Sorsby Fundus Dystrophy: Novel Mutations, Novel Phenotypic Characteristics, and Treatment Outcomes

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Submitted: September 24, 2014 Accepted: February 16, 2015

PURPOSE. To report novel TIMP3 mutations, and to characterize the ocular phenotype of Sorsby fundus dystrophy (SFD), including a novel early sign for the disease and to report the effect of anti-VEGF therapy.

METHODS. Twenty-one probands of three unrelated families with SFD were investigated using wide-field imaging, confocal laser scanning ophthalmoscopy with autofluorescence imaging, optical coherence tomography (OCT), indocyanine green-angiography (ICG-A), and molecular diagnostic for causative mutations.

RESULTS. Molecular genetic analysis revealed two novel (p.Tyr174Cys, p.Tyr177Cys) and one previously described (p.Tyr182Cys) missense mutations in TIMP3. In families with p.Tyr177Cys and p.Tyr182Cys, metamorphopsia and/or decrease in visual acuity were the initial symptoms occurring at approximately the sixth decade of life. The p.Tyr174Cys mutation carriers had first symptoms at approximately the third decade with dark adaptation problems and visual field defects. The ocular phenotype included drusen-like deposits, rapidly progressive geographic atrophy, choroidal neovascularization (CNV), and polypoidal choroidal neovascularization (PCV). Late disease manifestations were uniform with widespread chorioretinal atrophy, fibrosis, and choroidal thinning. Three asymptomatic young carriers of a TIMP3 mutation with otherwise normal findings on funduscopy and retinal imaging showed a characteristically reduced fluorescence on late-phase ICG-A images. This phenotypic sign was more pronounced and widespread in later disease stages. Patients with CNV or PCV showed a favorable response to therapy with intravitreally injected bevacizumab.

CONCLUSIONS. This study expands the spectrum of mutations in the TIMP3 gene and associated phenotypic findings. Imaging using late-phase ICG-A may be useful for early identification of individuals at risk for developing SFD. Intravitreal anti-VEGF therapy if initiated timely is effective in SFD patients with CNV.

Keywords: Sorsby fundus dystrophy, phenotype, genotype, therapy

Sorsby fundus dystrophy (SFD) is a rare retinal dystrophy with variable age of onset and autosomal-dominant inheritance that was first described by Sorsby in 1949.1 The disease is caused by mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) gene.2 In the eye, TIMP3 is mainly expressed and secreted by the RPE and is deposited in Bruch’s membrane.4 Functions of TIMP3 encompass regulation of turnover of the extracellular matrix (ECM) by inhibiting proteolytic enzymes,4 antiangiogenic properties by binding to the VEGF receptor 2 (VEGFR2),6 and regulation of inflammation.7 It has been suggested that mutant TIMP3 protein accumulates within Bruch’s membrane, thereby leading to a disturbed homeostasis in ECM remodeling, which might interfere with physiologic functions of Bruch’s membrane as well as of the adjacent choroid and RPE.8

The first visual symptoms in patients with SFD most commonly occur during the fourth or fifth decade of life, typically with sudden and progressive loss of vision due to the development of choroidal neovascularization (CNV) or with a delayed dark adaptation.9–12 Typical findings on fundus examination are multiple yellowish drusen-like deposits at the posterior pole, early-onset CNV, and chorioretinal atrophy. Late disease stages are characterized by widespread atrophy and fibrotic lesions at the posterior pole.1,11,12

Herein we report the clinical findings in two families with SFD with previously undescribed mutations in the TIMP3 gene as well as in several members of a SFD family previously shown to carry a p.Tyr182Cys TIMP3 mutation.

MATERIALS AND METHODS
This prospective case series was performed between October 2013 and May 2014 at the Department of Ophthalmology,
The study was in adherence with the declaration of Helsinki. Institutional review board approval (Ethics Committee, Medical Faculty, University of Bonn) and patients’ informed consent were obtained. All patients underwent a complete ophthalmologic examination including best corrected visual acuity (VA) and funduscopy with dilated pupils. All asymptomatic family members who underwent predictive genetic testing and/or predictive imaging underwent genetic counseling before testing. Pedigrees were designed using a dedicated software program (CeGaT Pedigree Chart Designer; CeGaT, Tübingen, Germany).

**Genetic Testing**

Genomic DNA was extracted from blood lymphocytes by a standard protocol. Coding exons and flanking splice junctions of the *TIMP3* gene were PCR amplified as described. The PCR products were analyzed by direct Sanger sequencing with the BigDye terminator kit 1.1 (Applied Biosystems, Weiterstadt, Germany) following the manufacturer’s instructions. The sequencing products were analyzed on an automated capillary sequencer 3130xl Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany) and evaluated with the software package Sequencing Analysis version 5.2 (Applied Biosystems).

Next-generation sequencing (NGS) for a gene panel covering 76 genes associated with autosomal-recessive and/or dominant RP and 44 genes known to be mutated in macular, cone-rod, or cone dystrophy was carried out on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Enrichment and filtering of data were carried out as described previously, and we applied quantitative readout of NGS reads, thereby excluding large structural rearrangements, such as exon deletions or duplications. Validation of identified putatively pathogenic variants and segregation analysis were carried out by Sanger sequencing. Bioinformatic analyses were performed using MutationTaster, SIFT, and Polyphen-2 programs. Nomenclature of mutations followed standards of the Human Genome Variation Society.

**Image Acquisition and Processing**

Patients underwent imaging with a confocal scanning laser ophthalmoscope (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany) with a dedicated imaging protocol. Images of 55° fundus autofluorescence (AF) and near-infrared (NIR) reflectance were acquired with central fixation and the high-resolution mode (1536 x 1536 pixels). Spectral domain optical coherence tomography (SD-OCT) volume scans covering a 25 x 30° field with 61 scans and at least nine images averaged, as well as vertical and horizontal line scans with 100 images averaged with central fixation were acquired. In addition, enhanced depth imaging (EDI) OCT horizontal and vertical line scans with 100 images averaged with central fixation were recorded. If required, the position to the foveal center was controlled manually. All OCT images were recorded in the high-speed mode with 768 A-scans per b-scan. Choroidal thickness was measured subfoveally based on horizontal EDI-OCT line scans. Measurement was performed manually with the Heidelberg Eye Explorer Software (HEYE, Heidelberg Engineering). Subjects with refractive error greater than ± 3 diopters (D) (spherical equivalent) as well as subjects with any pretreatment potentially affecting choroidal thickness (e.g., photodynamic therapy) were excluded from this analysis. A total of 64 healthy probands served as controls for choroidal thickness measures. Wide-field fundus images were acquired using an Optos 200Tx imaging system (Optos PLC, Dunfermline, United Kingdom). The Zeiss Visucam (Zeiss, Oberkochen, Germany) was used to perform 45° fundus color imaging. In patient II.4 from family 1, only parts of the imaging protocol were available.

Progression rate of geographic atrophy in patient II.1 of family 1 was calculated from measurements on 30° fundus AF images by using the Region Finder software (Heidelberg Engineering). In cases of difficult delineation of the atrophy border, other imaging modalities including IR or SD-OCT were consulted.

**RESULTS**

**Family 1**

In two index patients (II.1 and II.4; Fig. 1), SFD was suspected based on the phenotype (Table; Fig. 2) together with the relatively early onset of symptoms and the family history of severe vision loss in subsequent generations. Sanger sequencing of the *TIMP3* gene revealed a heterozygous c.530A>G (p.Tyr177Cys) mutation in exon 5 of both individuals. Subsequent genetic testing of six additional family members from two generations identified five further heterozygous carriers of the disease allele. A 71-year-old woman (II.2) without retinal disease was homozygous for the wild-type allele (Table; Fig. 1).

Investigated family members carrying the *TIMP3* mutation were between 23 and 78 years. The three youngest carriers of the mutation (patients III.1, III.2, and III.3; 23, 32, and 34 years) were asymptomatic and showed normal findings on

![Pedigree of family 1](image-url)
Three affected family members (II.1, III.4, III.8) between 54 and 61 years of age presented with macular drusen-like deposits, as well as yellowish deposits along the vascular arcades and nasal to the optic disk (Figs. 2E–L). Patients III.4 (54 years) and III.8 (56 years) were asymptomatic at first examination but both subsequently developed subfoveal CNV with progressive deterioration in VA and metamorphopsia (treatment outcome see below). Patient III.8 additionally presented with an asymptomatic polypoidal choroidal vasculopathy (PCV) and subretinal hemorrhage nasal to the optic disk. The 61-year-old female patient (II.1) showed progressive chorioretinal atrophy without signs for previous or currently active CNV (Figs. 2I–L). She complained about dark adaptation problems since the age of 47 and worsening VA over the past 2 years.

The oldest examined and affected individual of this family (II.4; 78 years) presented with widespread chorioretinal atrophy and fibrosis at the posterior pole. The atrophic changes reached beyond the vascular arcades and had multi-lobulated margins (Figs. 2M–O). Decline of VA started at the age of 57 years and was accompanied by dark adaptation problems. Visual acuity is now counting fingers in the right and 20/80 in the left eye.

### Family 2

Family members V.5, V.6, V.I.5, and V.I.6 have previously been shown to carry a heterozygous c.545A>G (p.Tyr182Cys) mutation in exon 5 of the TIMP3 gene, but detailed phenotyping was not reported. Five additional family members have now been tested for carrier status. Three subjects (age range, 52–79 years) were heterozygous for the c.545A>G (p.Tyr182Cys) mutation (V.1, V.4, VI.3) and two female members (VI.2, VI.4) without retinal disease were homozygous for the wild-type allele (Table; Fig. 3).

Patient VI.5 suffered from recurrent unilateral metamorphopsia and reduced vision since the age of 45 due to a unilateral subfoveal CNV in the absence of any additional fundus changes on funduscopy, fundus AF, and SD-OCT imaging (Figs. 4A–D; treatment outcome see below). Two family members carrying the disease allele (VI.3, 52 years; VI.6, 55 years) were asymptomatic except for slight metamorphopsia in patient VI.6, but funduscopy revealed drusen-like deposits within the central retina (Figs. 4E–H). Visual acuity in these three patients was 20/20 and none of them had dark adaptation problems.

### Family 3

Family members V.I.5, V.I.6, and V.I.7 have previously been shown to carry a heterozygous c.452A>G (p.Tyr174Cys) mutation in exon 5 of the TIMP3 gene. Only two female members (V.I.2, V.I.4) without retinal disease were homozygous for the wild-type allele. All investigated affected patients older than 70 years (V.1, V.4, V.6) had an onset of metamorphopsia and progressive loss of vision in the sixth decade. They all reported marked

### Table. Demographic Data and Clinical Findings of Included Patients With Sorsby Fundus Dystrophy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>VA, OD/OS</th>
<th>Age at Onset of Symptoms, y</th>
<th>First Symptoms</th>
<th>Age at Onset of Visual Loss, y</th>
<th>DA Problems</th>
<th>Phenotype (Funduscopy, FA, OCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II.1</td>
<td>61</td>
<td>HM; 20/63</td>
<td>47</td>
<td>DA</td>
<td>60</td>
<td>Yes</td>
<td>OU: midperipheral drusen-like deposits, central and midperipheral atrophy</td>
</tr>
<tr>
<td>II.4</td>
<td>78</td>
<td>CF; 20/80</td>
<td>57</td>
<td>Metamorphopsia</td>
<td>57</td>
<td>No</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
<tr>
<td>III.1</td>
<td>23</td>
<td>20/20</td>
<td>n/a</td>
<td>None</td>
<td>n/a</td>
<td>No</td>
<td>OU: normal</td>
</tr>
<tr>
<td>III.2</td>
<td>32</td>
<td>20/20</td>
<td>n/a</td>
<td>None</td>
<td>n/a</td>
<td>No</td>
<td>OU: normal</td>
</tr>
<tr>
<td>III.3</td>
<td>34</td>
<td>20/20</td>
<td>n/a</td>
<td>None</td>
<td>n/a</td>
<td>No</td>
<td>OU: normal</td>
</tr>
<tr>
<td>III.4</td>
<td>54</td>
<td>20/20</td>
<td>54</td>
<td>Metamorphopsia</td>
<td>54</td>
<td>No</td>
<td>OU: midperipheral drusen-like deposits OS: central CNV (treated)</td>
</tr>
<tr>
<td>III.8</td>
<td>56</td>
<td>20/20</td>
<td>56</td>
<td>Metamorphopsia</td>
<td>56</td>
<td>No</td>
<td>OU: central and midperipheral drusen-like deposits OS: eccentric PCV</td>
</tr>
<tr>
<td>Family 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.1</td>
<td>71</td>
<td>HM; CF</td>
<td>51</td>
<td>Visual loss</td>
<td>51</td>
<td>No</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
<tr>
<td>V.4</td>
<td>77</td>
<td>HM; 20/1000</td>
<td>58</td>
<td>DA</td>
<td>58</td>
<td>Yes</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
<tr>
<td>V.6</td>
<td>79</td>
<td>HM; CF</td>
<td>40</td>
<td>DA</td>
<td>48</td>
<td>Yes</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
<tr>
<td>VI.3</td>
<td>52</td>
<td>20/20</td>
<td>n/a</td>
<td>None</td>
<td>n/a</td>
<td>No</td>
<td>OU: central drusen-like deposits</td>
</tr>
<tr>
<td>VI.5</td>
<td>51</td>
<td>20/20</td>
<td>45</td>
<td>Metamorphopsia</td>
<td>45</td>
<td>No</td>
<td>OD: central CNV (treated)</td>
</tr>
<tr>
<td>VI.6</td>
<td>55</td>
<td>20/20</td>
<td>53</td>
<td>Metamorphopsia</td>
<td>n/a</td>
<td>No</td>
<td>OU: central drusen-like deposits</td>
</tr>
<tr>
<td>Family 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II.1</td>
<td>66</td>
<td>HM</td>
<td>30</td>
<td>DA</td>
<td>~30</td>
<td>Yes</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
<tr>
<td>III.3</td>
<td>58</td>
<td>20/100; 20/50</td>
<td>~40</td>
<td>DA</td>
<td>~45</td>
<td>Yes</td>
<td>OU: midperipheral atrophy, foveal region preserved, focal fibrotic changes</td>
</tr>
<tr>
<td>III.5</td>
<td>56</td>
<td>HM</td>
<td>31</td>
<td>DA</td>
<td>~30</td>
<td>Yes</td>
<td>OU: central and midperipheral atrophy, central fibrosis</td>
</tr>
</tbody>
</table>

CF: counting fingers; CNV, choroidal neovascularization; DA, dark adaptation problems; FA, fluorescein angiography; HM, hand movements; n/a, not applicable; OCT, optical coherence tomography; PCV, polypoidal choroidal neovascularization.

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**Novel Findings in Sorsby Fundus Dystrophy**

*IOVS* April 2015, Vol. 56, No. 4, 2666

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FIGURE 2. Clinical findings of family 1. Wide-field fundus (A, E, I, M), fundus color (B, F, J), fundus autofluorescence (C, G, K, N), and SD-OCT images (D, H, L, O) of representative members of family 1, illustrating increasing severity of clinical findings. Patient III.2 (32 years) showed normal findings (A–D). Patients III.8 (56 years) and II.1 (61 years) presented with drusen-like deposits along the vascular arcades, nasal to the optic disc and in the macular area (E–L). Patient II.1 additionally had central and midperipheral atrophic changes. Patient II.4 (78 years) showed widespread chorioretinal atrophy extending beyond the vascular arcades (M–O). Choroidal thickness in older patients was markedly reduced (L, O).
visual difficulties in dim light conditions later in the disease course and were now legally blind. On funduscopy, there was widespread chorioretinal atrophy with multilobulated borders and subretinal fibrosis at the posterior pole in these individuals (Figs. 4I–P).

**Family 3**

The index patient of family 3 (III.5; Fig. 5) presented with a widespread chorioretinal atrophy extending substantially beyond the vascular arcades (Figs. 6A–D). The family history was compatible with a dominant inheritance, but the very advanced disease stage was not immediately suggestive for SFD. Targeted NGS for a retinal dystrophy gene panel identified a heterozygous c.452A>G (p.Tyr174Cys) missense mutation in exon 5 of the *TIMP3* gene and a heterozygous c.1107G>A (p.Trp369*) nonsense mutation in the *RP1L1* gene. Segregation analysis of four family members revealed that the other genetically tested family member also affected by the retinal disease (II.1; see below) carried the *TIMP3* mutation, whereas the remaining (III.1, III.6, IV.2) were homozygous for the wild-type allele (Fig. 5). No other tested family member carried the *RP1L1* mutation.

Patient III.3 was not genetically tested but external ophthalmological examination revealed comparable changes as in patients II.1 and III.5 and he was therefore considered “affected.”

The age of the affected family members ranged from 56 to 66 years (Table; Fig. 6). Two of the three family members affected by the familial blindness had previously been diagnosed with “tapetoretinal degeneration” or “RP” (II.1, III.5). Impaired night vision and prolonged dark adaptation in the fourth decade of life were the first symptoms reported by all three affected family members, which were followed by progressive visual field defects. Funduscopy in the sixth (III.5) and seventh (II.1) decades of life revealed widespread central and peripheral chorioretinal atrophy ranging far beyond the vascular arcades with multilobulated borders and fibrotic changes at the posterior pole. Coarse pigmentations in the (mid-) periphery resembled bone spicule pigmentations (Fig. 6).

**Choroidal Thickness**

For measurement of choroidal thickness, four SFD patients were excluded due to refractive error greater than ± 3 D. The remaining 12 patients were compared with 64 healthy controls (range, 17–75 years). Young patients without visible fundus changes and patients with drusen-like deposits had subfoveal choroidal thicknesses within normal limits. Occurrence of CNV in the fellow eye had no impact on subfoveal choroidal thickness. Patients with chorioretinal atrophy showed a marked thinning of the choroid compared with controls (Supplementary Fig. S1).

**Indocyanine Green Late-Phase Angiography**

It has recently been suggested that pathological changes of Bruch’s membrane may be associated with reduced fundus fluorescence on late-phase indocyanine green-angiography (ICG-A). In healthy controls, there is a relatively uniform ICG fluorescence throughout the fundus at 30 to 40 minutes after intravenous dye injection, often with a slightly lower fluorescence centrally (Fig. 7A, Supplementary Figs. S2A–D).
Clinical findings of family 2. Wide-field (A, E, I, M) and conventional (B, F, J, N) fundus color images, fundus autofluorescence (C, G, K, O), and SD-OCT (D, H, L, P) images of representative members of family 2, illustrating increasing severity of clinical findings. Patient VI.5 (51 years) revealed normal findings except for recurrent unilateral subfoveolar neovascular activity (A–D). Patient VI.6 (55 years) showed central drusen-like deposits (E–H). The peripheral retina was unremarkable. Patients V.1 (71 years) and V.6 (78 years) showed widespread central and peripheral chorioretinal atrophy with focal subretinal fibrosis (I–P). The choroid was significantly thinned in areas of atrophy (L, P).
Indocyanine green–angiography late-phase images were recorded in nine patients carrying one of the three novel *TIMP3* mutations who had no marked chorioretinal atrophy or subretinal fibrosis. In three asymptomatic carriers of a *TIMP3* mutation without visible fundus changes (III.1, III.2, and III.3 of family 1), as well as in patient VI.5 of family 2 with unilateral CNV and normal findings in the contralateral eye, late-phase ICG-A showed a distinct central hypofluorescence with multilobulated borders (Fig. 7B, Supplementary Figs. S2E–G). All other available imaging modalities, including early- and late-phase fluorescein angiography, early ICG-A frames, SD-OCT, fundus AF, or NIR reflectance imaging showed normal findings. Patients with visible fundus changes (e.g., drusen-like deposits) showed a marked and more widespread hypofluorescence on

![Pedigree of family 3.](image)

**Figure 5.** Pedigree of family 3.

- ○ unaffected
- ● affected
- # Ophthalmological examination only
- + Not examined - Affected/unaffected according to family history

![Clinical findings of family 3.](image)

**Figure 6.** Clinical findings of family 3. Wide-field fundus (A, E), fundus color (B, F), fundus autofluorescence (C, G), and SD-OCT (D, H) images of patient III.5 (A-D) and patient II.1 (E-H) showing widespread central and peripheral chorioretinal atrophy with areas of pigment clumping and subretinal fibrosis. The choroid was markedly thinned (D, H).
late-phase ICG-A images at the posterior pole (Fig. 7C, Supplementary Figs. S2H–N).

**Progression of Chorioretinal Atrophy**

One patient (II.1, family 1, 61 years) developed chorioretinal atrophy without any signs for prior development or current presence of a CNV. Growth of the atrophic area was followed over a period of 26 months on fundus AF images (Fig. 8). Enlargement rates of the total area of atrophy were 7.0 \( \mu \text{m}^2 \) per year in the right eye, and 2.44 \( \mu \text{m}^2 \) per year in the left eye, respectively. Within the same 26 months interval, VA dropped from 20/40 in both eyes to 20/1000 in the right and 20/63 in the left eye.

**Treatment of CNV and PCV**

Two previously asymptomatic patients (III.4, 54 years, Figs. 9A–E; III.8, 56 years, Figs. 9F–J; both family 1) who were counseled about early symptoms for CNV presented with relatively sudden onset of metamorphopsia and mild drop in VA to 20/25 and 20/32, respectively. Examination revealed a small paracentral CNV with a cystoid macular edema and fluorescein angiographic leakage (Figs. 9A–C, F–H). One (patient III.4, Figs. 9D, 9E) or two (patient III.8; 4-week interval between treatments, Figs. 9I, 9J) intravitreal bevacizumab injections resulted in marked morphological improvement on SD-OCT, regression of metamorphopsia, and increase of VA to 20/20. There was no recurrent CNV activity within a 12-month follow-up period in patient III.4 (Fig. 9E), whereas patient III.8 was repeatedly treated with intravitreal bevacizumab injections for recurrent CNV activity for 8 months. Visual acuity was maintained at 20/20.

Another patient (IV.5, 51 years, family 2) has had recurrent CNV activity for 6 years. He had initially been treated with photodynamic therapy combined with intravitreal bevacizumab; however, signs of CNV activity recurred.\(^{19}\) He had received a total of 35 intravitreal injections of bevacizumab to date based on monthly OCT controls and recurrent symptoms (drop in VA and/or metamorphopsia). Under this pro re nata treatment regimen, foveal morphology largely remained intact and VA has remained at 20/20 (Figs. 9K–O).

Patient III.8 (family 1) also developed an asymptomatic PCV with subretinal hemorrhage nasal to the optic disc in the other eye that progressively enlarged during an initial observation over 2 months. Again, two intravitreal bevacizumab injections with a 4-week interval led to regression of the PCV (Figs. 9P–S).

**Treatment of Night Blindness With Vitamin A**

One subject (II.1, 61 years, family 1, Figs. 2I–L, Fig. 8) without widespread chorioretinal atrophy at time of initial investigation had marked difficulties in dim light conditions. High-dose vitamin A supplementation (15,000 IU per day) for 1 month as an off-label therapeutic had no effect on her visual symptoms.
Due to elevated liver enzymes, the dosage was not increased further and treatment was stopped.

**DISCUSSION**

Here we report a comprehensive clinical characterization of three German families affected by SFD carrying three different missense mutations in the *TIMP3* gene.

**Mutation Analysis**

The novel point mutations c.452A>G and c.530A>G and the previously described c.545A>G mutation in exon 5 of the *TIMP3* gene are segregating with the disease in the respective families and are predicted to introduce a tyrosine-to-cysteine substitution (p.Tyr174Cys, p.Tyr177Cys, and p.Tyr182Cys) in the C-terminal domain of the TIMP3 protein. This results in an odd number of cysteines in the mutant molecules, similar to the effect of most other SFD-related *TIMP3* mutations (Fig. 10A).2,21–30 Taken together, these data strongly support the causality of these mutations.

Gene panel diagnostics revealed an additional heterozygous nonsense mutation in the *RP1L1* gene (p.Trp369*) in the index patient of family 3 that did not cosegregate with the SFD phenotype. Most reported pathogenic *RP1L1* alleles are
heterozygous missense mutations, associated with autosomal-dominant occult macular dystrophy. A homozygous frame-shift mutation has been reported in a family with autosomal-recessive RP, and p.Trp369* may thus represent a recessive allele with no obvious effect on the course of the retinal disease in patient III.5 of family 3. Compatible with our interpretation, lack of cosegregation has previously been shown for a heterozygous RP1L1 nonsense mutation in a family with autosomal-dominant RP whose cause of disease had remained elusive after targeted NGS of known retinal dystrophy genes.

Phenotype and Differential Diagnosis

Intensive phenotyping of young asymptomatic TIMP3 mutation carriers showed no fundus changes on noninvasive fundus examination, including funduscopy, SD-OCT, and fundus AF consistent with previous reports. Herein, we describe reduced late-phase fluorescence on ICG-A as a novel early sign for SFD, which was observed up to approximately 20 years before the age at which first symptoms usually occurred in other affected family members. Similarities with observations in patients with pseudoxanthoma elasticum (PXE), a disease with a predominant pathology of Bruch’s membrane, suggest that reduced late-phase fluorescence on ICG-A could be a common sign in patients with pathological changes of Bruch’s membrane. The reduced ICG fluorescence might be due to decreased staining or permeability of Bruch’s membrane and/or subclinical damage of retinal pigment epithelium cells that normally actively take up ICG dye. In later disease stages, reduced ICG fluorescence is more pronounced and widespread.

Because SFD has a high penetrance later in life, predictive genetic testing is a means to distinguish those who will be affected from those who may be reassured that they will not develop the disease. Similar information may derive from late-phase ICG imaging many years before manifestation of any other signs or symptoms. Such predictive imaging may have specific implications on patient counseling similar to predictive genetic testing, and thus goes beyond the standard patient education on the risks of the invasive diagnostic imaging procedure. Among others, this may include information on the current lack of causative treatments, the potential psychological burden of being at high risk to loose vision, the risk of transmitting the disease to children, and potential consequences on insurability. However, further studies, including long-term prospective investigations, are needed to investigate the significance of late-phase ICG-A for early diagnosis of SFD.

The late manifestation of SFD was consistent in all three families; that is, a widespread chorioretinal atrophy extending beyond the vascular arcades with variable degree of fibrosis at the posterior pole, which is in agreement with former reports. However, the age of patients with a late phenotype differed between families: carriers of the p.Tyr174Cys mutation were at least 20 years younger than those with a p.Tyr182Cys and p.Tyr177Cys mutation. Accordingly, the earliest symptoms occurred approximately 20 years earlier in the family with the p.Tyr174Cys mutation.

Correlations between certain types of TIMP3 mutations and disease onset have previously been reported (Fig. 10B). Because the molecular pathomechanisms underlying SFD are largely unknown, the reasons for the different onset of disease manifestations and severity remain unclear. Mutation-specific factors, genetic interactions, and/or environmental factors might contribute to such differences. There is a strong similarity between the clinical phenotypes of AMD and SFD, suggesting that TIMP3 risk haplotypes that confer susceptibility to AMD might also contribute to phenotypic variability in SFD.

Although the end point of SFD appears relatively uniform, visual symptoms and morphological manifestation during the course of the disease may vary considerably between and within families. Dark adaptation may be compromised early or only after widespread photoreceptor damage in later disease stages, and there is a variable degree of drusen-like deposits. Choroidal neovascularization may develop at any time point in the disease course; that is, before the occurrence of any other funduscopically visible changes (patient VI.5, family...
laser photocoagulation, we treated an asymptomatic eccentric progressing subtype: 3.02 mm$^2$/y (Fastest progressing subtype: 0.45–1.79 mm$^2$/y$^{50}$) or Stargardt disease (O.94 ± 2.08).[53–1.58 mm$^2$/y$^{54}$]. Notably, AF is not consistently increased preceding growth of atrophy in SFD, suggesting that lipofuscin accumulation may not play a role in GA progression in SFD. Rather, SFD-related pathology might result in compromised RPE cell physiology and, eventually, cell death.

Consistent with reports on PXE,[55] late SFD disease stages were also associated with reduced choroidal thickness, suggesting similar pathogenetic mechanisms affecting the choroid–Bruch’s membrane complex.

The phenotypic similarity of SFD with AMD includes drusen-like deposits, CNV, and GA. However, diagnostic differentiation between the two diseases is particularly important because SFD patients are at high risk of also loosing substantial amounts of peripheral visual function, which affects counseling and prognostication. Dominant inheritance with functional deficits beyond those expected in AMD may be helpful in distinguishing SFD. Furthermore, late disease stages may be misinterpreted as late-stage RP or cone-rod dystrophies. Thus, for differentiation, examination of additional family members and a detailed (family) history may be helpful and molecular genetic diagnostics can confirm a suspected diagnosis of SFD. In one of the families reported herein, multigene panel diagnostics indicated SFD and specified the clinical diagnosis.

Therapeutic Approaches

Consistent with previous reports,[56–59] monotherapy with intravitreal injections of VEGF-inhibitors was beneficial in all three eyes treated for fovea-involving CNV. Because extrfoveal CNV has been shown to extend subfoveally, resulting in substantial visual decline if left untreated,[10] but also after laser photocoagulation,[60] we treated an asymptomatic eccentric PCV also. Subretinal hemorrhage and fluid was reversed without further growth of the lesion. In all treated eyes, VA was stabilized or improved and was 20/20 at last follow-up. This favorable functional outcome likely was achieved because treatment was initiated early on. Therefore, screening of family members to identify individuals at risk for developing CNV, regular examination even of asymptomatic SFD patients, and detailed counseling about early symptoms appears prudent.

Treatment with Vitamin A at 50,000 IU per day was shown to improve night blindness in early disease stages of SFD in a treatment trial over 4 weeks.[25] One patient with early GA was treated with 15,000 IU per day, a dosage shown to be safe for long-term usage.[61] The lack of functional improvement in this patient may for instance be due to an irreversible disease stage or a vitamin A dosage too low to achieve substantial effects.

Conclusion

This study expands the spectrum of mutations in the TIMP3 gene causing SFD and identifies a novel imaging tool for early SFD diagnosis. The detailed phenotyping of SFD family members reveals new insights into the pathogenesis of SFD and into the complex interactions among choroid, RPE, and Bruch’s membrane.

Acknowledgments

Supported by the ProRetina Deutschland, Aachen, Germany, and the BONFOR research program of the University of Bonn, Bonn, Germany. The Department of Ophthalmology, University of Bonn, receives research support from Heidelberg Engineering. No other conflicting relationships were reported. The sponsor or funding organization had no role in the design or conduct of this research. No sponsor or funding agency had any involvement in the design, collection, analysis, and interpretation of the data; manuscript writing; and the decision to submit the manuscript for publication.

None of the authors has a proprietary interest.

Disclosure: M. Gliem, None; P.L. Müller, None; E. Mangold, None; F.G. Holz, Heidelberg Engineering (C); H.J. Bolz, Bioscientia (E); H. Stöhr, None; B.H.F. Weber, None; P. Charbel Issa, None.

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