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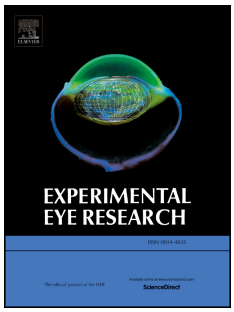


Sorsby fundus dystrophy – A review of pathology and disease mechanisms

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**Title: Sorsby Fundus Dystrophy – a review of pathology and disease mechanisms**

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**Abstract:**

Sorsby fundus dystrophy (SFD) is an autosomal dominant macular dystrophy with an estimated prevalence of 1 in 220,000 and an onset of disease around the 4$^{th}$ to 6$^{th}$ decade of life. Similar to age-related macular degeneration (AMD), ophthalmoscopy reveals accumulation of protein/lipid deposits under the retinal pigment epithelium (RPE), referred to as drusen, in the eyes of patients with SFD.

SFD is caused by variants in the gene for tissue inhibitor of metalloproteinases-3 (*TIMP3*), which has been found in drusen-like deposits of SFD patients. TIMP3 is constitutively expressed by RPE cells and, in healthy eyes, resides in Bruch's membrane. Most SFD-associated TIMP3 variants involve the gain or loss of a cysteine residue. This suggests the protein aberrantly forms intermolecular disulphide bonds, resulting in the formation of TIMP3 dimers. It has been demonstrated that SFD-associated TIMP3 variants are more resistant to turnover, which is thought to be a result of dimerisation and thought to explain the accumulation of TIMP3 in drusen-like deposits at the level of Bruch's membrane.

An important function of TIMP3 within the outer retina is to regulate the thickness of Bruch's membrane. TIMP3 performs this function by inhibiting the activity of matrix metalloproteinases (MMPs), which have the function of catalysing breakdown of the extracellular matrix. TIMP3 has an additional function to inhibit vascular endothelial growth

factor (VEGF) signalling and thereby to inhibit angiogenesis. However, it is unclear whether SFD-associated TIMP3 variant proteins retain these functions. In this review, we discuss the current understanding of the potential mechanisms underlying development of SFD and summarise all known SFD-associated TIMP3 variants.

Cell culture models provide an invaluable way to study disease and identify potential treatments. These allow a greater understanding of RPE physiology and pathophysiology, including the ability to study the blood-retinal barrier as well as other RPE functions such as phagocytosis of photoreceptor outer segments. This review describes some examples of such recent *in vitro* studies and how they might provide new insights into degenerative diseases like SFD.

Thus far, most studies on SFD have been performed using ARPE-19 cells or other, less suitable, cell-types. Now, induced pluripotent stem cell (iPSC) technologies allow the possibility to non-invasively collect somatic cells, such as dermal fibroblast cells and reprogram those to produce iPSCs. Subsequent differentiation of iPSCs can generate patient-derived RPE cells that carry the same disease-associated variant as RPE cells in the eyes of the patient. Use of these patient-derived RPE cells in novel cell culture systems should increase our understanding of how SFD and similar macular dystrophies develop.

**Keywords:**

Retinal pigment epithelium, induced pluripotent stem cells, tissue inhibitor of matrix metalloproteinases-3, Sorsby fundus dystrophy, AMD, cell culture, disease modelling

**Abbreviations:**

A disintegrin and metalloproteinases (ADAMs)

Age-related macular degeneration (AMD)

Bruch's membrane (BrM)

Choroidal neovascularisation (CNV)

Extracellular matrix (ECM)

Embryonic stem cells (ESCs)

Induced pluripotent stem cells (iPSCs)

Matrix metalloproteinases (MMPs)

Photoreceptor outer segments (POS)

Retinitis pigmentosa (RP)

Retinal pigment epithelium (RPE)

Sorsby fundus dystrophy (SFD)

Tumour necrosis factor-alpha converting enzyme (TACE)

Tissue inhibitor of metalloproteinases-3 (TIMP3)

Vascular endothelial growth factor (VEGF)

VEGF receptor 2 (VEGFR2)

## 1. Introduction

Sorsby Fundus Dystrophy (SFD) is an autosomal dominant macular dystrophy described by Orphanet as a rare disease, which they define as affecting not more than one person per 2000 in the European population (Capon et al., 1988; Sorsby et al., 1949). Southampton Eye Unit, currently has eight patients with SFD, from a population of 1.76 million people in Hampshire, suggesting a prevalence closer to 1 in 220,000 (Kaye and Lotery, 2017). Symptoms typically occur around the $4^{th}$ to $6^{th}$ decade of life (Sorsby et al., 1949) and progress of the disease leads to bilateral loss of central vision (Hoskin et al., 1981). Presenting symptoms commonly include nyctalopia, or night-blindness, typically caused by vitamin A deficiency, blurring, black spots, photopsia, metamorphopsia, reduced colour vision or loss of central vision in one eye (Burn, 1950; Capon et al., 1988; Hamilton et al., 1989). If nyctalopia occurs, it commonly precedes other symptoms (Hamilton et al., 1989), however not all patients develop night-blindness. Hence vitamin A has been trialled as a treatment for night-blindness but with no significant visual improvement, except at unsustainably high doses (Gliem et al., 2015; Jacobson et al., 1995). Ophthalmoscopy can reveal drusen-like deposits in the macula or yellow retinal flecks (Hamilton et al., 1989). In particular, drusen-like deposits (Fig. 1B and 1G) are a frequent characteristic of early disease and can predate other symptoms (Hoskin et al., 1981). The initial development and progression of these retinal changes as visualised by funduscopy are not always simultaneous (Fig. 1C and 1D), but consistently occur in both eyes (Capon et al., 1988). As the disease progresses, evidence of sub-retinal haemorrhage (Fig. 1G), oedema, exudates, retinal epithelial detachment (Fig. 1A and E-G) or macular scarring may become evident (Fig. 1C) (Carr et al., 1977; Sorsby et al., 1949). Patients may be incorrectly diagnosed as having idiopathic choroidal neovascularization or age related macular degeneration (AMD). A strong family history of macular degeneration may lead to the correct diagnosis.

SFD has generally a poor visual prognosis (Gemenetzi et al., 2011). This can be because of acute visual loss caused by choroidal neovascularisation (CNV) or, less commonly, progressive visual loss from atrophy of the retinal pigment epithelium (RPE) due to a reduced blood supply (Gourier and Chong, 2015). The latter SFD phenotype therefore has some similarities with the geographic form of AMD (Gourier and Chong, 2015). Previously, argon laser photocoagulation, photodynamic therapy and intravitreal steroids have been trialled for SFD patients with CNV, but recent reports suggest these to be ineffectual; often yielding poor visual outcomes (Puech et al., 2014; Sivaprasad et al., 2008). Inhibition of vascular endothelial growth factor (VEGF) with monoclonal antibodies has been shown to be an effective treatment, delaying visual loss due to CNV and reducing scarring (Gemenetzi et al., 2011; Gliem et al., 2015; Keller et al., 2014). Bevacizumab (Avastin) or less commonly ranibizumab (Lucentis) have shown moderate benefits when administered systemically but are more effective and safer when administered intravitreally (Balaskas et al., 2012; Gemenetzi et al., 2011; Keller et al., 2014). Some studies suggest the addition of a long-acting angiostatic steroid, in combination with bevacizumab, has beneficial effects due to the aggressive nature of CNV and its early onset (Sivaprasad et al., 2008). However, these drugs do not prevent disease, but merely delay irreversible end-stage blindness. Further studies are therefore required to fully evaluate the efficacy of existing treatments and their long-term safety (Gemenetzi et al., 2011; Sivaprasad et al., 2008).

## 2. TIMP3

SFD is caused by variants in the gene encoding Tissue Inhibitor of Metalloproteinases-3 (*TIMP3*) (Felbor et al., 1997; Felbor et al., 1996; Felbor et al., 1995; Jacobson et al., 1995; Weber et al., 1994). TIMP3 is one of four members of the TIMP family of matrix metalloproteinase (MMP) inhibitors, with MMPs being members of the metzincin family of protease enzymes (Brew and Nagase, 2010). The TIMP family of proteins has a highly conserved tertiary structure comprising six intramolecular disulphide bonds forming between 12 cysteine residues (Fig. 2A and 2B). All four members of the TIMP family inhibit extracellular matrix (ECM) degradation by MMPs primarily through their N-terminal domain (Fig. 2B) (Handsley and Edwards, 2005; Murphy et al., 1991; Williamson et al., 1990). However, residues throughout the length of the protein have been identified as being part of conserved metzincin-binding and hemopexin-domain-binding interfaces and a sequence alignment from NCBI's Conserved Domain Database demonstrates conservation of these regions across the TIMP family in humans and in other species (Fig. 2A and 2C) (Brew and Nagase, 2010; Marchler-Bauer et al., 2017). Interestingly, 9 of the 12 disulphide bond-

forming cysteine residues can be seen to be conserved within or close to metzincin-binding or hemopexin-domain-binding regions (Fig. 2C). It should be noted that in this review we have used modified nomenclature to conform to the Human Genome Variation Society (HGVS) guidelines, numbering from the start codon as 1, as first suggested by Bakall et al (2014).

The remodelling of the ECM by MMPs, and the regulation of this process by TIMPs is essential for a diverse range of activities including tissue remodelling, wound healing, angiogenesis, morphogenesis, and many other normal physiological processes. In contrast, loss of ECM regulation in general is associated with a number of conditions characterised by pathological tissue breakdown, including cancer, arthritis, chronic obstructive pulmonary disorder and cardiovascular disease (Malemud, 2006; Nagase and Woessner, 1999; Navratilova et al., 2016).

As well as inhibiting MMPs, TIMP3 has been found to inhibit the family of A Disintegrin And Metalloproteases (ADAMs), including Tumor necrosis factor-Alpha Converting Enzyme (TACE; also known as ADAM17) (Amour et al., 1998; Lee et al., 2001), aggrecanases (Kashiwagi et al., 2001), and binding of VEGF to its receptor VEGFR2 by binding to VEGFR2 through the C-terminal domain (Fig. 2B), thereby inhibiting angiogenesis (Qi et al., 2003). Interestingly, wild-type TIMP3 has also been found to induce apoptosis in a variety of cell-types, including rat smooth muscle cells, HEK293 cells, and human colon carcinoma cells (Baker et al., 1998; Bond et al., 2000; Smith et al., 1997). It is unknown whether TIMP3 has a role in increasing apoptosis of RPE cells in SFD. In contrast with the other TIMPs, which are soluble, TIMP3 is tightly sequestered in the ECM via interactions involving both the N- and C-terminal domains (Leco et al., 1994; Lee et al., 2007).

## 2.1 Accumulation of TIMP3 in drusen-like deposits

TIMP3 is constitutively expressed by RPE cells and it is a component of healthy Bruch's membrane (BrM) (Della et al., 1996; Fariss et al., 1997; Vranka et al., 1997). Levels of TIMP3 in BrM have been seen to increase with age in healthy tissue (Kamei and Hollyfield, 1999). However, it has been suggested that this is due to accumulation of the protein rather than from increased expression, as levels of TIMP3 mRNA remain unchanged with increasing age (Bailey et al., 2001). Similarly, studies have shown no increase in TIMP3 gene expression in retinas of SFD patients (Bailey et al., 2001; Chong et al., 2003), despite increased levels of TIMP3 being observed in the BrM of these patients (Chong et al., 2000; Fariss et al., 1998). Importantly, immuno-staining for TIMP3 is seen more strongly in the thickened BrM of patients with SFD than in healthy aged eyes (Fariss et al., 1998).

SFD-associated TIMP3 variants have been found to promote the formation of higher molecular weight protein complexes suggested to be a product of dimerisation/multimerisation between variant TIMP3 molecules (Langton et al., 1998; Langton et al., 2000; Yeow et al., 2002). Compared to wild-type TIMP3, SFD variant TIMP3 has been demonstrated to be more resistant to turnover, which is speculated to be a result of their aggregation; a reason thought to underlie variant TIMP3 accumulation in BrMs of SFD patients (Langton et al., 2000; Langton et al., 2005). The formation of such discrete TIMP3 in drusen-like deposits in the BrM could contribute to the thickening of this membrane with pathological outcomes including impaired vectorial transport and RPE atrophy (Langton et al., 2005).

It is important to note that TIMP3 accumulation is not exclusive to patients with variants in the *TIMP3* gene (Fariss et al., 1998). The accumulation of wildtype TIMP3 is reported in patients with AMD and Retinitis Pigmentosa (RP), as well as other retinopathies in which aberrant protein accumulation occurs in the BrM (Crabb et al., 2002; Fariss et al., 1998; Kamei and Hollyfield, 1999; Mullins et al., 2001). If SFD-associated variant TIMP3 leads to a lower turnover and therefore an accelerated accumulation of TIMP3, it could explain the earlier disease onset in SFD compared to AMD patients where wildtype TIMP3 aggregates at a slower pace. (Kamei and Hollyfield, 1999). Therefore an improved understanding of TIMP3-driven pathophysiology in SFD may also provide insights into related retinopathies such as AMD.

Authors have also suggested that TIMP3 accumulation could also be a consequence of intermolecular cross-linking due to oxidative reactions in the high photo-oxidative retinal environment (Beatty et al., 2000; Crabb et al., 2002). Another potential explanation is the dysregulation of the protein folding pathway which increases with age. Wild-type TIMP3 could therefore spontaneously misfold and become more resistant to turnover (Langton et al., 2005; Macario and Conway de Macario, 2002; Paschen and Frandsen, 2001). Interestingly, a binding partner of TIMP3, Fibulin 3, aberrantly accumulates in drusen-like deposits in the macular degenerative disease Malattia Leventinese, which suggests that accumulation of both proteins may be involved in the initiation of disease in different types of retinopathies (Klenotic et al., 2004; Marmorstein et al., 2002; Stone et al., 1999).

For a monogenic disorder, SFD displays a remarkable degree of heterogeneity; a complexity of disease phenotypes beyond a simple binary measure of whether TIMP3 accumulates or not. The nature and relative positions of variants in TIMP3 may underpin this heterogeneity, as they could impart different disease characteristics and phenotypes, which might affect the precise resistance to clearance and whether the protein retains its functions.

2.2 Association of phenotype with different TIMP3 variants

To improve development of future SFD therapies it will be necessary to determine whether there is a consistent mechanism of disease progression for all SFD-associated TIMP3 variants and how this leads to the heterogeneity of observed SFD phenotypes. Some studies have suggested that the severity of the disease, and in particular the age of onset, might be determined by certain characteristics of TIMP3 variants, such as whether the variant is in the N-terminal or C-terminal domains (Lin et al., 2006; Qi et al., 2009; Qi et al., 2013; Schoenberger and Agarwal, 2013; Warwick et al., 2016). Homology modelling has previously been used to produce a predicted 3D structure of TIMP3 and using UCSF Chimera 1.11.2, a graphic has been prepared for this review with SFD-associated variants highlighted (Fig. 3) (McCafferty and Sergeev, 2016; Pettersen et al., 2004). However, it is clear that although most SFD-associated TIMP3 variants are in the C-terminal domain, SFD can also be caused by the N-terminal domain variants Cys24Arg and Ser38Cys and severity is not easily correlated with the amino acid residue effected (Fig. 3 and Table 1) (Bakall et al., 2014; Carrero-Valenzuela et al., 1996; Felbor et al., 1997; Felbor et al., 1995; Fung et al., 2013; Meunier et al., 2016; Schoenberger and Agarwal, 2013; Tabata et al., 1998; Warwick et al., 2016).

Similarly, the majority of SFD-associated TIMP3 variants identified to date involve the gain of a cysteine residue, which is thought to allow intermolecular disulphide bonds and explain the dimerisation observed for several TIMP3 variants (Tables 1 and 2) (Barbazetto et al., 2005; Jacobson et al., 2002; Langton et al., 1998; Langton et al., 2000; Schoenberger and Agarwal, 2013; Weber et al., 1994). However, the gain or loss of a cysteine residue is not the sole cause of SFD, as shown by the TIMP3 variants Glu162Lys and His181Arg (Tables 1 and 2) (Lin et al., 2006; Saihan et al., 2009). There is also some disagreement in this area as Ser179Cys TIMP3 has both been demonstrated to form dimers and not to form dimers in ARPE-19 cells (Table 2) (Langton et al., 2005; Qi et al., 2002).

There is also some controversy as to whether SFD-associated TIMP3 variants retain their function to inhibit MMPs, although most studies have found that most variants tested thus far retained inhibitory capacity (Table 2) (Arris et al., 2003; Langton et al., 2000; Langton et al., 2005; Qi et al., 2002; Yeow et al., 2002). It has been suggested that loss of MMP inhibition is associated with earlier onset and a more severe phenotype, but it is not known with any certainty that this is the mechanism by which disease progression is driven (Langton et al., 2005; Saihan et al., 2009). Interestingly, it can be seen that 11 of the 16 residues at which SFD-associated variants have been identified are within or close to conserved metzincin-

and hemopexin-domain-binding regions (Fig. 2C). This might suggest that the capacity for SFD TIMP3 to bind to MMPs could be reduced, if not actually lost, in patients with these variants. There is also some controversy as to whether SFD-associated variant TIMP3 maintains its ability to inhibit the binding of VEGF to VEGFR (Fogarasi et al., 2008; Qi et al., 2009).

A recent comparison between the associated phenotypes for the variants Ser38Cys and Tyr191Cys, found earlier disease onset corresponding to a relatively larger change in the size of the amino acid as serine and cysteine are both small amino acids whereas tyrosine is larger. It was suggested that substituting a small amino acid with a large amino acid would have a greater effect on the protein and therefore lead to a more severe disease phenotype than substituting a small amino acid with another small amino acid (Meunier et al., 2016). Interestingly this study identified an associated pulmonary disease with a similar relationship between the amino acid substitution and the disease severity (Meunier et al., 2016). This suggests that a similar mechanism of disease development occurring in the lungs and lifestyle factors such as smoking could be a greater problem for SFD patients than for wildtype TIMP3 carrying counterparts (Meunier et al., 2016). However the early onset of disease associated with the Ser179Cys variant suggests that age of onset is not solely determined by the change in the size of the variant amino acid.

These studies demonstrate our current lack of understanding of how some variants in TIMP3 lead to disease. Thus far, all studies of SFD-associated variant TIMP3 function have been performed using exogenously expressed variant TIMP3 in ARPE-19 cells, using human cell-types other than RPE cells, or using animals or insects (Arris et al., 2003; Langton et al., 2000; Langton et al., 2005; Qi et al., 2009; Qi et al., 2013; Qi et al., 2002; Saihan et al., 2009; Weber et al., 2002; Yeow et al., 2002). The variability of cell-types used to study variant TIMP3 pathophysiology could have different outcomes due to inherent cell-specific differences. For instance, glycosylation differs between cell-types, and TIMP3 is known to be glycosylated at the C-terminal end of the protein (Fig. 2B) (Apte et al., 1995; Langton et al., 1998; Spiro, 2002). In fact, Ser179Cys TIMP3 expressed by an endothelial cell line has been reported to be predominantly in the glycosylated form, whereas wild-type TIMP3 produced by endothelial cells was predominantly unglycosylated (Qi et al., 2009). It will be important for future studies to be performed using endogenously-expressed variant TIMP3 in RPE cells in order to determine how these variants interact with potential binding partners, such as Fibulin 3, under physiological conditions. This will be possible with cell culture models using SFD patient-derived RPE cells and will hasten our understanding of TIMP3 in this blinding disorder.

## 3. Cell culture models to interrogate macular dystrophies

RPE cells have been isolated from porcine, murine, bovine as well as human source material in the past (Albert et al., 1972; Feeney and Mixon, 1976; Ho et al., 1997; Mannagh et al., 1973; Oka et al., 1984). RPE cells are now routinely cultured on porous supports to mimic the different apical and basal retinal environments (Dunn et al., 1996; Johnson et al., 2011; Ratnayaka et al., 2015). This has allowed studies into mechanisms by which the RPE cells perform their function as a barrier between the retina and the choroid (Ablonczy and Crosson, 2007; Ban and Rizzolo, 1997; Dunn et al., 1996; Rizzolo, 2014; Williams and Rizzolo, 1997).

A consequence of culturing RPE on a porous support is that cells display characteristic membrane specialisation as well as directional secretion of key proteins such as PEDF and VEGF which may be then quantified (Becerra et al., 2004; Blaauwgeers et al., 1999). Similarly, polarised expression of membrane proteins such as Aquaporin-1, Bestrophin-1, and $Na^+/K^+$-ATPase, which play critical roles in exchanging metabolites, may also be observed (Ames et al., 1992; Juuti-Uusitalo et al., 2013; Maminishkis et al., 2006; Stamer et al., 2003). The interaction between RPE cells and the choroidal endothelial cells can also be modelled in culture conditions in order to gain a better understanding of the blood-retinal barrier (Dardik et al., 2010; Hamilton et al., 2007). As well as forming a barrier and interacting with cells of the choroid, RPE cells have an important role in supporting the photoreceptors in the retinoid cycle and phagocytosis of photoreceptor outer segments (POS) and these processes can also be studied *in vitro* (Ablonczy et al., 2011; Dowling, 1960; Finnemann et al., 1997; Young and Bok, 1969).

By modelling these normal physiological functions of RPE cells, insights may be gained about ways in which breakdown of these processes contributes to disease. In addition, some specific disease-associated phenotypes can be similarly modelled in cell culture systems. For example, accumulation of known drusen components such as vitronectin, amyloid beta, and clusterin has also been observed in the pores of the supporting membrane, whilst deposition of complement proteins has been demonstrated (Johnson et al., 2011; Lynn et al., 2017). Such advances in *ex vivo* cultures make them highly amenable to studying drusen formation as well as related aspects of RPE pathobiology. These in-vitro cultures could take advantage of patient-derived RPE cells with the relevant disease-associated genetic variants and genetic background. This would also circumvent the need to directly obtain primary RPE from donor tissues, which are in scarce supply and often obtained as many as 24-48 hours after death.

3.1 Generation of retinal pigment epithelial cells from pluripotent stem cells.

A better source of RPE cells than primary animal or human tissue or the ARPE-19 cell line might be from differentiation of pluripotent stem cells (PSCs). Pluripotency is defined as the capacity to differentiate to produce cells from all three germ layers. The first PSCs to be obtained were mouse embryonic stem cells (ESCs) with human ESCs following 17 years later (Evans and Kaufman, 1981; Thomson et al., 1998). Differentiation to produce RPE-like cells was first shown with primate ESCs (Kawasaki et al., 2002) and since then human ESCs have been differentiated to produce RPE by directed and spontaneous differentiation (Idelson et al., 2009; Klimanskaya et al., 2004; Osakada et al., 2009; Vugler et al., 2008).

RPE derived from PSCs (PSC-RPE) are pigmented, phagocytose POS, form a strong barrier with a high transepithelial electrical resistance, and express characteristic RPE markers (Brandl et al., 2014; Buchholz et al., 2009; Idelson et al., 2009; Klimanskaya et al., 2004; Kokkinaki et al., 2011; Liao et al., 2010; Vugler et al., 2008). Importantly, comparisons suggest that PSC-RPE are a better model of native adult human RPE than cell lines such as ARPE-19 (Brandl et al., 2015; Carr et al., 2009; Garcia et al., 2015; Vugler et al., 2008). In fact, it has been suggested that PSC-RPE are morphologically similar to RPE cells of the human macula, meaning that they could be particularly useful for modelling macular dystrophies (Vugler et al., 2008). The potential for PSC-RPE to appropriately perform the functions of native RPE is underlined by their use in human clinical trials involving patients with AMD and Stargardt's macular dystrophy (Schwartz et al., 2012).

A more recent innovation is the ability to generate induced pluripotent stem cells (iPSCs). iPSCs are generated by reprogramming of somatic cells to produce an ESC-like cell with the same characteristics of pluripotency (Takahashi and Yamanaka, 2006). Human iPSCs have similarly been differentiated to produce RPE cells (Buchholz et al., 2009; Golestaneh et al., 2016; Hirami et al., 2009; Kokkinaki et al., 2011; Leach et al., 2016; Osakada et al., 2009; Vaajasaari et al., 2011). iPSCs have benefit over ESCs for modelling disease because they can be generated from patient-derived somatic cells, such as skin fibroblasts, allowing derivation of autologous RPE cells carrying the same variants as *in situ* RPE cells from the patient (Golestaneh et al., 2016; Parfitt et al., 2016; Singh et al., 2013). This enables studies to directly assess the effects of TIMP3 variants in the RPE cells of SFD patients. Use of ESCs and iPSCs presents unique challenges. For instance, it is possible that ESCs will be more useful for cell replacement therapies, as human leukocyte antigen-matched ESC lines could be used for multiple patients. Thereby being far cheaper and more practical than the requirement to generate an iPSC line for every individual patient. However, iPSCs will be

more useful for studying diseases because of the ability to investigate the effects of different disease-associated variants on cell function within the patient's genetic background, which might also have an effect on the patient's unique disease phenotype.

3.2 Use of pluripotent stem cell-derived retinal pigment epithelial cells to model disease.

Although thus far iPSCs have not been used to model SFD, they have been used to study similar diseases, including AMD, Best disease and RP (Golestaneh et al., 2016; Lukovic et al., 2015; Moshfegh et al., 2016; Singh et al., 2013), providing a proof of principle for this type of investigation. Best disease is characterised by accumulation of autofluorescent sub-retinal lipofuscin deposits and fluid (Blodi and Stone, 1990; Xiao et al., 2010). Similar accumulation of autofluorescent deposits was observed in patient-derived iPSC-RPE after physiologically stressing the cells by feeding with bovine POS (Singh et al., 2013). This demonstrates the ability to model the development of a disease phenotype *in vitro* in a far shorter time than the decades over which the disease develops *in vivo*. Importantly, in the absence of physiological stress, PSC-RPE derived from patients and from healthy siblings were functionally comparable, did not form deposits, and demonstrated similar levels of expression of characteristic RPE marker proteins in parallel. This reflects the fact that the disease develops over decades and patients can often retain vision until the $5^{th}$ to $6^{th}$ decade, suggesting that it would be unlikely to identify disease-associated phenotypes in patient-derived RPE cells after a short amount of time in culture in the absence of physiological stresses (Fishman et al., 1993; Singh et al., 2013). SFD patient-derived iPSC-RPE cells could be used to assess whether TIMP3 accumulates during early drusen formation. Another study of Best disease patient-derived iPSC-RPE used a biosensor to demonstrate a defect in chloride ion influx in patient-derived cells, which contributes to sub-retinal fluid accumulation (Moshfegh et al., 2016). iPSC-RPE from derived from patients with MERTK-associated RP failed to phagocytose POS under *in vitro* conditions, highlighting the importance of such models to recapitulate and study key features of disease (Lukovic et al., 2015).

Similar studies using iPSC-RPE sourced from different SFD patients (Fig. 4E-F) could be used to study how different TIMP3 variants affect this important monolayer in the retina. These studies should include assessment of TIMP3 function, determination of RPE function in terms of maintaining barrier integrity, polarised secretion of growth factors, and POS phagocytosis, as well as investigation of potential changes in the underlying ECM and the interaction between the RPE cell layer and the choroidal endothelial cells. Such experiments

will provide insights into the mechanisms by which SFD progresses and develops towards choroidal neovascularisation or geographic atrophy (Fig. 4A-D).

## 4. Conclusions

SFD is a poorly understood macular dystrophy. Although multiple disease-associated variants have been identified within a single protein, the heterogeneity of phenotypes and differences in findings between studies of SFD-associated TIMP3 function means that it is still unclear how the disease develops (Langton et al., 2005; Qi et al., 2002). Thus, it is still not known whether SFD-associated TIMP3 variants lead to a gain of function in which TIMP3 is more likely to accumulate, disrupting overlying RPE. Alternatively, the disease could develop via a loss of function in which the capacity for TIMP3 to inhibit MMP activity or VEGF signalling is reduced leading to ECM dysregulation and neovascularisation. Although it might be expected that all SFD-associated TIMP3 variants would lead to disease development via a shared mechanism, the observations that some, but not all, variants affect MMP inhibition and that there are contradictory results for some variants (Table 2) demonstrate a further need to investigate TIMP3. This is made more challenging by a long list of known SFD-associated variants, which are not all fully characterised (Table 1). The frequency with which new disease-associated TIMP3 variants are discovered makes it probable that more variants are still to be identified, particularly with the rise of next generation sequencing. Several of these variants have disproved previous ideas of how TIMP3 variants lead to disease. For example, the discovery of the N-terminal Cys24Arg and Ser38Cys variants demonstrated that the disease is not exclusively caused by C-terminal variants (Bakall et al., 2014; Schoenberger and Agarwal, 2013). Due to the similarity of phenotypes between AMD and SFD and the overlap in age of onset (Fig. 4G), it is possible that some SFD patients have been mis-diagnosed as having early onset AMD. Identifying these patients may contribute to the identification of novel TIMP3 variants that could help to elucidate the shared mechanisms in these diseases. Care should also be taken when considering AMD cohorts in early studies as they may contain some SFD patients. In this review, we have included a list of all known SFD-associated TIMP3 variants to demonstrate the range of ages of onset as well as the different types of variants that have been identified to date (Table 1). For clarity, we have included the previously commonly used nomenclature for amino acid residue numbering alongside the current nomenclature based on HGVS guidelines (Table 1), which we have used throughout and we would suggest that future publications employ this newer nomenclature to avoid confusion.

Until now, much of the work on SFD-associated variant TIMP3 function has been done in cell-types other than RPE cells or in non-human cells (Table 2), which is not ideal for the purpose of elucidating the mechanism by which specific TIMP3 variants could drive disease in human RPE cells. By exploiting advances in RPE cell culture techniques and the power of iPSCs to generate patient-derived cell-types that are otherwise difficult to obtain, there is the possibility to realistically re-create important aspects of SFD under *in vitro* conditions. Use of patient-derived iPSC-RPE will enable further studies of how specific disease-associated TIMP3 variants bring about different phenotypic changes (Fig. 4E and F). Potential insights could help not only our understanding of SFD but also related retinopathies such as AMD and RP, in which TIMP3 accumulation has also been observed, meaning that an improved understanding of SFD could be beneficial for the development of therapies for AMD.

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Figure Legends

**Figure 1.** Fundus images showing phenotypes associated with Sorsby fundus dystrophy. Fundus images show large pigment epithelial detachments in the macula of the right eye (A; white asterisks) and soft drusen in the macula of the left eye (B; white x) of a 55 year old man initially diagnosed with AMD, but subsequently being found to have a Sorsby fundus dystrophy-associated Ser38Cys TIMP3 variant. In the fundus images of a 37-year old female SFD patient with the Ser204Cys TIMP3 variant, a disciform scar can be seen in the right eye (C; arrowhead) and an early choroidal neovascular membrane in the macula of the left eye (D; outlined by a dashed line). Early (E) and late (F) frames of a fluorescein angiogram demonstrate increasing hyperfluorescence in the right eye of the 55 year old man, consistent with a fibrovascular pigment epithelial detachment. Optical coherent tomogram in the right eye of the 55 year old man (G) demonstrates a large epithelial detachment (white asterisk), with associated subretinal fluid (white dashed arrows) and drusen (white solid arrow). The optic disc is also shown (OD).

**Figure 2**. Predicted linear (A) and 3D (B) schematics of TIMP3 protein structure demonstrating the presence of intramolecular disulphide bonds between cysteine residues. The 3D schematic is derived from a pdb file produced by previously published homology modelling (McCafferty & Sergeev, 2016). Cysteine residues are represented by blue circles on the linear diagram (A) and with arrows on the 3D schematic (B) and disulphide bonds between cysteine residues are represented by black lines. Disulphide bonds are predicted to form between Cys24 (Cys1) and Cys91 (Cys68), Cys26 (Cys3) and Cys118 (Cys95), Cys36 (Cys13) and Cys143 (Cys120), Cys145 (Cys122) and Cys192 (Cys169), Cys150 (Cys127) and Cys155 (Cys132), and Cys163 (Cys140) and Cys184 (Cys161). Amino acid residues have previously been described using a different nomenclature, which is given in brackets above. Metzincin- and hemopexin-domain-binding domains, which are involved in binding to MMPs, are highlighted with boxes (A). The C-terminal glycosylation site is shown on the 3D schematic with the ribbon coloured grey (B). Alignments of the MMP-binding metzincin- and hemopexin-domain-binding domains for TIMP3, TIMP1, TIMP2, and Chicken TIMP3 amino acid sequences are shown with capitalised letters representing conserved features of the binding domains (C). Sequence alignments are derived from NCBI's Conserved Domain Database. Amino acid residues highlighted in red have been found to be substituted in SFD-associated TIMP3 variants. Underlined amino acid residues are cysteine residues that form disulphide bonds. Molecular graphics were developed with the UCSF Chimera 1.11.2 package. Chimera is developed by the Resource for Biocomputing, Visualisation, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

**Figure 3.** A representation of a theoretical model of the structure of TIMP3 based on previously published homology modelling (McCafferty & Sergeev, 2016). The chain is shown as a ribbon and coloured based on the proximity to the N-terminus (blue) or the C-terminus (red), such that the N-terminal domain is blue and green and the C-terminal domain is yellow, orange, and red. Arrows are used to identify and label the amino acid residues that have been found to be the sites of variants associated with Sorsby fundus dystrophy. A dashed line represents an approximate dividing point between the N-terminal domain which is involved in binding to MMPs and the C-terminal domain which is involved in binding to VEGFR2. Molecular graphics were developed with the UCSF Chimera 1.11.2 package. Chimera is developed by the Resource for Biocomputing, Visualisation, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

**Figure 4.** Schematic diagram of the outer retina representing a healthy eye (A) and disease progression in Sorsby fundus dystrophy (SFD; B, C, D). Sub-RPE drusen, likely to disrupt the RPE layer, and thickened Bruch's membrane have been observed in patients with the disease (B). This then progresses to either a choroidal neovascularisation (CNV) state (C), or geographic atrophy (GA) occurs (D), both resulting in photoreceptor loss. To further understand the pathogenesis of disease, induced pluripotent stem cells (iPSC) have been reprogrammed from somatic cells of SFD patients (E) and differentiated into RPE cells (F). The cells shown here (E, F) are derived from the 71 year old father of the patient who's retina is shown in Fig. 1C-F. Our studies will also help increase knowledge of age-related macular degeneration (AMD) with which SFD has a phenotypic and genetic overlap (G).

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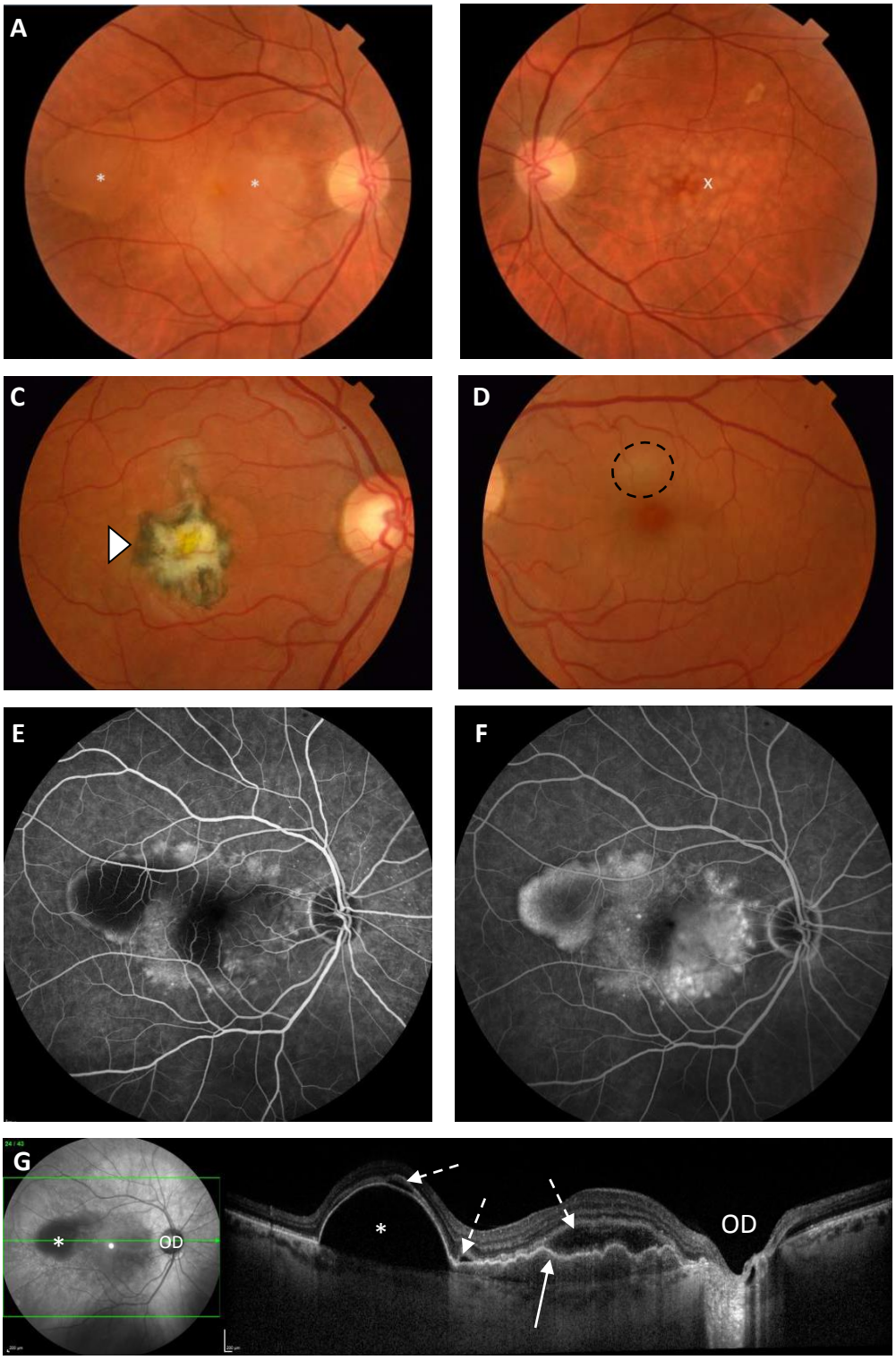
| TIMP3 Variants | | | Approximate Age of Onset (Years) | Original Publication |
|---|---|---|---|---|
| **Current Nomenclature (conforming to HGVS guidelines)** | **Old Nomenclature** | **ID Numbers** | 20 40 60 80 | |
| Cys24Arg | n/a | - | Early | Bakall et al, 2014 |
| Ser38Cys | n/a | CM128770 | | Schoenberger and Agarwal, 2013 |
| Intron 4/exon 5 splice site | n/a | - | | Tabata et al, 1998 |
| Tyr151Cys | n/a | - | Early | Bakall et al, 2014 |
| Glu162Lys | Glu139Lys | CM093568 | | Saihan et al, 2009 |
| Glu162STOP | Glu139STOP | NM_000362.4:c.484G>T | | Langton et al, 2000 |
| Tyr174Cys | n/a | - | | Gliem et al, 2015 |
| Tyr177Cys | n/a | - | | Gliem et al, 2015 |
| Ser179Cys | Ser156Cys | NM_000362.4:c.536C>G | | Felbor et al, 1995 |
| His181Arg | His158Arg | CM065486 | | Lin et al, 2006 |
| Tyr182Cys | Tyr159Cys | - | | Fung et al, 2013 |
| Gly189Cys | Gly166Cys | NM_000362.4:c.565G>T | | Felbor et al, 1997 |
| Gly190Cys | Gly167Cys | CM951214 | | Jacobson et al, 1995 |
| Tyr191Cys | Tyr168Cys | NM_000362.4:c.572A>G | | Felbor et al, 1996 |
| Ser193Cys | Ser170Cys | CM051655 | | Barbazetto et al, 2005 |
| Tyr195Cys | Tyr172Cys | CM022256 | | Jacobson et al, 2002 |
| Trp198Cys | n/a | - | Early | Bakall et al, 2014 |
| Ser204Cys | Ser181Cys | NM_000362.4:c.610A>T | | Weber et al, 1994 |

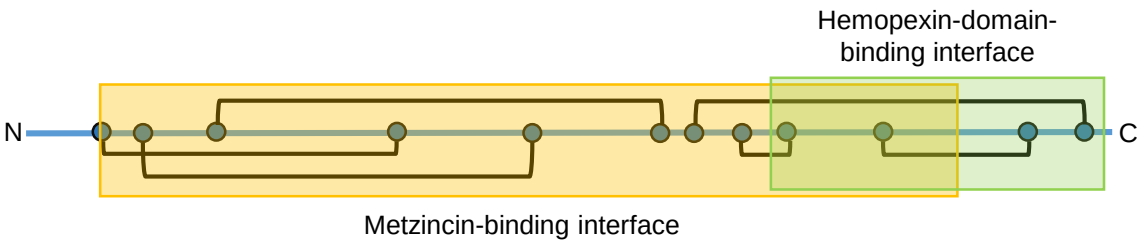
**Table 1**. Known TIMP3 genetic variants associated with Sorsby fundus dystrophy. Approximate age of onset of visual symptoms for each variant is included.

**Table 2. Summary of studies on the ability of SFD-associated TIMP3 mutants to dimerise or inhibit MMP activity.**

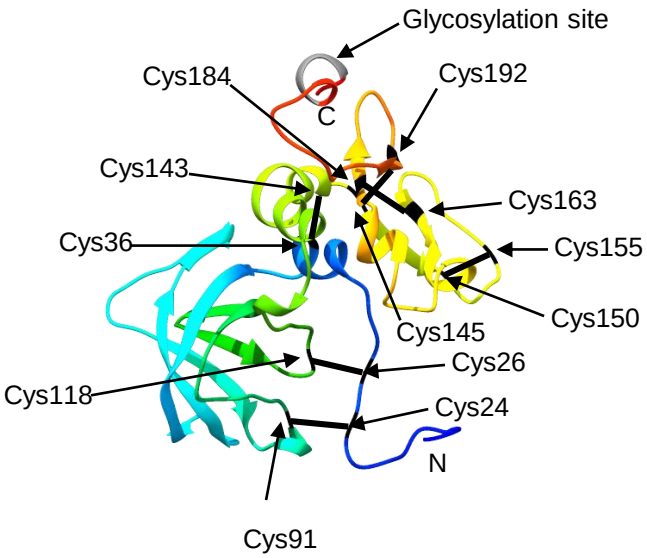
| Dimerisation | | MMP inhibition | | References |
|---|---|---|---|---|
| Glu162STOP | Dimers have been seen in transfected insect cells[1] and ARPE-19 cells[2]. Premature termination results in loss of 3 cysteine residues, leaving one unpaired cysteine residue, Cys145. | Glu162STOP | Mutant TIMP3 has been shown to retain MMP inhibitory function in transfected insect cells[1], but this function has been seen to be lost in patient-derived fibroblasts[1] and in ARPE-19 cells[2]. | 1. Arris et al, 2003.<br>2. Langton et al, 2005.<br>3. Langton et al, 2000.<br>4. Yeow et al, 2002.<br>5. Weber et al, 2002.<br>6. Qi et al, 2002.<br>7. Langton et al, 1998.<br>8. Saihan et al, 2009. |
| Ser179Cys | Dimers have been identified in COS-7[3], BHK[4], patient-derived fibroblasts[5] and mouse RPE cells[5].<br>However, another study found no presence of dimers or any higher molecular weight aggregates in ARPE-19 cells expressing mutant TIMP3[6]. | Ser179Cys | Mutant TIMP3 has been shown to retain MMP inhibitory function in COS-7[3], BHK[4], and ARPE-19 cells[2]. However, one study demonstrated loss of this function in ARPE-19 cells and even found an increase in secretion of MMP2 in ARPE-19 cells expressing this TIMP3 mutant[6]. | |
| Gly189Cys | Presence of dimers shown in COS-7 cells[3]. | Glu162Lys | MMP inhibitory function was retained in ARPE-19 cells[8]. | |
| Gly190Cys | Presence of dimers shown in BHK cells[4]. | Gly189Cys | MMP inhibitory function was retained in COS-7 cells[3]. | |
| Tyr191Cys | Presence of dimers shown in BHK cells[4]. | Gly190Cys | MMP inhibitory function was retained in BHK cells[4]. | |
| Ser204Cys | Presence of dimers shown in COS-7[7], BHK[4], and patient-derived fibroblast cells[1, 5]. | Ser204Cys | MMP inhibitory function was retained in COS-7[3], BHK[4], and patient-derived fibroblast cells[1]. | |
| Gly162Lys | Presence of dimers has been shown in ARPE-19 cells, despite no gain or loss of a cysteine residue. Slightly later onset than for some other mutations possibly due to this being a less stable dimer without a disulphide bond[8]. | | | |


A
C
D
E
F
G
OD
OD

**A**




**B**




**C**

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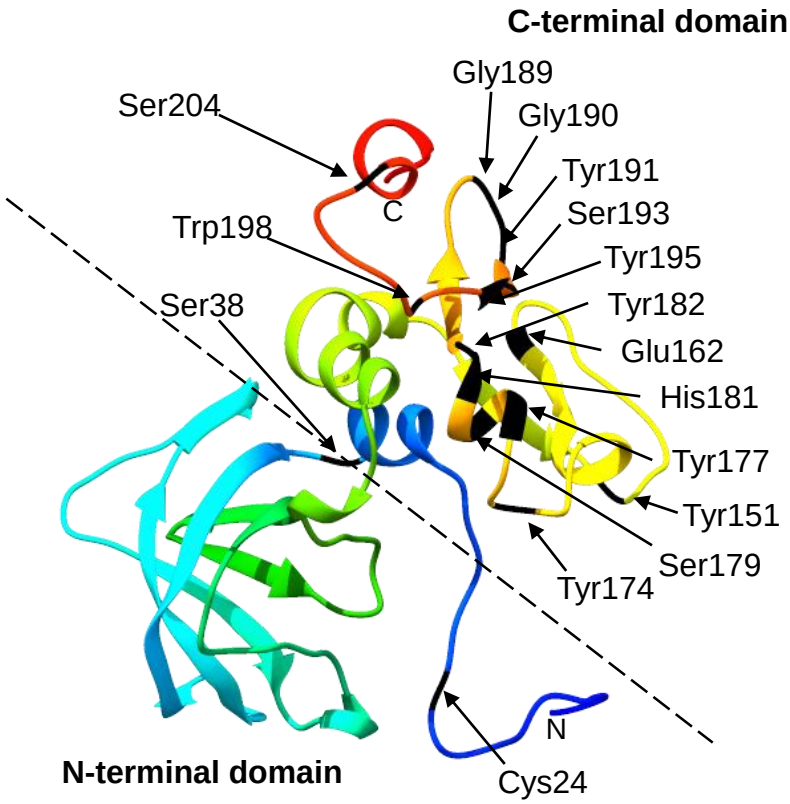
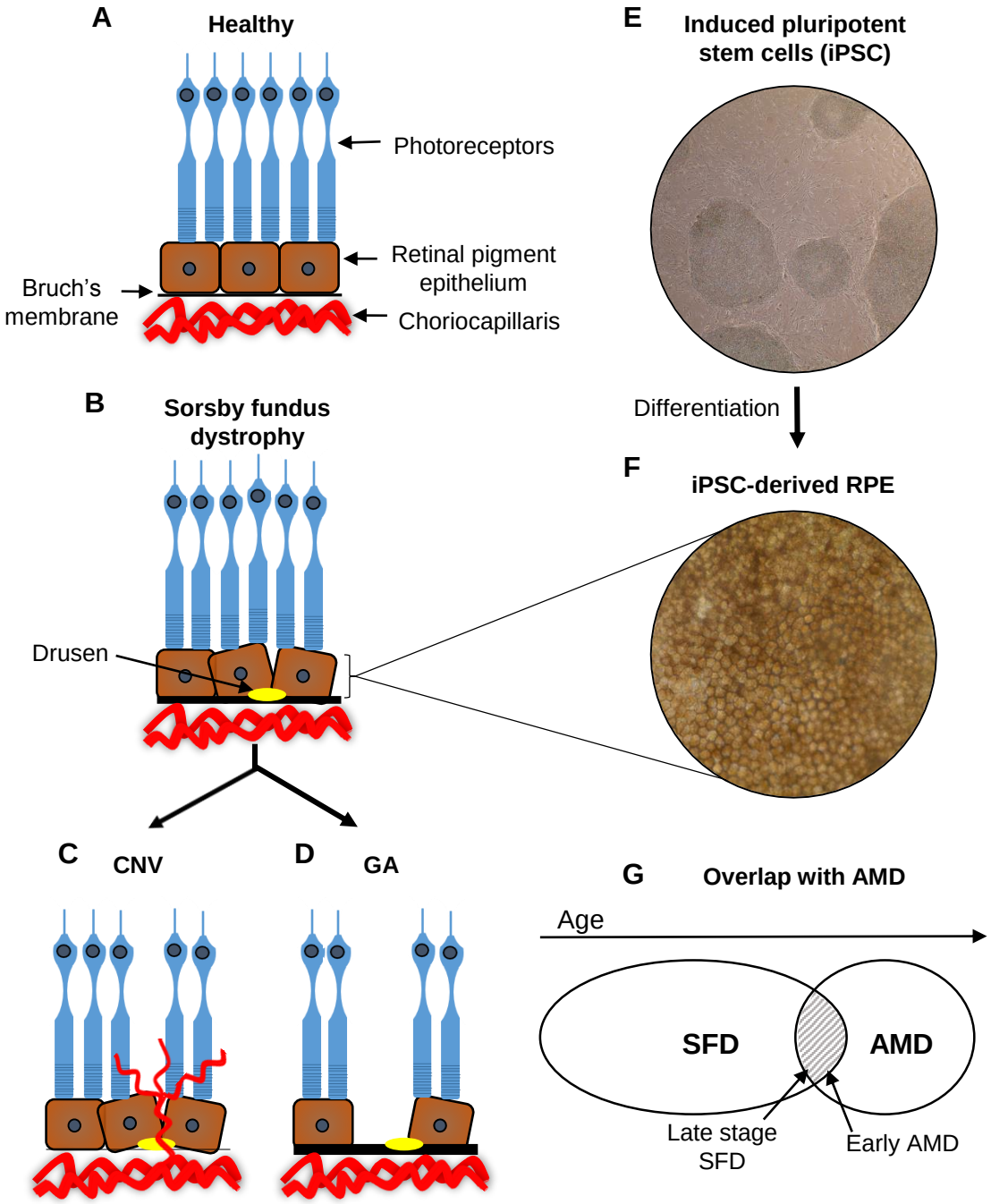
Metzincin-binding residues:
TIMP3: 24 CTCSP… 58 GT… 85 EASESLC… 107 R… 114 YtGLC… 149 ScYY… 168 M… 174 Y…
TIMP1:  1 CTCVP… 34 LY… 64 PAMESVC…  88 K…  95 HiTTC… 131 PcLS… 150 Q… 156 E…
TIMP2:  1 CSCSP… 41 KR… 66 APSSAVC…  89 K…  97 HiTLC… 132 RcPM… 151 W… 157 I…
Chicken
TIMP3: 25 CTCVP… 59 GT… 86 EASESLC… 108 R… 115 YtGLC… 150 PcYY… 169 M… 175 H…
Hemopexin-domain-binding residues:
TIMP3: 154 PCFVtsKnecLwTdmL… 182 YacirQkGgYCSWYRgwA…
TIMP1: 136 PCKLqsGthcLwTdqL… 164 LaclpRePgLCTWQSlrS…
TIMP2: 137 PCYIssPdecLwMdwV… 165 FacikRsDgSCAWYRgaA…
Chicken
TIMP3: 155 PCFAtsKnecIwTdmL… 183 YaciqRvEgYCSWYRgwA…
```


C-terminal domain
Gly189
Ser204
Gly190
Tyr191
C
Ser193
Trp198
Tyr195
Ser38
Tyr182
Glu162
His181
Tyr177
Tyr151
Ser179
Tyr174
N
N-terminal domain
Cys24


A
Healthy
Photoreceptors
Retinal pigment epithelium
Bruch's membrane
Choriocapillaris
B
Sorsby fundus dystrophy
Drusen
C
CNV
D
GA
E
Induced pluripotent stem cells (iPSC)
Differentiation
F
iPSC-derived RPE
G
Overlap with AMD
Age
SFD
AMD
Late stage SFD
Early AMD

**Highlights**

- Sorsby fundus dystrophy (SFD) is a dominant macular disease leading to blindness.
- We estimate the prevalence of SFD at approximately 1 in 220,000.
- SFD is driven by multiple different variations in TIMP-3, which are summarised.
- Mechanisms by which variants in TIMP3 might lead to disease are discussed.
- We propose that patient-derived stem cells may provide a new model to study SFD.