A second independent Tyr168Cys mutation in the tissue inhibitor of metalloproteinases-3 (TIMP3) in Sorsby’s fundus dystrophy

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Abstract
Sorsby’s fundus dystrophy (SFD) is a rare autosomal dominant macular disorder with age of onset usually in the fourth decade. It is characterised by loss of central vision owing to subretinal neovascularisation and disciform macular degeneration. In an effort to identify the SFD gene, the disease locus was first mapped to chromosome 22q13-ter by genetic linkage analysis, the same chromosomal region as the gene encoding the tissue inhibitor of metalloproteinases-3 (TIMP3). Subsequently, two separate mutations in TIMP3 were found in affected members of two unrelated SFD pedigrees (Tyr168Cys and Ser181Cys). More recently, two additional SFD related mutations, Ser156Cys and Gly167Cys, have provided further confirmation that heterogeneous mutations in TIMP3 are causally responsible for the SFD phenotype. We now report the occurrence of the Tyr168Cys mutation in an SFD patient of Austrian descent and show that this mutation found earlier in an American SFD family arose independently. The new findings add to an emerging pattern of SFD mutations which all seem to affect the C-terminal region of the mature TIMP3 protein. In addition, all known mutations cause a change of an amino acid to a cysteine residue. This suggests a critical role for the additional C-terminal free thiol group in SFD pathogenesis.

Key words: Sorsby’s fundus dystrophy; mutational analysis; tissue inhibitor of metalloproteinases-3 (TIMP3).

The macular dystrophies represent a large group of distinct disorders that affect central vision. In this group, age related macular degeneration (AMD) has an estimated prevalence of almost 20% in the population over 65 years of age in developed countries. To facilitate the development of adequate therapeutic treatment, it is important to gain insights into the basic pathogenesis of these macular disorders. Clinical similarities in the early disease processes of some forms of macular degeneration have often been noted and are of particular interest for the understanding of the genetically complex AMD. Based on some common histopathological features, particularly at the level of Bruch’s membrane, autosomal dominant Sorsby’s fundus dystrophy (SFD) is thought to provide an excellent genetic model for the study of AMD.

SFD is a fully penetrant autosomal dominant macular disorder characterised by loss of central vision from subretinal neovascularisation or atrophy of the retina and choroid. Early features of SFD include a delayed choriocapillary filling on fluorescein angiography and a deposition of confluent, lipid containing material between the basement membrane of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch’s membrane. These early manifestations in SFD appear very similar to findings in a large subgroup of patients with AMD.

In an effort to determine the molecular basis of SFD, we first mapped the disease locus to chromosome 22q13-ter. This same region was shown to contain the gene encoding the tissue inhibitor of metalloproteinases-3 (TIMP3), the most recently isolated member of a family of proteins that regulate the proteolytic activity of ECM degrading matrix metalloproteinases (MMPs). Subsequently, we detected two point mutations in TIMP3 in affected members of two SFD pedigrees suggesting TIMP3 as the SFD gene.

More recently, two additional TIMP3 mutations, Ser156Cys and Gly167Cys, have been identified in SFD patients. However, the pathophysiological consequences of these mutations are still unknown. The characterisation of additional mutations in the SFD gene and, in particular, in vitro mutagenesis is likely to provide new clues to the mechanism by which such mutations may cause the clinical features in SFD.

Case report
At the age of 33, our patient experienced a sudden loss of central vision in the right eye resulting from subretinal macular haemorrhage with glial cicatisation of the neuroretinal layer. The visual acuity was 20/400 right eye with -3.0 sph and 20/25 left eye with -4.5 sph, -0.75 cyl axis 170°. On fundoscopy of the right eye, there was a large discolimon plantation scar of the macula surrounded by accumulations of pigment and atrophic zones of the RPE and choriocapillaris. In the posterior pole of the left eye, the optic disc appeared normal, but there was RPE atrophy peripheral to the fovea with...
regional atrophy of the choriocapillaris. Fine drusen-like deposits of the RPE layer were irregularly scattered throughout the posterior poles and the midperipheral regions bilaterally. Regions of RPE atrophy with pigment clumping could be detected in all quadrants. On static perimetry, there was an absolute central scotoma of the right eye and a relative central scotoma of the left eye, both extending to about 15° peripherally. Standard electroretinography (ERG) showed normal rod and cone signals in both eyes.

At the age of 37, the patient presented with an abrupt decrease in central vision in the other eye owing to fresh choroidal neovascularisation with slight subretinal haemorrhage and serous retinal detachment. Dark adaptation was 2 log units above normal bilaterally. Electro-oculography (EOG) showed subnormal light/dark ratios (1.54 RE and 1.58 LE). Standard scotopic ERG showed a selective rod response within normal limits whereas standard flash and photopic ERG were subnormal.

The family history remains unclear as the patient's mother and two brothers (aged 34 and 32) showed no characteristic ophthalmological features of SFD, while the father and the paternal grandmother died early in life. The father's sibs had been adopted by various families.

Materials and methods

The human TIMP3 gene consists of five coding exons distributed over approximately 55 kb of genomic DNA. PCR primer pairs flanking the exons are listed in the table. The conditions for PCR amplification have been reported elsewhere. Briefly, after an initial denaturation of one minute at 94°C, 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C (exon 1 and 5) or at 52°C (exons 2, 3, and 4), and 30 seconds at 72°C were carried out followed by a final extension of five minutes at 72°C.

To increase sensitivity of the single stranded conformational polymorphism (SSCP) analysis, the PCR products were digested with various restriction enzymes to yield fragments <200 bp (table). A combination of two gel running conditions was applied using 6% non-denaturing polyacrylamide gels with (5%) and without glycerol at 4°C, 25 W constant power for four to seven hours.

Exon 5 PCR fragments were cloned into the pCR II vector (Invitrogen). Recombinant clones were sequenced using the dideoxy nucleotide chain termination method (USB, Cleveland) and sequencing primers M13(–40)F (5'-GTT TTC CCA GTC ACG A-3') and M13(–40)R (5'-CAG GAA ACA GTG ATG AC-3'). Genotyping was performed using (GA), dinucleotide repeat markers at loci D22S275 and D22S281 and PCR conditions as described. The intragenic polymorphism in exon 3 of the TIMP3 gene is the result of a silent mutation in codon 60 and 64, respectively, and can be analysed by SSCP of exon 3 PCR fragments amplified with primers ex3/ex3R (table).

Results

To test the Austrian patient for mutations in TIMP3, we performed SSCP of the entire

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**Figure 1 (A) A SSCP mobility shift in exon 5 of the TIMP3 gene in Austrian SFD patient G. For comparison, two controls are shown (DS = double strand). (B) Sequencing of exon 5 of TIMP3 shows a heterozygous A to G transition in the second base of codon 168 resulting in a Tyr168Cys alteration in the mature TIMP3 protein.**
coding sequence of the gene. Analysis of exon 5 showed a band shift (fig 1A) which was not found in her two brothers or in 270 unaffected controls. However, this mobility shift was similar to a TIMP3 mutation in exon 5 previously identified in an American SFD family.9

Sequencing of the mutant allele showed an A to G transition in the second position of codon 168 changing a highly conserved tyrosine residue13 to a cysteine (fig 1B). These alterations are identical to the findings in the American SFD family.9

To ascertain whether the Tyr168Cys mutation in the American SFD family9 and our Austrian SFD patient originated from a common ancestor, we genotyped the critical subjects with (CA)n dinucleotide repeat markers at D22S275 and D22S281 as well as a frequent intragenic SSCP in exon 3 of the TIMP3 gene. The Austrian patient does not carry the particular alleles at D22S275 and D22S281 associated with SFD in the American family (fig 2A). In addition, the intragenic SSCP in exon 3 shows that the band shift segregating with SFD in the American family is not shared by the Austrian SFD patient (fig 2B).

Discussion

To date, four different mutations have been identified in TIMP3 in patients diagnosed with Sorsby’s fundus dystrophy, including a Ser156Cys mutation in a German-Czech family with an atypical early onset and a rapid progression of the disease,10 a Gly167Cys mutation,11 a Tyr168Cys mutation in an American family,9 and a Ser181Cys mutation in a Canadian family of Irish descent.7 In addition, we report here a second Tyr168Cys mutation in an Austrian patient and unambiguously show its independent occurrence from the identical American mutation.

Despite this relatively small number of known mutations in SFD, a mutational pattern underlying the disease appears to emerge. So far, all mutations have been identified in exon 5 of the TIMP3 gene, thus affecting the C-terminal region of the mature protein. Although little is known about the genetics of TIMP3 action, the C-terminal domains of TIMP1 and TIMP2 are thought to increase binding stability between the inhibitor and its matrix metalloproteinases and, thus, to influence the rate of inhibition through low affinity interactions.17,18 In addition, the N-terminal domains of TIMP1 and TIMP2 alone retain metalloproteinase inhibitory function and appear not to require their C-terminal domains for full activity.19,20 This suggests that the mutational changes in the C-terminal domain of TIMP3 may not fully interfere with TIMP3 inhibitory function and could explain, at least in part, the late onset of symptoms usually in the fourth decade of life.3 Similarly, partial activity of the mutated TIMP3 protein could also account for the absence of pathological features in other body tissues with high TIMP3 expression.7,15

It is striking that all known TIMP3 mutations lead to the introduction of an additional cysteine residue in the peptide sequence suggesting that the mechanism underlying the SFD pathology may be directly related to the presence of a free thiol group in the C-terminal part of the peptide. This raises the question of whether other types of mutations in the TIMP3 gene could be responsible for other as yet unknown clinical conditions different from SFD. The study of transgenic animals carrying various types of mutations in the TIMP3 gene may provide some clues to the effect of specific mutations on the resulting clinical phenotype.

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