# A novel *TIMP3* mutation associated with a retinitis pigmentosa-like phenotype

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**Abstract:**

Sorsby Fundus Dystrophy is an inherited macular degeneration caused by pathogenic variants in the *TIMP3* gene. In this study we describe a father and son initially diagnosed with retinitis pigmentosa of unknown genetic origin. More recent genetic testing of the patients, identified a novel c.410A>G; p.Tyr137Cys variant of uncertain clinical significance in the Tissue Inhibitor of Metalloproteinase-3 (*TIMP3*) gene. The atypical clinical findings led us to compare the theoretical molecular effects of this variant on the TIMP3 protein structure and interactions with other proteins using homology modeling and machine learning predictions.

**Introduction:**

Sorsby Fundus Dystrophy is a rare, early onset autosomal dominant retinal dystrophy first described in 1949.[1] Symptoms generally first appear in the third to fourth decade of life as central visual blurring, metamorphopsia, central scotomas, nyctalopia and/or reduced color vision, with progressive worsening over time.[2-4] The slow progression of vision loss is generally a consequence of progressive geographic atrophy while a rapid onset of central blurring vision suggests choroidal neovascularization that develops in a majority of patients with this disease[5-8]. Typical clinical exam findings include drusen-like deposits between the basal lamina of the retinal pigment epithelium (RPE) and inner collagenous layer of Bruch's membrane or reticular pseudo drusen deposits between the photoreceptor outersegments and the apical surface of the RPE.[9,10] Histopathological examination of SFD eyes show thick, widespread, confluent lipid-enriched amorphous deposits in the basal lamina of RPE.[9,11-13] Choroidal neovascularization is a common occurrence in these patients and can lead to intraretinal edema, subretinal fluid accumulation as well as subretinal hemorrhage.[14,15] Chorioretinal atrophy can often be widespread late in the disease. The presentation of SFD is similar to age-related macular degeneration (AMD), Malattia Leventinese (familial dominat drusen,or Doyne honeycomb retinal dystrophy), pattern dystrophy and Best disease. SFD is caused by pathogenic variants in the *TIMP3* (Tissue Inhibitor of Metalloproteinase-3) gene that encodes a protein that inhibits enzymes that degrade matrix components.[16,17] Simulation of retinitis pigmentosa has not been emphasized and could be a source of inaccurate clinical diagnosis as in the present report.

**Case 1 (Proband):**

This is a 74 year-old Caucasian male patient with a clinical diagnosis of retinitis pigmentosa. He is of Dutch ancestry on his maternal side, and Northern Irish and English on his paternal side. He first became symptomatic at the age of 40 years with complaints of a blind spot and glare. He was evaluated by two different ophthalmologists with expertise in inherited retinal dystrophies, and was diagnosed with retinitis pigmentosa based on clinical examination. His vision loss has continued to progress with nyctolopia developing in his 50's. He had bilateral cataract surgery at 60. His review of systems was negative for features consistent with a syndromic retinal dystrophy.  His medical history was significant for eczema, hypertension, age-related hearing loss, a hemorrhagic stroke at the age of 71, hepatic steatosis, and a renal tumor newly diagnosed at his current age of 74.

On his most recent eye exam (at age of 74), his visual acuity was hand motion in both eyes. His intraocular pressures were normal. Slit lamp examination revealed anterior chamber intraocular lenses in place in both eyes. His fundus examination displayed drusen and RPE changes in the macula with normal appearing vessels (no hemorrhages or exudates). Bone spicule pigmentation with extensive RPE dropout was observed in the fundus periphery of both eyes (Figure 1a).

His family history  is significant for other family members also diagnosed with retinitis pigmentosa. They include his son, his sister, his brother, his mother, his maternal grandmother, and a maternal great-uncle, consistent with autosomal dominat inheritance pattern. His sister is reportedly being treated for choroidal neovascularization at the age of 73. His brother, 67 years of age, also has glaucoma.

The patient had prior limited genetic testing in 2012 with no pathogenic variants detected in the following genes: *RHO, PRPH2/RDS, RP1, IMPDH1, PRPF31, PRPF, PRPF8, NR2E3, SNRNP200, TOPORS, KLHL7, RPGR,* and *RP2.*

**Case 2 (Proband's son):**

This is a 49 year old Caucasian male who was diagnosed with retinitis pigmentosa based on eye examination at the age of 47 and early symptoms of nyctalopia. He was diagnosed with pigment dispersion syndrome and macular degeneration of both eyes and continues to take AREDS vitamins, Omega 3's and Lutein. He reports normal peripheral vision. He has no systemic features consistent with a syndromic retinal dystrophy. His medical history is significant for congenital cataract of his left eye, eczema, and a deep vein thrombosis at age 49.

His most recent eye exam (at age 49) measured his visual acuity to be 20/20 OD and 20/400 OS. His visual fields were full to confrontation in both eyes. Intraocular pressures were normal. He had a nuclear sclerotic cataract in both eyes, and a dense posterior subcapsular cataract in his left eye. His dilated fundus exam showed drusen and RPE changes, normal vessels, and granular RPE changes in the periphery (Figure 1b).

**Results**

Genetic testing was repeated using an 856 gene retinal dystrophy panel via next-generation sequencing technology at a CLIA-certified laboratory. A sample from the proband as well as both his affected son (case 2) and unaffected son were also tested. The father and the affected son were heterozygous for a novel c.410A>G; p.Tyr137Cys variant of uncertain significance in the *TIMP3* gene. The proband's unaffected son did not carry this variant. This

variant had not previously been reported as pathogenic or as benign. It had not been detected in large population cohorts and results in a non-conserved amino acid substitution, which is likely to impact secondary and tertiary protein structure.

Protein Modeling of Y137CTIMP3: Due to the absence of a crystal structure covering the relevant protein domains of TIMP3, we generated a homology model for the full-length TIMP3 protein with the software SWISS-MODEL[18,19], using a crystal structure for the ortholog TIMP2 as template (PDB: 1GXD, chain D). Figure 3a is a surface representation of this homology model with residue 137 highlighted in red, created using the software Chimera[20-22]. The residue is exposed at the surface, with the mutant residue being smaller and more hydrophobic than the wild-type residue, suggesting that the mutation may result in the loss of external interactions with other functional molecules.

We also used the homology model to predict potential effects of the mutation on protein stability. Among three prediction approaches (mCSM, SDM and DUET) none predicted a destabilizing effect, and all predicted a slight but probably not significant stabilizing effect (with less than 0.5 kcal/mol $\Delta\Delta G$, i.e. suggesting there is no significant change in protein stability). Unless the mutation disrupts protein stability via the disruption of disulfide bonds (see discussion below), this further suggests that the mutation affects interactions with other biomolecules, rather than the protein stability. Since the residue is located in a region of the protein, which mediates the interaction with the protein EFEMP1 (residues 105 - 188, see section "Family & Domains" on UniProt: https://www.uniprot.org/uniprot/P35625), this could be a potential interaction weakened or lost due the mutation.[23]

**Discussion**

Since many of the SFD-associated mutations introduce a new cysteine residue into the TIMP3 protein, one hypothesis that has been out forward is that these mutations could affect disulfide bridges. For some of the mutated residues such as S38C, the disruption of disulfide bonds has been confirmed [24-31] whereas other reports suggest no effect[32]. However, the exact structural effect of altered disulfide bonding on TIMP3 function (MMP inhibition, apoptosis, anti-angiogenesis), glycosylation status or binding to (pro)MMPs, VEGFR2 or EFEMP1 is unknown and is difficult to predict, the question whether this mutation results in a gain of function or loss of function phenotype remains open.

While altered disulfide bonds can affect both the global and local protein structure, and predictions on the precise effects are impossible, non-synonymous mutations in residues exposed on the protein surface would at least be expected to affect interactions with other biomolecules known to the corresponding protein domain.

Figure 3b depicts the location of the majority of TIMP3 mutations (highlighted in red) associated with SFD that result in amino acid substitutions: p.(Ser38Cys)[33], p.(Glu162Lys) [29], p.(Tyr151Cys) [4], p.(Asp167Asn) [34], p.(Tyr177Cys) [4], p.(Ser179Cys) [35], p.(His181Arg) [36], p.(Tyr182Cys) [4], p.(Gly189Cys) [3], p.(Gly190Cys) [37]. p.(Tyr191Cys) [38], p.(Ser193Cys) [39], p.(Tyr195Cys) [40], and p.(Ser204Cys) [38]. Most of them are exposed on the surface, and most of them are located in a region between the positions 160 to 200. The residue at position 137 lies on the same side of the protein, but not adjacent to most of the other mutated residues.

The protein region that mediates the interaction with EFEMP1 is annotated for the positions 105 to 188, according to UniProt[41,42] (Figure 3c, highlighted in green). This domain only partially

overlaps with the region that contains most of the mutated residues, but most of the other

mutated residues, between positions 189 to 195, are still located in close proximity to the

EFEMP1 domain and could therefore affect a molecular interaction in this region via altered

steric or electrostatic properties. Overall, in-silico analyses, including homology modeling and

evolutionary conservation support a deleterious effect of this novel variant.

The present cases illustrate the importance of genetic testing for the accurate diagnosis of

patients diagnosed with an inherited retinal disease.  The phenotype of these patients, at least at

one point in time simulated that of retinitis pigmentosa. Prior studies have demonstrated the

importance of monitoring SFD patients for choroidal neovascularization and the use of

intravitreal anti-VEGF therapy as effective treatment.[4] Other studies have also investigated the

use of vitamin A 50,000 IU per day as a treatment to improve night blindness in early disease

stages of SFD. [37,43] We also emphasize the importance of genetic counseling and further

investigation into variants of uncertain clinical significance, especially when detected in a gene

that is clinically consistent with the patient's phenotype.

**Figure Legends**

Figure 1: Fundus photographs of a) Proband and b) Proband's son . Fundus of the proband (a)
displays drusen and RPE changes in the macula with normal appearing vessels (no hemorrhages
or exudates). Bone spicule pigmentation with extensive RPE dropout can be seen in the fundus
periphery of both eyes. Fundus of the Proband's son (b) shows drusen and RPE changes, normal
vessels, and granular RPE changes in the periphery.

Figure 2: Pedigree chart of Proband's family. Squares and circles depict males and females
respectively. Filled symbols represent affected individuals, and symbols with lines through them
depict deceased individuals.

Figure 3: Molecular surface representations of the homology model for TIMP3, derived from the template crystal structure for the ortholog TIMP2 (PDB: 1GXD, chain D). a) Molecular surface with internal ribbon representation of the secondary structure, and the residue 137 highlighted in red; b) Molecular surface representation highlighting the location of the majority of known TIMP3 mutations (amino acid substitutions) in red; c) Molecular surface representation highlighting the protein domain that mediates the protein interaction with EFEMP1 (positions 105 to 188, green color).

**Declaration of interest**

**Funding**

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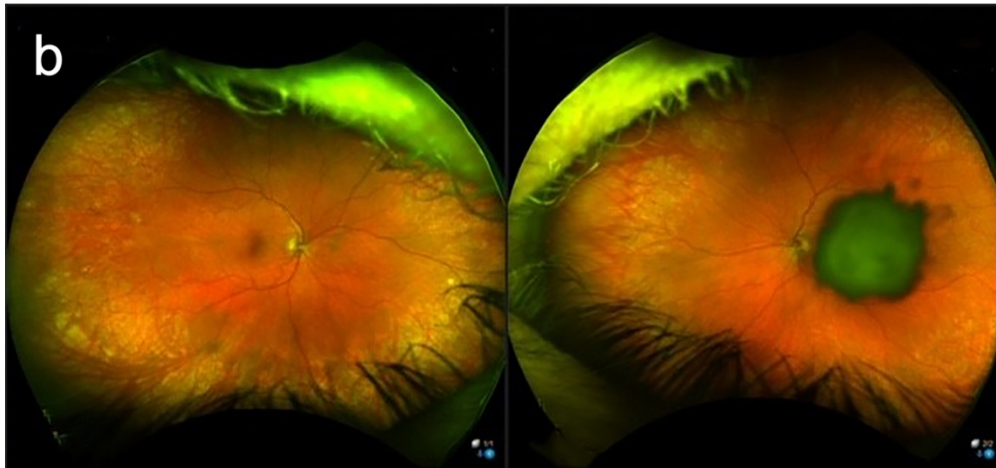
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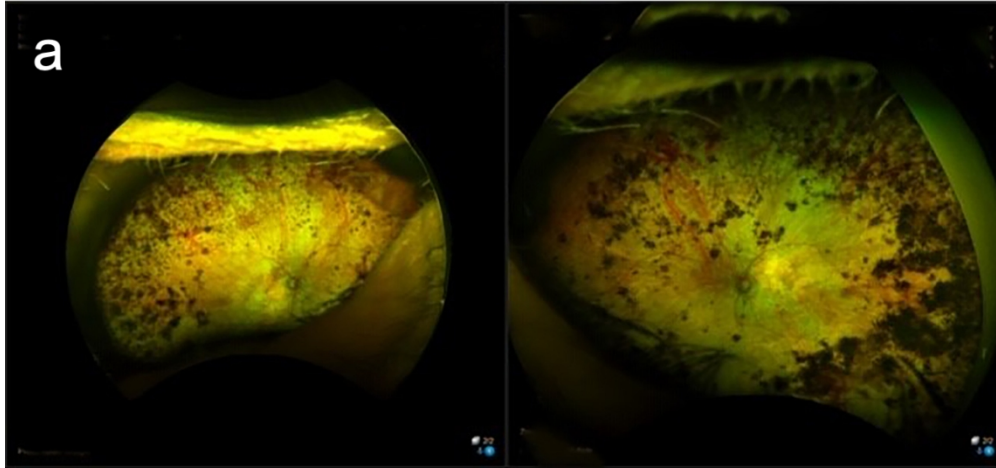


# Proband's Son



Figure 1: Fundus photographs of a) Proband and b) Proband's son . Fundus of the proband (a) displays drusen and RPE changes in the macula with normal appearing vessels (no hemorrhages or exudates). Bone spicule pigmentation with extensive RPE dropout can be seen in the fundus periphery of both eyes. Fundus of the Proband's son (b) shows drusen and RPE changes, normal vessels, and granular RPE changes in the periphery.
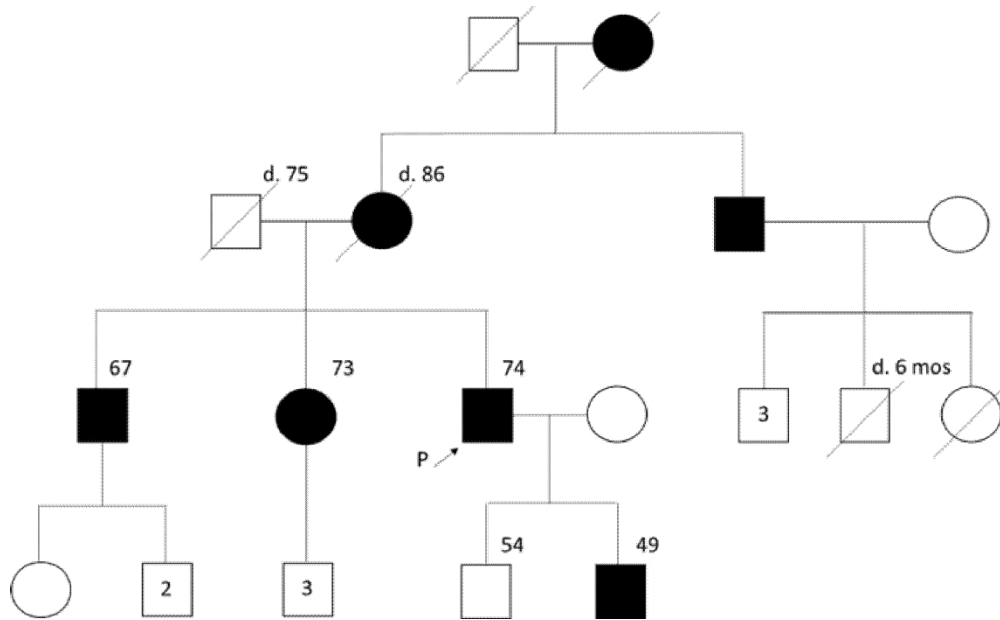
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# Pedigree



Figure 2: Pedigree chart of Proband's family. Squares and circles depict males and females respectively. Filled symbols represent affected individuals, and symbols with lines through them depict deceased individuals.
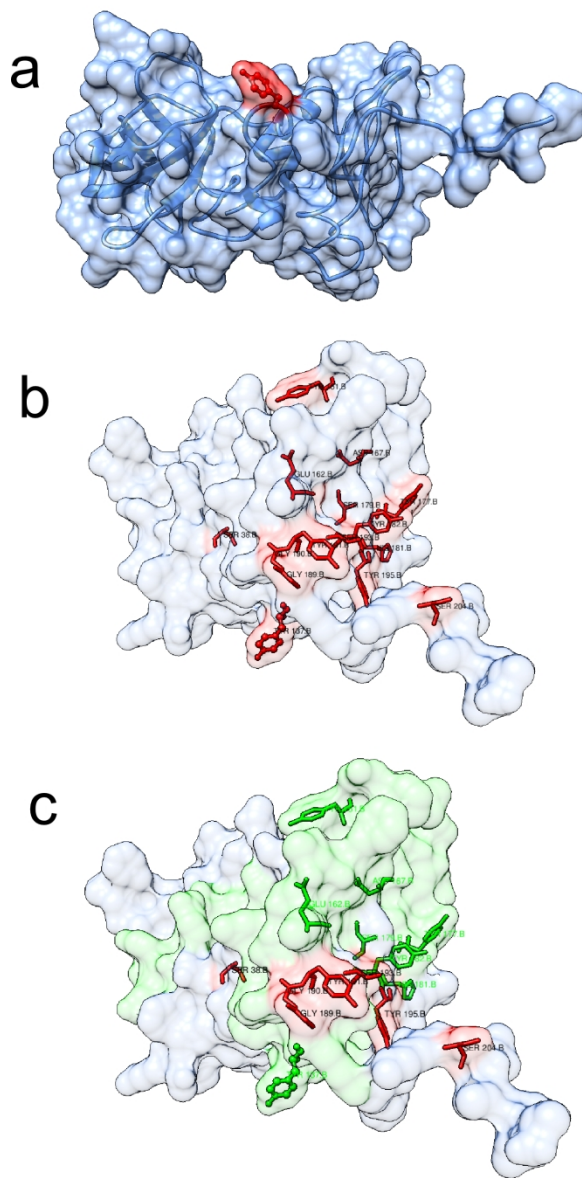
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Figure 3: Molecular surface representations of the homology model for TIMP3, derived from the template crystal structure for the ortholog TIMP2 (PDB: 1GXD, chain D). a) Molecular surface with internal ribbon representation of the secondary structure, and the residue 137 highlighted in red; b) Molecular surface representation highlighting the location of the majority of known TIMP3 mutations (amino acid substitutions) in red; c) Molecular surface representation highlighting the protein domain that mediates the protein interaction with EFEMP1 (positions 105 to 188, green color).

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