Novel Mutation in the TIMP3 Gene Causes Sorsby Fundus Dystrophy

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Objective: To determine the molecular basis of a retinopathy previously described as dominant macular subretinal neovascularization with peripheral retinal degeneration.

Methods: The TIMP3 gene was analyzed in family members, and 4 mutation-positive patients were studied using psychophysics and electroretinography.

Results: Cosegregating with disease in the family was a single base pair change in the TIMP3 gene, altering a conserved tyrosine to cysteine at amino acid position 172 (Y172C). There was psychophysical and electroretinographic evidence of rod dysfunction greater than cone dysfunction. Dark adaptometry showed abnormalities with regional retinal variation in degree.

Conclusions: The Y172C mutation in the TIMP3 gene is another cause of Sorsby fundus dystrophy. The expression of this form of the disease, as in other C-terminal TIMP3 mutations, is speculated to be secondary to mutant TIMP-3, causing a decreased turnover of the extracellular matrix.

Clinical Relevance: The molecular clarification of inherited retinal degeneration involving abnormal extracellular matrix turnover in and around Bruch’s membrane should provide clues to the pathogenesis of not only these particular diseases but also forms of age-related macular degeneration.

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Among many monogenic retinal degenerative diseases, only Sorsby fundus dystrophy (SFD) commonly manifests as a hemorrhagic maculopathy secondary to choroidal neovascularization (CNV).1 This feature of SFD is interesting because of its clinical similarity to some forms of age-related macular degeneration (AMD). The hypothesis that there may be both molecular and clinical similarity to AMD became testable when it was determined that SFD was caused by mutations in the gene encoding tissue inhibitor of metalloproteinases-3 (TIMP-3).2 An extracellular matrix (ECM) protein. However, candidate gene screening with the TIMP3 gene in AMD populations did not reveal a simple genetic relationship between the rare inherited disease and the common age-related cause of CNV.3,4

More than a decade ago, a family was described as having “dominant macular subretinal neovascularization with peripheral retinal degeneration.”9 Among the diagnostic possibilities considered was SFD, but the age at disease onset was thought to be too early compared with other reports in the literature at that time.10,11 We reevaluated the family and report that this retinopathy is a severe form of SFD caused by a novel mutation in the TIMP3 gene.

RESULTS

The PCR product from exon 5 of the TIMP3 gene amplified from the proband (patient III-5) migrated in an aberrant electrophoretic pattern compared with normal control DNA samples run in parallel during SSCP analysis. Direct sequencing of the PCR product revealed a single base pair change, altering a conserved tyrosine to cysteine at amino acid position 172. This Y172C TIMP3 gene mutation coseg-
PATIENTS AND METHODS

Informed consent was obtained from the study participants for all procedures. Mutation screening of the TIMP3 gene was performed. Venous blood samples were collected from 13 family members (generations III and IV in Figure 1A), and DNA was extracted. Techniques for single-strand polymorphism (SSCP) analysis and direct sequencing of polymerase chain reaction (PCR) products have been described elsewhere. A subset of 4 patients (III-4, 5, and 6 and IV-14), all positive for a TIMP3 gene mutation, were assessed with routine ophthalmic examinations and specialized tests of visual function. Static threshold perimetry in dark-adapted (500- and 650-nm stimuli) and light-adapted (600-nm stimulus on a 10 candela/m² white background) states was performed using a modified automated perimeter and analyzed for rod and cone threshold elevation. Dark adaptometry was tested with 500- and 650-nm stimuli after a retinal exposure of 7.8 log scotopic-troland-seconds, estimated to bleach about 99% of the rhodopsin present, and recovery was measured until prebleach baseline dark-adapted (>3-hour) thresholds were attained. Details of these procedures have been published previously.13-15 For the patients with central scotomas, bleaching and testing were performed using infrared visualization of the fundus with a modified fundus photoperimeter. Electroretinogram (ERG) photoresponses were evoked in the dark-adapted state with high-energy blue (2.3-4.6 log scotopic-troland-seconds) and red (1.4-3.6 log photopic-troland-seconds) stimuli and in the light-adapted (3.2 log troland white background) state with red (2.2-4.1 log photopic-troland-seconds) stimuli. A model of phototransduction activation consisting of the sum of rod and cone components was used to quantify the leading edges of dark-adapted waveforms; a model of cone phototransduction was used to quantify the leading edges of light-adapted waveforms. Details of recording and analysis methods have been published previously.16,17

Rod- and cone-mediated thresholds throughout the field of vision were shown for a 39-year-old patient (III-6) with a large central scotoma. Throughout the peripheral visual field were significant rod threshold elevations (mean, 2.0 log units); beyond the central scotoma, most cone thresholds were within normal limits. Patients III-3 and III-5 also showed more rod than cone threshold elevations in the peripheral visual field beyond their central scotomas. An asymptomatic 20-year-old heterozygote (patient IV-14) had normal results on eye examination and visual function tests. Rod and cone ERG photoresponses suggest that the abnormalities on psychophysical testing have a photoreceptor basis (Figure 2B). Patient IV-14 had normal rod and cone responses, whereas 3 older patients (patients III-3, 5, and 6) had rod maximum amplitudes reduced to approximately half of the mean normal value and normal or slightly reduced cone maximum amplitudes. Rod photoresponse sensitivities were within normal limits in 2 patients (III-6

regates with the disease in generation III, which has the only living clinically affected members at this time; there were both mutation-positive and mutation-negative members in generation IV (Figure 1B).

The fundus photograph of patient III-5 at age 45 years (Figure 1C) is representative of the central retinal scarring from hemorrhagic macular degeneration found in affected members in the fourth and fifth decades of life. Fundus appearance and fluorescein angiography in patients III-3, 4, 6, and 7 documenting RPE abnormalities in the macula and midperipheral retina were previously reported.5 Five of the 6 affected patients had an acute loss of central vision in one eye between ages 26 and 29 years, with the second eye losing vision within 2 years of the first event. The affected half brother (patient III-1) lost central vision between ages 35 and 36 years. In generation IV, 4 younger asymptomatic family members (ages 7-21 years) carry the mutation and are at risk for developing the disease (Figure 1A).

Visual function studies in a subset of heterozygotes for the Y172C TIMP3 mutation are shown in Figure 2.
Figure 2. Functional phenotype of heterozygotes with the Y172C TIMP3 mutation. A, Static threshold dark- and light-adapted perimetry in a patient representing a late stage of the disease. Elevation of rod and cone thresholds across the visual field are shown as gray-scale maps, with 16 levels representing 0 to 3 log units of threshold elevation. Black squares indicate no detection of stimuli. T, N, I, and S indicate temporal, nasal, inferior, and superior visual fields, respectively. B, Rod- and cone-isolated photoresponses (symbols connected by thin black lines) fitted with a model of phototransduction activation (thicker gray lines). Photoresponses evoked with blue 4.6 log scotopic-troland-second (ROD) and red 4.1 log photopic-troland-second (CONE) flashes are shown for each patient. Arrows on the y-axis denote the lower limit (mean − 2 SD) of normal maximum amplitude. C and D, Dark adaptation with 500-nm stimulus after a full bleach exposure in patients (symbols and thin lines) compared with the normal range (gray, thicker lines). Prebleach thresholds are shown preceding time zero; numbers represent test location in degrees in the T and N visual fields.
and IV-14), whereas 2 other heterozygotes (patients III-3 and 5) had sensitivity losses of about 0.4 log units. Cone photoreceptor sensitivities were within normal limits for all 4 patients.

Dark adaptation was tested at several different retinal loci in the 4 heterozygotes; representative functions obtained at ≥30° eccentric to the anatomical fovea are shown (Figure 2C and D). In patients IV-14 and III-6, dark-adaptation functions at 3 different loci were normal; in patient III-5, dark adaptation was borderline normal at 4 loci; and in patient III-3, it ranged from normal to abnormal at 4 loci tested. The abnormality, when present, consisted mainly of threshold elevation (Figure 2D).

Following the first association between a TIMP3 mutation and SFD, 2 several reports of other TIMP3 mutations in SFD have appeared. 8,9 Almost all mutations documented in SFD to date, including Y172C, would be expected to alter residues in the C-terminal domain of the molecule. There is still no biological explanation of how mutation in an inhibitor of matrix metalloproteinase may be leading to decreased ECM turnover and extreme thickening of Bruch’s membrane, the histopathological hallmark of SFD. 10,19 Recent in vitro studies of 4 SFD mutations suggest that the pathogenesis is probably not caused by haploinsufficiency but by persistent increased function. Evidence has appeared that mutant TIMP-3 may be accumulating as a dimer and functionally contributing to a slowed turnover of the ECM. 6 Immunocytochemical studies in a donor retina with SFD previously demonstrated an accumulation of TIMP-3 in Bruch’s membrane. 19 Recently, the need for a greater understanding of ECM biology to interpret disease was further emphasized when another counterintuitive observation was made in patients with multicentric osteolysis and arthritis syndrome, disorders characterized by an enhanced ECM breakdown. Causative mutations were found in the gene encoding matrix metalloproteinase-2, 20 suggesting that like SFD this was another ECM disorder with a complex mechanism. 21

Considering the onset ages reported for hermaphroditic macular degeneration in SFD families with other causative mutations, 8 the family with the Y172C TIMP3 mutation is among those with an earlier onset of macular disease. The clinical manifestations in SFD are all likely to be secondary to the slowly progressive thickening of Bruch’s membrane from the ECM imbalance discussed previously. Until the disease threshold is reached, the retinas of these patients are clinically and functionally normal. Choroidal neovascularization could be secondary to a hypoxic stimulus from the retina, which becomes separated by a critical distance from its choriocapillaris blood supply by ECM buildup. The photoreceptor abnormalities leading to extramacular vision loss may be secondary to RPE dysfunction. 13 The finding of increased TIMP-3 content of Bruch’s membrane with normal aging and in AMD 22 raises the suspicion that this molecule may be a marker for pathological processes that lead to a disturbed ECM turnover. Abnormal binding and activity of TIMP-3 may be one of the aberrant pathways leading to many diseases of Bruch’s membrane.

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