Review: Mechanisms of TIMP-3 accumulation and pathogenesis in Sorsby fundus dystrophy

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Sorsby fundus dystrophy (SFD) is a rare, inherited form of macular degeneration caused by mutations in the gene encoding tissue inhibitor of metalloproteinases 3 (TIMP-3). There are 21 mutations currently associated with SFD, with some variants (e.g., Ser179Cys, Tyr191Cys, and Ser204Cys) having been studied much more than others. We review what is currently known about the identified SFD variants in terms of their dimerization, metalloproteinase inhibition, and impact on angiogenesis, with a focus on disparities between reports and areas requiring further study. We also explore the potential molecular mechanisms leading to the accumulation of extracellular TIMP-3 in SFD and consider how accumulated TIMP-3 causes macular damage. Recent reports have identified extraocular pathologies in a small number of SFD patients. We discuss these intriguing findings and consider the apparent discrepancy between the widespread expression of TIMP-3 and the primarily retinal manifestations of SFD. The potential benefits of novel experimental approaches (e.g., metabolomics and stem cell models) in terms of investigating SFD pathology are presented. The review thus highlights gaps in our current molecular understanding of SFD and suggests ways to support the development of novel therapies.

Sorsby fundus dystrophy: Sorsby fundus dystrophy (SFD) is an autosomal dominant macular dystrophy that progresses to bilateral vision loss. It was originally identified by Sorsby and Mason, who described multiple families with members presenting with central vision loss in their early 40s, with a similar fundal presentation to that observed in patients with age-related macular degeneration (AMD) [1]. The prevalence of SFD is estimated to be 1 in 220,000 [2], with onset typically occurring between 40 and 60 years of age, although this depends on the particular mutation involved (Table 1).

Sorsby fundus dystrophy is caused by mutations in the gene encoding tissue inhibitor of metalloproteinases 3 (TIMP-3) [3], an endogenous inhibitor of metalloproteinases. To date, 21 mutations associated with SFD have been identified (Figure 1, Table 1). The mutations were originally named based on residue numbering, which excluded the 23-amino-acid signal peptide of TIMP-3; however, naming now complies with the Human Genome Variation Society nomenclature and includes the signal peptide. The first identified SFD mutation was Ser204Cys [3], and this remains the most studied mutation. The majority of SFD mutations are in the C-terminal region of TIMP-3 and involve substitution to a cysteine residue, or the generation of an unpaired cysteine residue via the truncation (e.g., Glu162STOP) or mutation of a previously paired cysteine (e.g., Cys24Arg). A few SFD mutations do not conform to this pattern. For example, the Leu10His and Gly12Arg mutations occur in the TIMP-3 signal peptide [4], and the Cys24Arg and Ser38Cys mutations occur in the N-terminal domain. Also, not all mutations result in the generation of an unpaired cysteine residue (e.g., Leu10His, Gly12Arg, Glu162Lys, and His181Arg).

Mutations in TIMP-3 have also been identified in other macular dystrophies [5,6]. DeBenedictis and colleagues present a case study of a father and son who both received a clinical diagnosis of retinitis pigmentosa, with a strong family history of this disease [5]. Upon genetic testing, it was found that these patients had a Tyr137Cys variant of TIMP-3. Moreover, Warwick and colleagues reported a 56-year-old male, a 64-year-old male, and a 61-year-old female who had been diagnosed with neovascular age-related macular degeneration (AMD) but were found to carry a Cys113Gly mutation in the TIMP-3 gene [6]. These studies highlight the importance of genetic testing, as physical symptoms such as night blindness and neovascularization are not exclusive to a particular macular dystrophy and may result in the underdiagnosis of SFD.

Sorsby fundus dystrophy shares clinical and pathological characteristics with AMD [7], with a key hallmark of both being the presence of drusen deposits in the retinal extracellular matrix (ECM), termed Bruch's membrane (BrM). In SFD and AMD, the drusen contains large amounts of lipid and accumulated protein, and in the case of SFD, this accumulated protein is mutant TIMP-3 [8,9]. Interestingly, the accumulation of TIMP-3 has also been observed in AMD and retinitis pigmentosa [9-11], and a binding partner of

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Table 1. Tissue inhibitor of metalloproteinases 3 (TIMP-3) variants and their ability to dimerise.

Mutation	Age of onset (years)	Original publication	Dimerization and/or multimerization
Leu10His & Gly12Arg	Early (2nd Decade)	[4]	Not clear. Dimeric species shown in cytoplasmic, membrane, nuclear extracts and ECM (for Gly12Arg) but no difference compared to wild-type TIMP-3 in HEK293FT cells [4].
Cys24Arg	Early	[117]	Not known.
Ser38Cys	40-50	[118]	Yes, in ARPE-19 cells [79] but only monomeric in patient fibroblasts [119].
Intron 4/ Exon 5 Splice Site	50-80	[120]	Yes, in ARPE-19 cells [79].
Ser149Cys	30-50	[121]	Not known.
Tyr151Cys	Early	[117]	Yes, in ARPE-19 cells [79].
Glu162Lys	30-50	[80]	Yes, in ARPE-19 cells [80].
Glu162STOP	20-40	[75]	Yes, in transfected insect cells [74] and ARPE-19 cells [70]. Dimer molecular weight is ~26 kDa, which is similar to wild-type TIMP-3.
Tyr174Cys	30-40	[122]	Not known.
Tyr177Cys	50-60	[122]	Yes, in ARPE-19 cells [79].
Ser179Cys	20-30	[123]	No in ARPE-19 cells [69]. Yes, in ARPE-19 cells [70], BHK cells [77], COS-7 cells [75], porcine aortic. endothelial cells [81] mouse RPE, and human fibroblasts. Good in vivo evidence of dimer formation in mice [76].
His181Arg	50-70	[97]	Yes, in ARPE-19 cells [79] and skin fibroblasts (not explicitly concluded but 39-50 kDa species observed) [97].
Tyr182Cys	50-60	[124]	Yes, in ARPE-19 cells [79].
Gly189Cys	20-40	[125]	Yes, in COS-7 cells [75].
Gly190Cys	30-40	[17]	Yes, in BHK cells [77].
Tyr191Cys	30-40	[126]	Yes, BHK cells [77], porcine aortic endothelial cells [81].
Ser193Cys	30-40	[127]	Not known.
Tyr195Cys	20-40	[128]	Not known.
Trp198Cys	Early	[117]	Not known.
Ser204Cys	30-60	[3]	Yes, COS-7 cells [33], BHK cells [77], patient-derived fibroblasts [76], patient-derived fibroblasts [74], ARPE-19 cells [70], hiPSC-RPE cells [73], porcine aortic endothelial cells [81].

TIMP-3, fibulin-3 [12], accumulates in Malattia Leventinese [13], potentially suggesting role for TIMP-3 in this degenerative macular disease [2]. In the retina, the cells expressing TIMP-3 include the retinal pigment epithelial (RPE) cells [14] and the choroidal endothelial cells [15]; both cell types enclose and are adjacent to BrM (Figure 2). Thus far, the overwhelming majority of research has been conducted in RPE cells; however, choroidal endothelial cells are also an important source of TIMP-3, so the responses of these cells to SFD TIMP-3 warrant further investigation. Novel human choroidal endothelial cell lines have been developed [16] and may be a suitable model for such studies.

Advanced SFD manifests in two forms: (i) acute visual loss due to choroidal neovascularization (CNV), in which new leaky vessels develop from the choroid and invade the outer retina, and (ii) progressive loss of central vision because of RPE-cell atrophy and the death of the photoreceptors that RPE cells support. Vitamin A was initially used to treat a common symptom of SFD, night blindness [17]; however, not all patients presented with this symptom, and the treatment did not address or target the underlying pathology. Prior to the development of anti-vascular endothelial growth factor (VEGF) therapy for the treatment of CNV, there was not a positive outlook for SFD patients with CNV. Photodynamic therapy and argon and krypton laser photocoagulation were available options for treating retinal neovascularization, but these approaches had limited effectiveness and high rates of CNV recurrence [18-20]. The advent of anti-VEGF therapy in the 2000s drastically improved the visual prognosis for patients with CNV [21-24]. For RPE-cell atrophy, there are

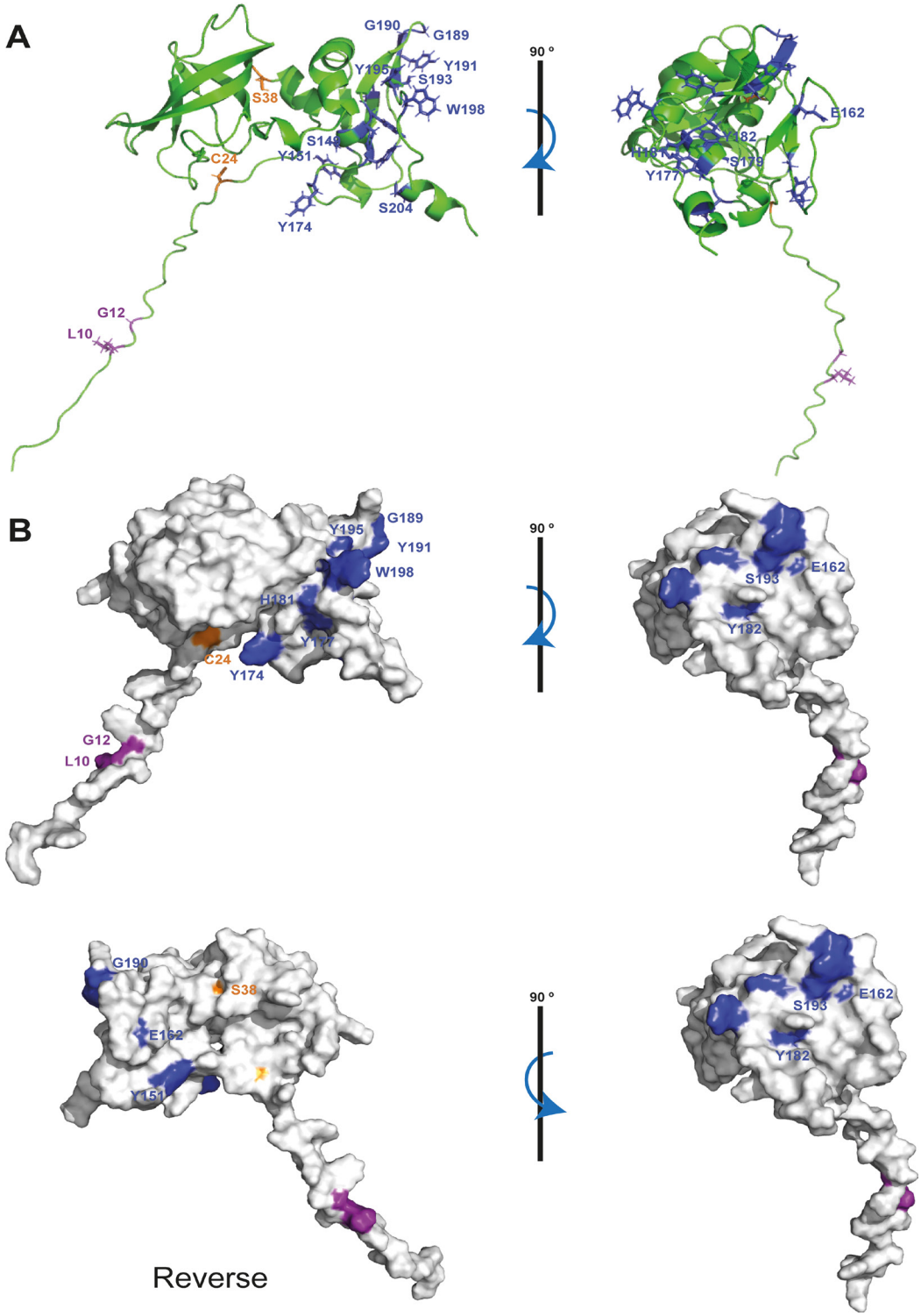

Figure 1. Predicted tertiary structure of tissue inhibitor of metalloproteinases 3 (TIMP-3), showing residues mutated in Sorsby fundus dystrophy (SFD). A model of human TIMP-3 was generated using AlphaFold and annotated using PyMOL. Residues located in the signal peptide are indicated in purple, residues in the N-terminal domain are indicated in orange, and those in the C-terminal region are indicated in blue. **(A)** depicts the ribbon structure of TIMP-3, and **(B)** depicts the surface of TIMP-3, showing surface residues mutated in SFD.

currently no treatments or cure; however, there are ongoing clinical trials that aim to target the enzymes involved in the phototransduction cascade of the visual cycle or utilize neuroprotective agents to protect neuro-retinal tissue against cellular injury. Cell-based approaches and gene therapy are also being screened [25,26]. Furthermore, in February 2023, the complement-C3 inhibitor pegcetacoplan was approved by the Food and Drug Administration in the US for the treatment of dry AMD. This treatment is not yet available in other countries but is undergoing trials [25]. Given that dry AMD shares remarkable similarities with RPE-cell atrophy [7], it seems possible that this treatment could be tested for use in SFD. However, the specific disease mechanisms of SFD and AMD are likely to differ somewhat, so the proposed treatments for AMD may not be applicable to SFD. Complement-system dysregulation plays a clear role in AMD pathogenesis [27,28], but this has not been established in SFD, although more recent evidence implies a role on the part of inflammation in the development of SFD (see Section 4.4).

TIMP-3:

TIMP-3 structure—Tissue inhibitor of metalloproteinases 3 is a member of the tissue inhibitor of metalloproteinases family, of which there are four members in mammals. These TIMPs were originally named based on their ability to inhibit matrix metalloproteinases (MMPs) [29], but they also inhibit related metalloproteinases of the adamalysin (ADAM) and adamalysin with thrombospondin motifs (ADAMTS) families. Human TIMP-3 is synthesized as a 211-amino-acid precursor protein, with a signal peptide of 23 amino acids that aids in the secretion of the protein and is subsequently cleaved off [30]. When separated by electrophoresis, mature TIMP-3 is commonly seen as two bands: unglycosylated TIMP-3 has a mass of ~24 kDa, while N-glycosylation of TIMP-3 at position Asn184 near the carboxyl terminus generates a ~27 kDa form [31-33]. The N-terminal ~120 amino acids of TIMP-3 fold to form the N-terminal domain, with the remaining amino acids forming the C-terminal domain, which cannot

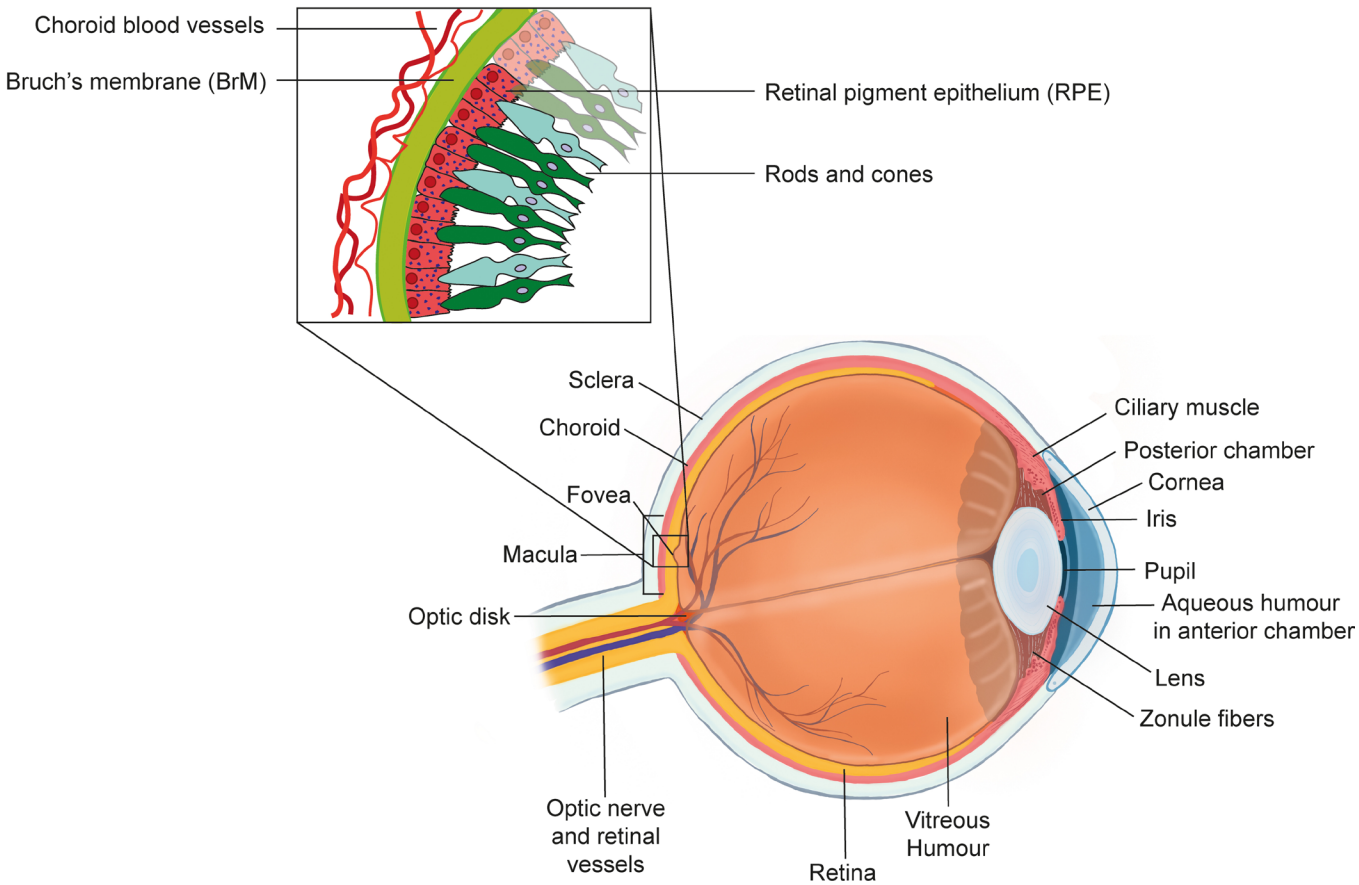

Figure 2. Structure of the eye, showing key layers of the retina. Drusen-like deposits composed of accumulated lipid and protein deposits in Bruch's membrane (BrM). The BrM also greatly thickens and increases the diffusion distance from the choroid to the retinal pigment epithelium (RPE).

fold independently and packs against the N-terminal domain. The tertiary structure of TIMP-3 is maintained by six disulfide bonds that form between twelve cysteine residues. There is high sequence and structural homology between the four mammalian TIMPs, which form a wedge-like structure with an inhibitory ridge that fits into the active sites of target metalloproteinases. The crystal structure of N-terminal TIMP-3 has been fully resolved [34], but the structure of full-length TIMP-3 remains elusive due to challenges involving its recombinant expression. This makes it difficult to predict the effects of many of the known SFD mutations on the protein's structure, as most of the mutations occur in the C-terminal region. However, a predicted model of full-length TIMP-3's structure has been generated based on its homology with TIMP-2 [35], and this agrees well with the structure predicted by AlphaFold (Figure 1). These models are likely to be useful in modeling SFD mutants and their interactions with potential regulatory molecules (e.g., heparan sulfate proteoglycans HSPGs) in the ECM. It is also important to note that due to differences between TIMP-2's and TIMP-3's sequences, the last ten residues of TIMP-3, from Asp202 onward, are unresolved in the model, so the impact of the Ser204Cys mutation on TIMP-3's structure is particularly difficult to predict.

TIMP-3 functions—Among the TIMP family, TIMP-3 is unique in several respects. First, TIMP-3 has the broadest inhibitory capacity, being able to inhibit all MMPs, as well as a range of ADAMs and ADAMTSs [29]. The MMPs, ADAMs, and ADAMTSs that are particularly relevant in the retina and, thus, in SFD include MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, MMP-14, ADAM10, ADAM17, ADAMTS-4, and ADAMTS-5 based on published studies [36-43]. The inhibition of MMPs is important for the normal maintenance and turnover of a healthy ECM, and the disruption of this process can result in fibrosis, cancer, and cardiovascular disease [29,44]. In the retina, BrM turnover is important for maintaining a healthy substratum for the RPE, as well as serving as a selective barrier for nutrient exchange between the RPE and choroid. Additionally, imbalances in MMP activity have been implicated in retinal dystrophy [45]. The roles of ADAMs and ADAMTSs in the retina are less well defined, although ADAMs are important in retinal development [46,47] and ADAM10 and ADAM17 have been implicated in AMD pathogenesis [48]. Among the ADAMs, ADAM10, and ADAM17 are well known sheddases [49,50], meaning they can cleave cell surface receptors (e.g., low-density lipoprotein receptor-related protein 1 (LRP1) [51]) and other proteins (e.g., tumor necrosis factor (TNF) [52]) that are present on the cell surface. Thus, TIMP-3's ability to regulate these ADAMs gives it an important role in the regulation of processes such as inflammation. It is also worth noting that at high levels, TIMP-3 can induce apoptosis in a variety of cell types, including RPE cells [53,54], by stabilizing death receptors, including TNF receptor-1, FAS, and TNF-related apoptosis-inducing ligand receptor-1 [55], through the inhibition of ADAM17-mediated shedding. A range of ADAMTS members (including ADAMTS-1, 2, 4, 5, 8, 9, 12, and 13) have been implicated in angiogenesis regulation [56] and their expression at an mRNA level can be regulated by TNF in adult retinal pigment epithelial 19 (ARPE-19) cells [37], leading to the suggestion that ADAMTS family members may be involved in the development of CNV. Moreover, mutations in the gene encoding ADAMTS-18 have been shown to result in severe retinal dystrophy linked to Knobloch syndrome, which affects the development of the eye and occipital skull [57].

Secondly, TIMP-3 is the only TIMP that can bind to the ECM, while the other TIMPs are soluble. It has been shown that TIMP-3 can bind to a variety of sulfated glycosaminoglycans in the ECM, as shown by Yu et al., who extracted TIMP-3 from rat uterine tissue via the addition of heparin, heparan sulfate (HS), or chondroitin sulfate [58]. They also showed via confocal microscopy that TIMP-3 co-localized with HS and that immunostaining for TIMP-3 was lost after the digestion of tissue sections with heparinase III or chondroitinase. Lee et al. subsequently identified a highly basic region on TIMP-3, which they proposed binds to sulfated ECM components. Residues from the N- (Lys26, Lys27, Lys30, and Lys76) and C-terminal (Arg163 and Lys165) domains were found to be necessary for ECM binding, as mutations of these residues to Glu or Gln ablated TIMP-3 binding to the ECM and rendered it soluble [59]. Moreover, the ECM binding of TIMP-3 has been shown to enhance its inhibition of glycosaminoglycan-binding metalloproteinases, such as ADAMTS-4 and ADAMTS-5 [60]. Both heparan and chondroitin sulfate are present in the retinal ECM, with heparan sulfate being abundant in the nerve fiber layer, choroid, BrM, and RPE and chondroitin sulfate being present throughout the retina, with moderate and stronger staining in the nerve fiber layer, ganglion cell layer, retinal vasculature, sclera, and interphotoreceptor matrix [61]. Heparan and chondroitin sulfate are commonly found attached to core proteins to form proteoglycans–perlecan, agrin, versican, and aggrecan are abundant in the human retina [62]–but their relative contributions as ECM reservoirs of TIMP-3 are unknown. Additionally, the interphotoreceptor matrix contains two retinal-specific proteoglycans, interphotoreceptor matrix proteoglycan 1 and 2 (IMPG1 and IMPG2), which are both rich in chondroitin sulfate [63]. Given that TIMP-3 is expressed in the interphotoreceptor matrix [64],

it is also worth considering the contributions of IMPG1 and IMPG2 as ECM reservoirs of TIMP-3.

Additionally, TIMP-3 also has anti-angiogenic activity [65]. Recombinant TIMP-3 has been shown to inhibit the chemotaxis of human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner in a modified Boyden chamber assay and also to inhibit basic-fibroblast-growth-factor-2-induced blood vessel formation in a chorioallantoic membrane (CAM) assay [65]. Further work has revealed that TIMP-3 can competitively block VEGF binding to VEGF receptor 2 (VEGFR2) and reduce the VEGF-mediated phosphorylation of VEGFR2 [66].

Key functions of TIMP-3 are thus metalloprotease inhibition, protection of the ECM, and inhibition of angiogenesis. These important functions will be revisited when we discuss how SFD mutants give rise to pathology (Section 4)

TIMP-3 turnover—Protein levels of TIMP-3 are regulated post-translationally by the balance between its endocytosis via the scavenger receptor LRP1 [67] and its binding to sulfated glycosaminoglycans in the ECM [60]. Scilabra et al. used an LRP1-knockout cell line (PEA-13) to show that TIMP-3 is predominantly endocytosed by LRP1 in mouse embryonic fibroblasts. There are also LRP-independent pathways that account for a sizeable portion of TIMP-3 endocytosis and have yet to be molecularly defined [67]. Low-density lipoprotein receptor-related protein 1 can be proteolytically shed from cell membranes to act as a decoy receptor that inhibits TIMP-3 endocytosis. Mutagenesis studies showed that TIMP-3 preferentially binds to the N-terminal domain of ligand-binding cluster II of LRP1 [68], and lysine residues ~21 angstroms apart (e.g., K26A/K45A or K42A/K110A) on a highly basic region of TIMP-3 drive its binding to LRP1 [35]. This basic region also mediates TIMP-3 binding to sulfated glycosaminoglycans and the ECM [59], so the binding of TIMP-3 to glycosaminoglycans or the ECM prevents its endocytosis and lysosomal degradation [60].

Initial characterization of SFD TIMP-3 mutants: This section summarizes the main evidence and conclusions from research conducted on several SFD mutants over the last 20–30 years (Table 1 and Table 2, and Appendix 1 and Appendix 2). Some of the mutants (e.g., Ser179Cys, Tyr191Cys, and Ser204Cys) have been investigated in much more depth than others (e.g., Tyr174Cys, Tyr177Cys, Tyr182Cys, Ser193Cys, Tyr195Cys, and Trp198Cys). The majority of initial studies between 1990 and 2005 were performed in cell lines such as baby hamster kidney (BHK) and CV-1 origin SV40-carrying 7 (COS-7) cells, probably because their ease of transfection provided a tractable model in which to study these proteins. Follow-up studies have been performed in ARPE-19 cells [69,70], and more recently, there has been a trend toward performing such studies in human induced pluripotent stem-cell-derived RPE (hiPSC-RPE) models [71-73], which are thought to recapitulate human physiology and SFD pathogenesis more accurately.

Dimerization: The consensus is that SFD mutants have a propensity to form dimers, multimers, and higher-molecular-weight complexes [70,74-77] (Table 1). This has not been seen by all groups (e.g., Qi et al. [69] did not observe the dimerization/multimerization of Ser179Cys), but most studies on most mutations have shown the formation of dimers and multimers. The generally accepted premise is that SFD mutants with a free cysteine can form intermolecular disulfide bonds with other mutant monomers to generate dimers. However, this process would be self-limiting and would not result in the formation of multimers, as there would be a lack of free cysteines to form further intermolecular disulfide bonds. Li et al. suggest that dimers could be formed between SFD TIMP-3 monomers and wild-type TIMP-3, which would generate a dimeric species and cause a disulfide bond(s) of wild-type TIMP-3 to be rearranged, generating an unpaired cysteine and resulting in the formation of higher-molecular-weight complexes [78]. It is also plausible that SFD TIMP-3 mutants may form disulfide bonds with higher-molecular-weight proteins, such as cell surface receptors or ECM components, via disulfide exchange.

Multiple groups have compared the Western blot migration of SFD TIMP-3 under non-reducing and reducing conditions and observed that the addition of reducing agents, such as β-mercaptoethanol or dithiothreitol, caused dimeric bands to disappear and increased the intensity of monomeric bands [70,74,75,79]. These data support the interpretation that SFD dimers are a product of intermolecular disulfide bond formation driven by the free cysteine residues of SFD mutants. These data could be supported by the alkylation of samples after reduction to eliminate the possibility of aberrant disulfide bond formation as samples re-oxidize during electrophoresis.

It is also worth noting that SFD mutants without a free cysteine residue, for example, Glu162Lys and His181Arg, have also been reported to form dimers [79,80] and that dimers of wild-type TIMP-3 can also sometimes be seen in Western blots and zymograms [70,77]. This indicates that dimerization can also occur via a mechanism that is independent of intermolecular disulfide bonding. While technically challenging given the low yield of recombinant TIMP-3 production [35], it would be ideal to analyze dimer formation using techniques other than Western blotting or reverse zymography because the unfolding of proteins in

Table 2. Tissue inhibitor of metalloproteinases 3 (TIMP-3) variants and their inhibitory ability.

Mutation	Matrix metalloproteinases (MMP) inhibition (relative to wild-type TIMP-3)
Leu10His	
Gly12Arg	Not known.
Cys24Arg	Not known.
Ser38Cys	Not known.
Intron 4/ Exon 5 Splice Site	Not known.
Ser149Cys	Not known.
Tyr151Cys	Not known.
Glu162Lys	Retains inhibition of MMP-2/9 [80].
Glu162STOP	Retains inhibition of MMP-2/9 [68], with MMP-2 inhibition demonstrate for monomer [74].
	Reduced MT1-MMP inhibition [70].
Tyr174Cys	Not known.
Tyr177Cys	Not known.
Ser179Cys	Retains (monomer) inhibition of MMP-2/9 [83] [70].
	2–3-fold lower K_{on} values for MMP-2/3/9/13 [77]. Reduced MMP-2/9 inhibition [81,82], increased MMP-9 activity in spleen tissue isolated from Ser179Cys$^{+/+}$ mice [82], reduced inhibition of MT1-MMP [69,70].
	Increased secretion and activation of pro-MMP-2/9 [69].
His181Arg	Proposed molecular modeling suggests that His181Arg have altered pro-MMP-2 activation [97].
Tyr182Cys	Not known.
Gly189Cys	Retains inhibition of 'MMPs' in transfected COS-7 cells [75].
Gly190Cys	Retains inhibition of MMP-2/9 [77].
Tyr191Cys	Retained [77] or reduced inhibition of MMP-2/9 [81].
Ser193Cys	Not known.
Tyr195Cys	Not known.
Trp198Cys	Not known.
Ser204Cys	Retains inhibition of 'MMPs' [73], MMP-2/9 [33,70,74,77] and MT1-MMP [70].
	Reduced inhibition of MMP-2/9 [81]. Due to increased accumulation, overall inhibition of MMP-2/9 was unaltered [72].

SDS may favor artifactual aggregation. Approaches such as nuclear magnetic resonance (NMR), circular dichroism, or light scattering analysis could be useful for analyzing the multimerization of native SFD mutants.

Linear ranges of detection for reverse zymography and Western blotting are seldom reported, and inadvertent overexposure may lead to overestimating the abundance and, thus, the physiologic significance of dimers. Artifactual dimer formation is also a consideration in experiments in which recombinant overexpression from plasmids exceeds physiologic levels. It would thus be highly beneficial to use validated linear-detection methods to analyze the relative abundance of monomers, dimers, and multimers in primary tissue isolated from SFD patients.

Metalloproteinase inhibition: It is also widely agreed that SFD mutants retain their ability to inhibit MMP-2 and MMP-9 (Table 2), and this has been extrapolated to the claim that MMP inhibition in general is retained. The inhibition of MMP has largely been analyzed via the reverse zymography of SFD mutants expressed in a range of cell types not directly relevant to the eye, such as patient-derived fibroblast cells or non-human cell lines, such as BHK or COS-7 cells [74,75,77,81], although some studies have used ARPE-19 cells [70] and hiPSC-RPE cells [73]. There has been some disagreement in this area in the literature, with some researchers reporting that the Ser179Cys mutant does not retain the ability or has only a reduced ability to inhibit MMP-2 and MMP-9 [69,81,82] and others finding that this function is retained, along with the inhibition of MMP-14-dependent pro-MMP-2 activation [70,83]. Most studies have focused on MMP-2 and MMP-9, perhaps because these are readily detectable by zymography, but RPE cells also express and secrete several

other MMPs, such as MMP-1 and MMP-3 [36,41,42], and analysis of these is lacking. Techniques other than reverse zymography are required to analyze MMPs that cleave gelatin less readily than MMP-2 and MMP-9 do. For example, Yeow and colleagues investigated MMP inhibition by Ser179Cys using a fluorescent substrate and found that this variant had lower kon values than wild-type TIMP-3 for MMP-2, MMP-3, MMP-9, and MMP-13 [77]. This does not necessarily mean that Ser179Cys has decreased inhibitory capacity, because it could also have a slower k_{off} rate and, thus, a similar K_i to that of wild-type TIMP-3. Further kinetic analyses are thus warranted. Furthermore, the inhibitory capacities of only a few SFD mutants have been investigated, so more extensive research is required to understand whether all SFD mutants universally retain the ability to inhibit MMPs.

An important limitation of reverse zymography is that the identity of an inhibitory species is inferred solely from its position on the gel. For example, several studies have concluded from reverse zymography that both monomeric and dimeric forms of SFD mutants (e.g., Ser179Cys, Tyr191Cys, and Ser204Cys) retain their ability to inhibit MMP-2 and MMP-9 [33,70,74,77,81]. Many of these experiments are done by transfecting cells that also endogenously express wild-type TIMP-3, which could be responsible for the inhibition observed. Considering the model proposed by Li et al. [78], a dimer could potentially be composed of mutant and wild-type TIMP-3, with the latter being responsible for the bands observed upon reverse zymography. Appropriate controls, such as the parallel analysis of untransfected cells, are thus critical in such experiments. Alternatively, hiPSC-RPE models, which only express mutant TIMP-3 [73], provide a clearer model system. Perhaps the most appropriate control is exhibited by Engel et al. [72], who used clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 to edit hiPSC-RPE cells from a patient expressing Ser204Cys, thus generating isogenic wild-type and mutant cells with all the regulatory elements in place. Furthermore, Hongisto et al. [73] used hiPSC-RPE models to show that Ser204Cys was significantly more monomeric than wild-type TIMP-3. Conditioned media from hiPSC-RPE cells expressing Ser204Cys effectively inhibited collagenase activity [73], indicating that monomeric forms of this mutant retain MMP inhibitory capacity. Similar analyses of other SFD variants are warranted.

Little is known about whether SFD mutants retain the capacity to inhibit ADAMs or ADAMTSs. Fogarasi et al. [84] showed that the Ser179Cys variant retained the ability to inhibit ADAM17, ADAMTS-4, and ADAMTS-5 to the same degree as wild-type TIMP-3. The ability of other SFD TIMP-3 mutants to inhibit these and other relevant ADAMs and ADAMTSs has not been profiled, so additional work in this area is needed.

Another potentially relevant protease is high-temperature requirement protein A1 (HTRA1), a serine protease that has been associated with AMD development [85], although its role remains unclear [86,87]. Interestingly, EFEMP1, a binding partner of TIMP-3, has been identified as a target of HTRA1 [88], but there is no evidence to suggest that TIMP-3 or any SFD TIMP-3 mutant interacts with or inhibits HTRA1.

TIMP-3 accumulation in SFD:

Evidence for TIMP-3 accumulation—As mentioned above, TIMP-3 is expressed by both RPE and choroidal endothelial cells [14,15], and TIMP-3 protein levels have been shown to increase in the extracellular BrM with age [11]. The extracellular accumulation of mutant TIMP-3 protein (e.g., Glu162STOP, Ser179Cys, Tyr191Cys, and Ser204Cys) has been identified in SFD patients [8], as well as in vitro (cell lines and hiPSC-RPE) and in mouse models of SFD [70-72,76,77,83,89]. Interestingly, the intracellular accumulation of mutant TIMP-3 protein has been observed in a hiPSC-RPE model (for Ser204Cys TIMP-3 [73]) and in HEK-293 cells (for Leu10His and Gly12Arg [4]). It is not known whether the intracellular accumulation of mutant TIMP-3 protein occurs in vivo in SFD patients. It would be useful to obtain more evidence regarding the abundance and localization of TIMP-3 in SFD eyes using immunohistochemical techniques on retinal sections, for example.

Proposed mechanisms of TIMP-3 accumulation—Intriguingly, it has been shown that TIMP-3 mRNA levels are not elevated in SFD patients [90], indicating that TIMP-3 accumulates because of altered post-translational regulation. Several mechanisms for this accumulation have been proposed.

Langton et al. proposed that SFD mutants of TIMP-3 accumulate in BrM because the dimers and multimers of TIMP-3 that form are “resistant to turnover” [70]. In this study, ARPE-19 cells were transfected with plasmids encoding Glu162STOP, Ser179Cys, Ser204Cys, or wild-type TIMP-3 and cultured to confluency to allow them to deposit a TIMP-3-containing ECM. Transfected cells were then removed and replaced with untransfected ARPE-19 cells, with the loss of SFD TIMP-3 variants from the ECM being monitored via Western blotting. The authors found that wild-type TIMP-3 was almost completely removed from the ECM, with ~1% remaining after 96 h. Higher-molecular-weight species of SFD mutants (i.e., dimers and multimers)

were initially removed at the same rate but then cleared more slowly so that over a period of 96 h, between 20 and 30% of the SFD mutants remained in the ECM, indicating that the cellular uptake and turnover of the mutants are defective. Interestingly, Soboleva et al. [83] have previously shown that Ser179Cys TIMP-3 accumulated to a greater degree in the ECM of fibroblasts, although its turnover over 48 h was the same as that of wild-type TIMP-3. This corroborates the observations of Langton et al., who saw the decreased turnover of this mutant only between 48 and 96 h [70]. Given that TIMP-3 is now known to be endocytosed by LRP1 [67], the resistance to turnover observed may indicate that the LRP1-mediated endocytic clearance of SFD mutants is impaired, leading to the accumulation of extracellular TIMP-3. Non-naturally occurring mutations in TIMP-3 impair its binding to LRP1 and reduce its endocytic turnover [35], so it is plausible that SFD mutations could similarly impair binding to LRP1 and reduce turnover.

Dimers of wild-type TIMP-3 have been isolated from the BrM of a healthy retina [91], demonstrating that dimer formation is not a property unique to SFD TIMP-3 variants. Instead, spontaneous misfolding or a disruption in the protein folding pathway may occasionally give rise to dimers, and SFD mutants may have a higher propensity to misfold than wild-type TIMP-3 due to the mutations affecting the formation of the six intramolecular disulfide bonds that stabilize the three-dimensional structure of TIMP-3. The mutants Ser179Cys, Gly190Cys, Tyr191Cys, and Ser204Cys TIMP-3 have all been shown to be more thermodynamically stable than wild-type TIMP-3 [89], indicating altered protein folding. Such disruptions in protein folding pathways have been implicated in neurodegenerative diseases, such as Alzheimer's disease [92], and β-amyloid deposits accumulate in the drusen in AMD [93], suggesting that similar misfolding-driven accumulation of TIMP-3 variants may also occur in SFD.

Several studies have shown that SFD TIMP-3 mutants have increased ECM binding (Appendix 1) [70,76,77,83], and this is likely to lead to extracellular accumulation. Wild-type TIMP-3 is thought to interact primarily with sulfated glycosaminoglycans, such as heparan sulfate, in the ECM [58]. The affinity of SFD mutants of TIMP-3 for sulfated glycosaminoglycans is not known, but several of the SFD mutations are localized at or near the basic face of TIMP-3, which is known to bind to sulfated glycosaminoglycans [59]. A key study performed by Keenan et al. found that the amount and sulfation of HS in BrM significantly decreases with age [94]. Heparan sulfate is a major binding partner for complement-factor H (CFH) in BrM, and the authors demonstrated that a decrease in HS levels and sulfation disrupted binding to CFH, particularly the 402H form that is associated with AMD and causes the overactivation of the complement [94]. The same could apply to TIMP-3, with a decrease in HS abundance and sulfation disrupting TIMP-3 trafficking and, potentially, activity. The average age of donors in the 'old' group in this study was 82 years, so these age-dependent changes in BrM may be more relevant for the accumulation of SFD mutants that present later in life, such as the intron 4/exon 5 splice site mutation, Tyr177Cys, His181Arg, and Tyr182Cys (Table 1). Heparan-sulfate abundance and sulfation is also known to change in other cell types in response to inflammation and pathology (e.g., diabetes and osteoarthritis), potentially providing a mechanism by which co-morbidities could influence the age of SFD onset.

Alternatively, SFD variants may bind more strongly to ECM proteins, potentially through aberrant disulfide bonds. Intriguingly, fibulin-3, a binding partner of TIMP-3 [12], is expressed at elevated protein levels in an hiPSC-RPE Ser204Cys model of SFD [73] and in choroid-RPE samples from AMD patients [95]. Fibulin-3 also accumulates in Malattia Leventinese [13], another macular dystrophy, and it has been suggested that the accumulation of TIMP-3-fibulin-3 complexes could act as a barrier to transport [13], leading to the accumulation of other proteins in the SFD BrM.

Other mechanisms suggested to cause TIMP-3 accumulation in SFD include altered glycosylation (the decreased glycosylation of Ser179Cys, Tyr191Cys, and Ser204Cys has been shown to increase their aggregation) [81], disrupted secretion from cells (Leu10His and Gly12Arg mutations in the TIMP-3 signal peptide disrupt its trafficking out of cells) [4], and intermolecular crosslinking (due to oxidative reactions in the high photo-oxidative retinal environment) [2,96]. The molecular mechanisms behind accumulation of SFD TIMP-3 are thus not fully resolved. It is likely that a combination of the above factors, potentially along with other as-yet-unidentified mechanisms, drives accumulation and that mechanisms may differ for individual SFD mutations.

How do accumulated SFD TIMP-3 mutants give rise to SFD pathology?: This section examines the evidence published thus far regarding how SFD TIMP-3 mutations affect TIMP-3 functions and how these changes may be responsible for the pathology observed in SFD. It has been hypothesized that SFD mutants retain the functions of wild-type TIMP-3 and their accumulation results in an 'enhanced' TIMP-3 effect, but it has also been suggested that SFD pathogenesis could be the result of a loss of TIMP-3 function [81].

Changes to MMP inhibition resulting in increased BrM thickness: As discussed in Section 2.2 above, the majority of studies examining the MMP-inhibitory activity of SFD

TIMP-3 mutants have concluded that this property is not altered in SFD (Table 2) [70,97]. Given the large accumulation of SFD mutants extracellularly in the BrM, the inhibition of MMPs in the extracellular environment is thought to be increased, leading to reduced ECM turnover and, consequently, the substantial thickening of the BrM that is observed in SFD [98]. In turn, this could greatly increase the diffusion distance between the choroid and the metabolically active RPE and thus impair the diffusion of fresh nutrients and waste between these cells, leading to the accumulation of sub-RPE deposits and geographic atrophy.

A caveat of this model is that most studies have focused on the SFD TIMP-3 inhibition of MMP-2, MMP-9, or MT1-MMP (MMP-14), but the choroid and RPE express many more MMPs that can potentially contribute to BrM turnover. Still, the SFD TIMP-3 inhibition of these is unexplored. There is also considerable scope for the investigation of the SFD TIMP-3 inhibition of ADAMTSs, which are also likely to contribute to BrM turnover.

4.2 Apoptosis induced by SFD TIMP-3 variants: Soboleva et al. [83] report that mouse fibroblasts expressing Ser179Cys TIMP-3 are multipolar and have a higher cytoplasmic-to-nuclear ratio than wild-type TIMP-3-expressing fibroblasts. Such observations have led to investigations of whether the expression of SFD mutants directly damages cells and thus causes geographic atrophy in SFD.

The overexpression of wild-type TIMP-3 induces apoptosis in a variety of cell types, including Henrietta Lacks (HeLa) cells [53], vascular smooth muscle cells, and even RPE cells [54]. It has been proposed that TIMP-3 promotes cell death by inhibiting ADAM17 and thereby stabilizing TNF receptors on the cell surface, with downstream signaling pathways increasing the susceptibility to apoptosis [99]. Subsequently, it was shown that recombinant TIMP-3, N-terminal TIMP-3, and adenovirally expressed TIMP-3 result in the stabilization of various death receptors, including TNF receptor-1, FAS, and TNF-related apoptosis-inducing ligand receptor-1 (TRAIL) [55].

Surprisingly, there are few studies examining the apoptotic properties of SFD variants of TIMP-3. Majid and colleagues report a modest but significant increase in the apoptosis of primary RPE cells overexpressing Ser179Cys, Gly190Cys, Tyr191Cys, and Ser204Cys TIMP-3 as compared to wild-type TIMP-3 [54]. The authors suggest that this small increase in apoptosis is unlikely to fully account for the SFD phenotype. However, small differences over the time course of an experiment may lead to substantial biologic effects in vivo over 30–40 years, and even if SFD TIMP-3 mutants do not have an inherently different apoptotic ability as compared to wild-type TIMP-3, their accumulation in BrM [8] may increase the apoptosis of neighboring cells. Further insight may be gained by longer-term experiments, although it would be important to validate levels of TIMP-3 expression, which we have observed can vary in vitro from mutant to mutant and over time.

Altered inhibition of angiogenesis: Marked CNV is commonly seen in late-stage SFD, along with the proliferation and invasion of blood vessels into the outer retina, which is indicative of dysregulated angiogenesis. Tissue inhibitor of metalloproteinases 3 has anti-angiogenic activity [65], which is thought to be due to its ability to competitively block VEGF from binding to VEGF receptor 2 [66]. Reports on SFD's effects on the inhibition of angiogenesis differ in their conclusions, with the decreased inhibition [69,82] and unchanged inhibition [84] of angiogenesis being reported.

Two independent studies performed by the same group [69,82] using different methods report that Ser179Cys TIMP-3 induces angiogenesis, which is noteworthy given the anti-angiogenic properties of wild-type TIMP-3. The first study reported that the application of conditioned medium derived from RPE cells expressing Ser179Cys TIMP-3 induced the migration of human dermal endothelial cells. Moreover, using a chick chorioallantoic membrane (CAM) assay, the authors found that conditioned medium containing Ser179Cys TIMP-3 induced greater neovascularisation than wild-type TIMP-3 and that wild-type TIMP-3 could inhibit the angiogenic response induced by Ser179Cys TIMP-3 [69]. Ser179Cys-expressing endothelial cells exhibited more of the binding of VEGF to VEGF receptor 2 [82] and formed tubular networks in 3D collagen gels in response to VEGF, whereas wild-type TIMP-3-expressing endothelial cells had a compromised ability to form tube-like structures [82]. These experiments were performed in porcine endothelial cells, so they may not be directly applicable to the human retina. Western blotting and immunofluorescent-staining experiments have demonstrated that mice homozygous for Ser179Cys TIMP-3 showed the increased expression of VEGF receptor 2 in the retina/choroid, corroborating observations in donor-eye tissue from a 77-year-old SFD patient and providing a potential mechanism for the increased angiogenesis seen in SFD. More recent evidence from the same group [81] suggests that cells expressing SFD mutants have upregulated VEGF receptor 2 on their cell surfaces. In response to VEGF treatment, Ser179Cys TIMP-3-expressing endothelial cells showed the enhanced autophosphorylation of VEGF receptor 2 as compared to cells expressing wild-type TIMP-3. In a Boyden-chamber assay, endothelial cells expressing Ser179Cys, Tyr191Cys, and Ser204Cys TIMP-3

variants migrated more than wild-type TIMP-3 in response to VEGF. Interestingly, the deglycosylation of these SFD mutants further increased their angiogenic potential.

Other studies support the conclusion that SFD variants may increase angiogenesis. Hongisto et al. [73] used an agnostic mass spectrometry approach to examine the proteome of hiPSC-RPE expressing Ser204Cys and wild-type TIMP-3, followed by a membrane-based antibody array. The expression of Ser204Cys increased the expression of proteins involved in angiogenesis-related pathways, including monocyte chemoattractant protein 1, platelet-derived growth factor AA, and angiogenin, despite there being no increase in VEGF secretion. Additionally, a recent report by Alsaffar et al. [79] showed that several SFD mutants (Ser38Cys, Glu162Lys, Glu162STOP, Ser179Cys, His181Arg, and Ser204Cys) inhibited VEGF receptor 2 signaling less than wild-type TIMP-3.

In contrast, Ser179Cys TIMP-3 has also been reported to inhibit angiogenesis as effectively as wild-type TIMP-3 [84]. Fibrin bead assays using mice aortic endothelial cells expressing wild-type or Ser179Cys TIMP-3 indicated that tubule formation was not significantly different in cells expressing wild-type and Ser179Cys TIMP-3. Moreover, a competitive enzyme-linked immunosorbent assay (ELISA) showed that Ser179Cys TIMP-3 blocked the binding of VEGF to VEGF receptor 2 as effectively as wild-type TIMP-3.

Inflammation: Damage to retinal cells is known to initiate an inflammatory response, with, for example, RPE cells releasing monocyte chemoattractant protein-1 (MCP1/CCL2) under conditions of cell stress and thus attracting macrophages expressing chemokine receptor type 2 (CCR2) to sites of retinal damage [100]. There is mounting evidence that such inflammatory responses play a role in SFD.

Spaide [101] reported the successful use of an anti-TNF monoclonal antibody, adalimumab, in the treatment of an SFD patient with the Ser204Cys mutation who presented with CNV in 2003. Initially, the patient was treated with intravitreal injections of triamcinolone, followed by anti-VEGF therapy with bevacizumab. Both these treatments had undesirable results, with triamcinolone leading to the development of a corticosteroid-related cataract and glaucoma, requiring surgery, and bevacizumab being ineffective in preventing exudation and hemorrhage in the eye. The patient was subsequently treated with adalimumab for 18 months, during which time they showed the cessation of CNV disease activity and had 20/20 visual acuity in the right eye.

The effectiveness of anti-TNF therapy in this patient is interesting because TIMP-3 is the primary physiologic inhibitor of ADAM17 and thus inhibits the ADAM17-dependent release of soluble TNF from its membrane-bound precursor [52]. The impaired inhibition of ADAM17 by SFD variants may thus potentially increase TNF release and result in inflammation. However, the ability of SFD TIMP-3 mutants to inhibit ADAM17 has not been extensively profiled. Fogarasi et al. [84] treated wild-type mouse liver extracts with increasing concentrations of recombinant wild-type TIMP-3 and Ser179Cys TIMP-3, which were expressed from *E. coli* and refolded in vitro, and found that both proteins inhibited the ADAM17 hydrolysis of a fluorogenic substrate to a similar extent. The authors point out that their recombinant TIMP-3 preparations may contain some incorrectly refolded material and that this may undermine their conclusions, but they also demonstrated the similar inhibition of ADAM17 in liver extracts from wild-type mice and mice heterozygous or homozygous for Ser179Cys. Thus, ADAM17 inhibition by additional SFD variants requires investigation.

Recent studies on hiPSC-RPE models expressing Ser204Cys TIMP-3 indicate the increased expression of several inflammation-related genes, including CCL2 and complement genes such as C1R, C1S, and C3 [71,73], at the mRNA and protein levels. While the mechanism behind this increased expression has not been elucidated, inflammatory stimuli, such as TNF, are known to alter the RPE expression of CFH [102].

Changes in metabolism: Retinal pigment epithelial (RPE) cells are very metabolically active to support their essential turnover of photoreceptor outer segments [103], and it has been suggested that the expression of SFD mutants could alter RPE cells' metabolism. The expression of Ser179Cys TIMP-3 decreases overall mitochondrial activity and lactate production in fibroblast cells in vitro, indicating a decrease in overall metabolic activity [83]. Broader alterations of metabolic activity have been observed in hiPSC-RPE expressing Ser204Cys TIMP-3 [72], including changes in metabolites such as tyrosine, aspartate, guanosine-5'-triphosphate (GTP), and flavin adenine dinucleotide (FAD), as well as a reduction in the flux from malate to pyruvate. Pyruvate generation via this pathway is necessary for glutathione generation, so a reduction could indicate that cells are oxidatively stressed. Glutathione was significantly decreased, rendering hiPSC-RPE more susceptible to oxidative damage; this has also been observed in an SFD mouse model [104] and could significantly contribute to the loss of RPE and geographic atrophy observed in late-stage SFD. While only a few studies have investigated changes in metabolism, the available data are compelling and indicate that further research in this area is warranted.

Are there any extraocular effects of SFD mutations?: Given that TIMP-3 is expressed widely, with particularly high expression in the lung, adipose tissue, and synovium [31,105], it seems likely that SFD patients would also present with effects in other tissues. The phenotype of TIMP-3-null mice may be a useful starting point when considering which extraocular tissues and biologic processes may be affected in SFD patients. TIMP-3-null mice exhibit dysregulated MMP activity, leading to a range of effects, including abnormal vascularization in the choroid [106], air-space enlargement [107], impaired bronchiolar branching [108], and dilated cardiomyopathy [109]. Abnormal vascularization in the choroid is observed in SFD patients, with late-stage disease resulting in CNV, but abnormal vascularization in other organ systems has not, thus far, been reported in SFD patients. Members from two families carrying the Tyr191Cys TIMP-3 variant have been reported to exhibit pulmonary pathology, with emphysema that was either moderate, with asymptomatic air trapping, or severe, with severe obstruction and chronic respiratory failure [110]. This potentially reflects the air space enlargement seen in TIMP-3-null mice. However, normal lung function has been reported for two sibling SFD patients with the Ser204Cys mutation [111]. It is unclear whether the reported lung pathologies are rare events in SFD patients, being potentially related to the Tyr191Cys mutation and/or epigenetic effects, or sub-clinical lung changes occur in a larger percentage of SFD patients than has hitherto been realized.

TIMP-3-null mice also exhibit dysregulated ADAM activity, with increased ADAM17 activity giving rise to increased TNF release in several models of inflammation [112-115]. As discussed above, there is a single report of clinical improvement in an SFD patient treated with anti-TNF therapy, suggesting that a systematic analysis of TNF responses in SFD patients may be warranted. The loss-of-function effects of TIMP-3 mutation would be expected to increase TNF release and systemic inflammation, while the gain-of-function effects would dampen inflammatory responses.

Lastly, TIMP-3-null mice exhibit dysregulated ADAMTS activity, leading to an increase in ADAMTS-dependent cartilage degradation and the development of osteoarthritis [116]. Altered osteoarthritis susceptibility has not been reported in SFD patients, possibly because of the high prevalence of osteoarthritis in the general population. As with altered ADAM activity, the loss-of-function effects of TIMP-3 mutation would be expected to increase osteoarthritis susceptibility, while the gain-of-function effects would be expected to protect against the disease.

Conclusions: Sorsby fundus dystrophy is a rare inherited macular dystrophy that is caused by mutations in the gene encoding TIMP-3. Despite being caused by a mutation in a single gene, SFD exhibits marked heterogeneity, with individual mutations having different ages of symptom onset and variable presentations of CNV. A potential mechanism for this heterogeneity was proposed by Meunier et al. [110], who suggested that there is a correlation between the size of the introduced amino acid residues and the severity of SFD, with the introduction of larger residues correlating with earlier onset and a more severe disease phenotype, but this is based on the analysis of a limited number of SFD mutations.

The majority of SFD mutants involve the addition of an unpaired cysteine, leading to the suggestion that this feature is essential for TIMP-3 accumulation and SFD pathology. However, mutations such as Glu162Lys and His181Arg, which do not generate an unpaired cysteine, also form dimers and multimers (Table 1) and cause SFD pathology, indicating that TIMP-3 multimerization via an unpaired cysteine residue is not the only molecular mechanism underlying SFD. These two variants were discovered comparatively recently, in 2009 and 2006, respectively, which has perhaps shifted the focus from the theory of unpaired cysteines being the sole cause of SFD TIMP-3 dimerization. Further investigation of the mechanisms of dimer formation for individual mutants may clarify this apparent discrepancy.

Two key questions are whether SFD mutations lead to a gain or loss of TIMP-3 function and whether this differs for individual mutations. For example, whether SFD mutants retain their ability to inhibit angiogenesis or, rather, promote angiogenesis is a contentious issue that warrants further investigation. The literature largely agrees that SFD mutants retain their ability to inhibit MMP-2 and MMP-9, but the inhibitory activity of many SFD mutants has not been studied, and similarly, the inhibition of a large number of MMPs has not been investigated. The inhibitory capacity of SFD mutants for ADAMs and ADAMTSs also largely remains unknown. Many MMPs, ADAMs, and ADAMTSs are expressed in the retina, and many of them have non-redundant functions, so a more comprehensive analysis of their inhibition by SFD mutants is justified.

It is not clear what broad effects SFD mutants have on RPE cells in terms of gene expression, metabolism, or apoptosis. Perhaps approaches such as that taken by Hongisto et al. [73], in which mass spectrometry was used to agnostically identify the differentially regulated proteins released by hiPSC-RPEs expressing Ser204Cys TIMP-3, may be usefully applied to other SFD variants. Metabolomic approaches, such as that used by Engel et al. [72] could similarly be employed

to gain a better understanding of whether all SFD mutants affect cellular metabolism in the same way or at all. The increased use of hiPSC-RPE models is likely to aid such future studies and improve our understanding of SFD pathogenesis. Key questions that must be addressed with regard to SFD pathophysiology thus include the following:

a. What effect do SFD mutations have on choroidal endothelial cells and other cells of the retina? Most studies have been performed using RPE cells.

b. What changes in TIMP-3's three-dimensional structure are induced by these mutations?

c. What does the introduction of an unpaired cysteine do to the default pattern of intramolecular TIMP-3 disulfide bonds?

d. What extraocular effects occur, and how common are these in SFD patients?

e. Do SFD mutants retain inhibitory activity for ADAMs and ADAMTSs?

f. What predominant biological effect do SFD TIMP-3 mutants have on cells in the retina?

There is a marked lack of therapies available to treat those with SFD, with anti-VEGF therapy currently being the only effective option. A greater understanding of the mechanism behind SFD pathogenesis, looking beyond the select few that have been extensively studied (e.g., Ser179Cys, Tyr191Cys, and Ser204Cys), is required to develop effective therapies.

APPENDIX 1. SUPPLEMENTARY TABLE 1.

To access the data, click or select the words "Appendix 1." Angiogenic and extracellular matrix (ECM) binding properties of Sorsby fundus dystrophy (SFD) tissue inhibitor of metalloprotaineases 3 (TIMP-3) variants.

APPENDIX 2. SUPPLEMENTARY TABLE 2.

To access the data, click or select the words "Appendix 2." Other properties of Sorsby fundus dystrophy (SFD) tissue inhibitor of metalloproteinases 3 (TIMP-3) variants.

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