SFD have hemorrhage and CNVM. The original families with SFD had punctate drusen to greater or lesser degree in the temporal macula, as does the family we describe herein. Slow filling of the choriocapillary layer is seen in some individuals with SFD, and we observed similar slow filling in the affected macula of the 3 affected sisters. Some families with SFD are also described as having pigmentary dispersion. Nevertheless, all families with SFD described thus far have mutations in the coding or splice region of exon 5 of the TIMP3 gene. The present family studied fits within this SFD-like phenotype but is genetically excluded from the TIMP3 locus.

Second, is this age-related macular degeneration (AMD)? Age-related macular degeneration is a genetically complex disorder. A recent report of an autosomal dominant family with AMD with atrophic disease shows linkage to markers on chromosome 1. Vision loss from AMD typically occurs in the seventh to eighth decades of life, rather than the earlier changes of the family studied herein. In contrast, the family studied herein has characteristics of hemorrhagic AMD, with an apparently single Mendelian trait. The relation of this non-TIMP3 gene hemorrhagic macular dystrophy with the exudative form of AMD is curious, as TIMP3 was excluded as the candidate gene for AMD. This family may provide a genetic model to study some aspects of the exudative form of AMD once the gene is identified.

Third, several family members showed anterior segment abnormalities with unusual zonular-like processes ending toward the central area of the anterior lens capsule. They also showed iris transillumination defects (Table 1). This is clinically similar to a pigmentary dispersion syndrome, but distinct differences are noted by
the absence of posterior iris bowing and the absence of midperipheral iris transillumination defects. Pigmentary dispersion or glaucoma results from mechanical breakdown of the iris pigmented epithelium by the zonularlike bundles, with dispersed pigment that congests the trabecular meshwork. In the family studied herein, we propose that close proximity between the centrally inserted zonularlike bundles and the pupillary ruff leads to mechanical breakdown of the iris pigmented epithelial cells with the release of pigment. Glaucoma has also been observed in families with SFD. In the family studied herein, cosegregation of the maculopathy and the lens changes is inconclusive at this time.

It is interesting to consider similarities between the ciliary epithelium and the RPE. The lens zonules are of noncollagenous glycoprotein elastic material, elaborated by the ciliary epithelium; the Bruch membrane also contains glycoprotein elastic fibrils and is elaborated by the RPE. Both epithelia are of neuroectodermal origin, and a defect common to both may account for the maculopathy and the anterior segment abnormality. We are examining additional family members to learn whether the lenticular changes cosegregate with the maculopathy and may facilitate gene identification. We anticipate using the lens changes to guide our effort toward mapping the gene.

Accepted for publication July 8, 1999.

This study was supported by grants R01EY10259, RO1-6094, and R01EY7003-core from the National Institutes of Health, Bethesda, Md; the Foundation Fighting Blindness, Hunt Valley, Md; and a Senior Scientific Investigator Award from Research to Prevent Blindness Inc, New York, NY (Dr Sieving).

We thank all the patients and their family members for their participation.

Reprints: Radha Ayyagari, PhD, W. K. Kellogg Eye Center, 1000 Wall St, Ann Arbor, MI 48105 (e-mail: ayyagari@umich.edu).

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