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Autosomal Recessive Sorsby Fundus Dystrophy Revisited: Molecular Evidence for Dominant Inheritance

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Summary

Sorsby fundus dystrophy (SFD) originally was characterized as an autosomal dominant disorder in which patients lose central vision during the 4th or 5th decade of life. Since Sorsby's initial description, interfamilial phenotypic variations have been noted and have given rise to controversy as to whether SFD constitutes more than one nosologic entity. In addition, several reports have proposed the existence of a recessively inherited form of SFD. The recent identification of the tissue inhibitor of metalloproteinases-3 (TIMP3) as the disease-causing gene in SFD has made it possible to address the questions of clinical and genetic heterogeneity. In this study, we reinvestigated a large, highly consanguineous Finnish family previously diagnosed as having early-onset autosomal recessive SFD. We identified a novel heterozygous Gly166Cys mutation in TIMP3 in all affected individuals and provide strong evidence for an autosomal dominant inheritance of the SFD phenotype in this family. Our results, in conjunction with a critical review of the reported cases, render the existence of a recessive mode of inheritance in SFD questionable. Considering all available data, we suggest that SFD is a genetically homogeneous, autosomal dominant condition.

Introduction

Sorsby fundus dystrophy (SFD) was described by Sorsby, Mason, and Gardener in 1949, as an autosomal dominant disorder in which patients experience a rapid decline of central vision during their 40s and lose ambulatory vision 3–4 decades later. In his original report, Sorsby noticed bilateral macular "hemorrhage and exudates developing into generalized choroidal atrophy with massive pigment proliferation" (Sorsby et al. 1949, p. 67). Follow-up studies of the families initially characterized by Sorsby emphasized the occurrence of interfamilial phenotypic variation and raised the possibility of genetic heterogeneity in this condition. For instance, affected members of the Kempster family reported progressive difficulties with night vision, for as long as 25 years before loss of visual acuity (Capon et al. 1988), whereas the Carver family had difficulties in adapting to sudden changes in ambient light (Polkinghorne et al. 1989), and the Ewbanks were asymptomatic prior to loss of visual acuity (Hoskin et al. 1981). Affected individuals in the latter family experienced a sudden decrease in central vision, triggered by subretinal macular neovascularization. In contrast, two patients from the Kempster family revealed atrophic macular disease without choroidal neovascularization, whereas the Carvers predominantly demonstrated a slow progression of the disease, associated with chorioretinal atrophy.

The existence of an autosomal recessive form of SFD was first suggested in 1958 by François, who had observed two affected brothers of healthy parents. Another study reported several affected members in a large family residing in the isolated parish of Lavia in southwestern Finland (Forsius et al. 1982). Genealogical studies revealed numerous consanguineous marriages in the ancestry. To account for the absence of any signs of SFD in the ancestors whereas a consanguineous couple and all their eight children were found to be affected, an autosomal recessive mode of inheritance of SFD was proposed (Forsius et al. 1982). Accordingly, the affected parents were assumed to be homozygotes giving rise only to affected children. In agreement with a recessive mode of inheritance, the manifestation of SFD was significantly earlier than that generally seen in the autosomal dominant SFD families (Hoskin et al. 1981; Capon et al. 1988; Polkinghorne et al. 1989). In a follow-up study (Eriksson et al. 1990), the 25-year-old daughter of an affected female also was diagnosed with SFD and has led the authors to discuss the possibility of autosomal dominant or pseudodominant inheritance of the disease trait in the Finnish family.

Recently, the tissue inhibitor of metalloproteinases-3 (TIMP3) has been identified as the gene causing autosomal dominant SFD (Weber et al. 1994). Thus far, five independent heterozygous missense mutations, all intro-

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ducing an additional cysteine residue into the C-terminal region of TIMP3, have been detected in Caucasian families of different geographic origins (Weber et al. 1994; Felbor et al. 1995, 1996; Jacobson et al. 1995) (table 1). Furthermore, mutational and haplotype analyses of 15 SFD pedigrees from disparate parts of the British Isles have demonstrated that all British SFD patients carry the same Ser181Cys mutation, which can be traced back to a single founder (Wijesuriya et al. 1996). The latter study included the Kempster, Carver, and Ewbank families originally described by Sorsby and later shown to be clinically heterogeneous. Despite the striking clinical variability, it therefore appears that the autosomal dominant form of SFD is genetically homogeneous.

To further clarify the question of genetic heterogeneity in SFD, we have reinvestigated the Finnish autosomal recessive SFD family that has been followed by Forsius and colleagues for >30 years (Forsius et al. 1982; Eriksson et al. 1990). We have identified additional affected individuals in the most recent generation and provide genetic evidence for a heterozygous disease-causing mutation in TIMP3, which strongly suggests an autosomal dominant mode of inheritance of the SFD phenotype in this family. On the basis of these results, together with a thorough review of the literature, we conclude that there is no satisfactory evidence for an autosomal recessive mode of inheritance in SFD. Thus, we propose that SFD represents a genetically homogeneous, autosomal dominant condition.

Subjects and Methods

Family Data

A detailed genealogical and ophthalmological description of the consanguineous Finnish SFD family has been published elsewhere (Forsius et al. 1982; Eriksson et al. 1990). For the present study, 25 family members >18 years of age underwent clinical reinvestigation and were included in the genetic analysis. In this family, the age at onset of visual loss varied from the 2d to the 4th decade. Progressive myopia and iris atrophy were diagnosed in the eight affected sibs and their mother. Glaucoma was seen in five sibs. Moreover, close follow-up studies during the past 3 decades have demonstrated that uneven or absent pigmentation in the extreme fundus periphery was the first sign seen in young family members who later developed SFD. Consequently, individuals were diagnosed as suspect for the disease if they were found to have an uneven pigment layer in the fundus periphery when studied with Goldmann's three-mirror contact lens. The present clinical follow-up confirmed the previous diagnoses of SFD in all eight affected sibs of generation III, as well as in one affected grandchild in generation IV (Eriksson et al. 1990). In addition, another five grandchildren were classified as suspect for developing SFD.

DNA Analysis

Ten-milliliter EDTA-blood samples were obtained from nine affected individuals, five grandchildren suspect for the disease, four unaffected family members, and seven unrelated spouses. Genomic DNA was isolated according to standard procedures. On the basis of the genomic exon/intron structure of TIMP3, PCR primers were designed flanking the five coding exons of TIMP3 and a 516-bp fragment that includes part of the 5′ UTR, as well as CpG islands, putative binding sites for SP1, and a possible TATA box (Stöhr et al. 1995). The oligonucleotide primers and the conditions for PCR amplification are as given by Felbor et al. (1996), except for the 5′ UTR fragment, which was assessed by use of primers PR2-F (5′-AGG GGT AGC AGT TAG CAT TC-3′) and PR1-R (5′-AGG AGG AGG AGA AGC CGT C-3′) and an annealing temperature of 56°C.

PCR products were tested for mobility shifts by single-stranded conformational analysis (SSCA) as described elsewhere (Orita et al. 1989). To increase the sensitivity of the assay, PCR products were digested with various restriction enzymes prior to their electrophoretic separation on polyacrylamide gels, as reported elsewhere

Table 1

Independent TIMP3 Mutations in SFD

Mutation	Exon	Sequence	Amino Acid Change	Expected Structure	Geographic Origin	Reference
1	5	TCC→TGC	Ser156Cys	Missense	German-Czech	Felbor et al. (1995)
2	5	GGC→TGC	Gly166Cys	Missense	Finnish	Present study
3	5	GGC→TGC	Gly167Cys	Missense	United States	Jacobson et al. (1995)
4	5	TAC→TGC	Tyr168Cys	Missense	Massachusetts	Weber et al. (1994)
5	5	TAC→TGC	Tyr168Cys	Missense	Austrian	Felbor et al. (1996)
6	5	AGC→TGC	Ser181Cys	Missense	Irish-Canadian	Weber et al. (1994)
					South African	Peters and Greenberg (1995)
					British Isles	Wijesuriya et al. (1996)
					Oregon	Carrero-Valenzuela et al. (1996)

(Felbor et al. 1996). The 516-bp PCR fragment containing the putative promotor region was digested with *Sma*I, yielding restriction fragments of 228 bp, 194 bp, and 94 bp in size.

Forward and reverse dideoxy sequencing was performed by use of the Sequenase PCR-product sequencing kit (United States Biochemical). To confirm the identified sequence variant in exon 5, we designed a mismatch-primer Mut-G166C (5′-GTA CCA GCT GCA GTA GCG GC-3′), which introduces a *Hae*III restriction-enzyme site in the control allele (5′-GTA CCA GCT GCA GTA GCG GCC-3′) but not in the mutant allele (5′-GTA CCA GCT GCA GTA GCG GCA-3′). Consequently, when used with primers 2FF and Mut-G166C, *Hae*III cleaves a 180-bp PCR product from control DNA into two fragments, of 161 bp and 19 bp, whereas the mutant allele remains undigested.

Haplotype Analysis

The Finnish SFD family was genotyped by use of the three highly polymorphic $(CA)_n$-dinucleotide–repeat markers D22S273, D22S280, and D22S281 (Gyapay et al. 1994). These markers have been localized closely to the SFD locus (Gregory et al. 1995). In addition, we analyzed a frequent intragenic polymorphism in exon 3 of the TIMP3 gene, which is due to a silent mutation at the third position of codon 60 (CAT/CAC) and codon 64 (TCC/TCT) (U. Felbor, D. Doepner, U. Schneider, E. Zrenner, and B. H. F. Weber, unpublished data).

Results

Mutational Analysis

To analyze the TIMP3 gene in the Finnish SFD family, we first performed single-stranded conformational analyses (SSCA) of the entire coding and the putative promotor region of the TIMP3 gene. An aberrant mobility shift was observed in exon 5 in affected individuals (fig. 1*A*) but not in 270 controls (data not shown). No other disease-associated band shifts were found in the remaining gene fragments analyzed. Subsequently, sequencing of the aberrant exon 5 PCR product revealed a heterozygous G→T transversion in the first position of codon 166, changing a glycine residue to a cysteine (fig. 1*B*). By enzymatic assay, the Gly166Cys mutation was shown to cosegregate with the disease phenotype (fig. 2). The restriction enzyme *Hae*III cleaves a 180-bp PCR product into fragment sizes of 161 bp and 19 bp in unaffected sibs and spouses, whereas all nine affected individuals, as well as the five grandchildren clinically classified as suspect for the disease, revealed heterozygous fragments of 180 bp, 161 bp, and 19 bp, thus confirming the mutational change in the affected individuals (only the relevant 180-bp and 161-bp fragments are depicted in fig. 2*C*).

Haplotype Analysis

To determine the segregation of the disease haplotypes in the Finnish pedigree, we genotyped the affected and unaffected members, using three DNA markers tightly linked to the disease locus and one polymorphism occurring in exon 3 of TIMP3 (fig. 2*B*). D22S273 and D22S281 were fully informative, whereas D22S280, as well as the intragenic marker, were only partially informative but consistent with genetic linkage to the disease locus (maximum LOD score, Z_{max}, of 3.03 at $\theta = .001$ for D22S281, if the clinical status of grandfather, II-4, is assumed to be unknown) (fig. 2*B*). On the basis of the order of loci as D22S273-D22S280-TIMP3/exon 3-D22S281 (Gregory et al. 1995), our results demonstrate that the Gly166Cys mutation segregates with haplotype 3-2-2-1 in the Finnish family. In addition to the disease-associated haplotype, the eight affected sibs of generation III inherited another two distinct haplotypes. Whereas individuals III-6, III-7, III-8, III-10, and III-14

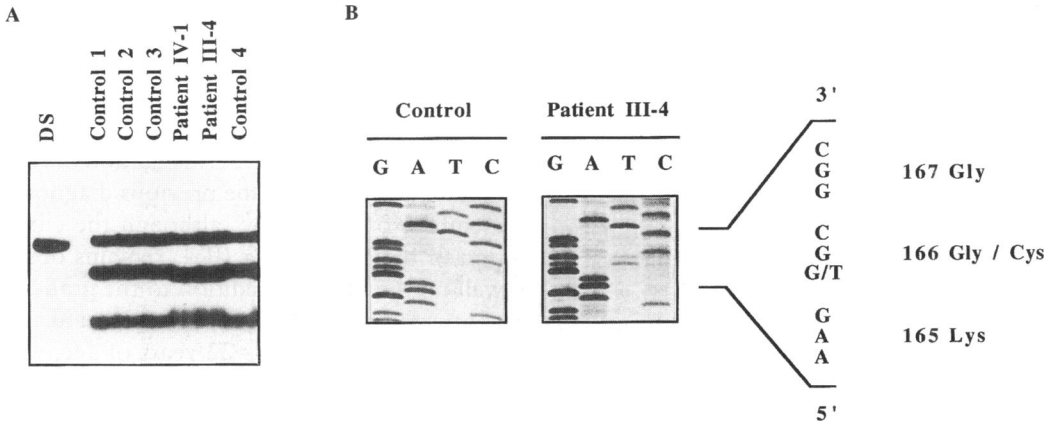

Figure 1 *A*, SSCP analysis of PCR-amplified exon 5 of TIMP3, demonstrating mobility shifts in Finnish patients IV-1 and III-4 (DS = double strand). *B*, Direct PCR sequencing, revealing a heterozygous G→T transversion in patient III-4, changing a glycine residue to a cysteine, at position 166.

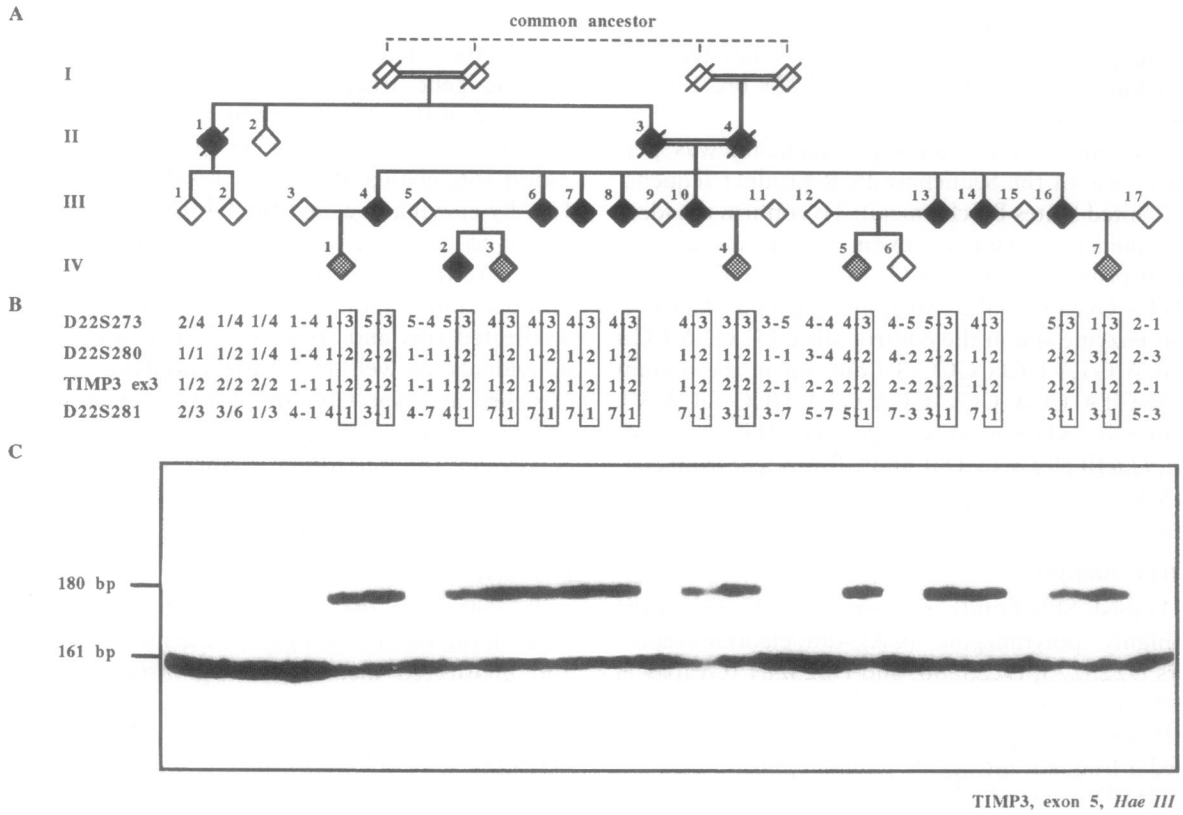

Figure 2 *A*, Pedigree of Finnish SFD family. The grandparents (II-3 and II-4), as well as their parents, are related in many ways. To assure confidentiality, the family members are represented by diamonds: blackened diamonds denote affected individuals; gray-shaded diamonds denote individuals clinically suspect for the disease; and unblackened/unshaded diamonds denote unaffected individuals. *B*, Haplotype analysis of Finnish SFD family. The haplotype associated with the SFD phenotype was determined as 3-2-2-1 (D22S273-D22S280-TIMP3/exon 3-D22S281) (*boxed*). *C*, Verification of Gly166Cys mutation by enzymatic restriction analysis. The mutation abolishes a *Hae*III restriction site and segregates with the disease, as a heterozygous 180-bp fragment.

carry a 4-1-1-7 haplotype, III-4, III-13, and III-16 share the 5-2-2-3 haplotype (fig. 2*B*). In generation IV, none of the affected individuals who all inherited the disease haplotype 3-2-2-1 share a same second haplotype (fig. 2*B*).

Discussion

In this study, we have reinvestigated a large, consanguineous Finnish SFD family that has been followed closely by Forsius and colleagues for >30 years (Forsius et al. 1982; Eriksson et al. 1990). At first examination in 1966, only three of the eight sibs in generation III (fig. 2*A*) were affected, and their mother (II-3) was believed to have autosomal dominant SFD, whereas the father (II-4) was treated for choroiditis disseminata by local ophthalmologists. However, in a follow-up study 16 years later, several findings have led those authors to reconsider the mode of inheritance in this family (Forsius et al. 1982). Striking similarities in the parents' fundus appearances, genealogical evidence for several consanguineous marriages in the pedigree, and the fact that apparently none of the ancestors were symptomatic were best explained by an autosomal recessive transmission of the disease. In addition, the observation that all eight children of the consanguineous marriage of the two affected parents were clinically affected was consistent with recessive inheritance, if genetic homozygosity of the disease locus in the parents is assumed (Forsius et al. 1982; Eriksson et al. 1990).

The present clinical reinvestigation of the Finnish SFD family has confirmed the previous diagnoses in all living members of the family, although the clinical presentation of the grandfather (II-4) remains obscure and may well not be SFD. In addition to the grandchild reported as affected in 1990 (IV-2) (Eriksson et al. 1990), another five grandchildren, 18–22 years of age, were then characterized as suspect for the disease, because of scarce pigmentation in the fundus periphery. Since previous observations in the Finnish family have shown that members with this abnormality all later developed SFD, it was considered to be an early and reliable diagnostic

sign for the disease. The identification of several affected individuals in the third generation was a first clue that the mode of inheritance in this family might not be autosomal recessive.

Strong evidence for dominance of the SFD phenotype in the Finnish kindred was then provided by the genetic analyses. We demonstrated a single heterozygous Gly166Cys mutation in the TIMP3 gene, segregating with the disease in two generations. We have not been able to detect a second mutation. In order to exclude the possibility that such a mutation may have remained undetected because of technical difficulties, we analyzed the segregation of haplotypes constructed with markers tightly linked to or within the TIMP3 locus. Assuming recessive transmission, we would have expected that all affected individuals share no more than four different haplotypes. We have constructed one common haplotype that was associated with the Gly166Cys mutation and was identified in all affected family members, as well as in the five grandchildren clinically classified as suspect for the disease (fig. 2*B*). However, another eight second haplotypes were found in the affected individuals of generations III and IV. In addition to the Gly166Cys-associated haplotype, the eight affected sibs of generation III revealed another two additional haplotypes, and the six affected grandchildren showed another six individual haplotypes clearly distinct from each other (fig. 2*B*). It is important to note that the various haplotypes cannot be derived from each other even if several meiotic recombination events are assumed to have occurred. From these findings we conclude that the Gly166Cys alteration is the only disease-causing mutation segregating as an autosomal dominant trait in the Finnish SFD family.

The identification of the Finnish Gly166Cys mutation strikingly fits into the observed pattern of heterozygous missense mutations that elsewhere have been identified in autosomal dominant SFD pedigrees (Weber et al. 1994; Felbor et al. 1995, 1996; Jacobson et al. 1995). In all cases, the mutational change introduces an additional cysteine residue in the C-terminal region of TIMP3, ≤ 26 amino acids from each other (table 1). Although the functional significance of these similar changes remains unknown, it appears likely that the pathological phenotype in SFD is due to a specific disease mechanism common to all mutations known so far. Nevertheless, the observed clinical phenotypes in SFD are highly variable. Inter- and intrafamilial phenotypic variations, in particular in early manifestations such as nyctalopia, the age at visual loss, the progression of the disease, and funduscopic signs can be demonstrated best in the British SFD patients, all of whom have been shown to carry an ancestral Ser181Cys mutation (Wijesuriya et al. 1996). Similarly, we have observed extensive intrafamilial, as well as intraindividual, differences in the Finnish kindred. For instance, one sib experienced a bilateral decrease in visual acuity in <1 mo at the age of 13 years, whereas another retained good vision until the 4th decade of life. In addition, the right eye of one patient was blind at the age of 24, whereas the left eye still had good vision 9 years later (Forsius et al. 1982; Eriksson et al. 1990). Given the variable expressivity in the British and Finnish kindreds, the early onset of symptoms in the Finnish SFD family cannot be, per se, an indication of the mode of inheritance. Further support for this notion is our previous finding of a Ser156Cys mutation in a German-Czech family, a mutation that is associated with dominant SFD but is manifesting at an unusually early age strikingly comparable to that observed in the Finnish kindred (Felbor et al. 1995).

Besides the Finnish pedigree, only one other documented case has suggested the occurrence of an autosomal recessive mode of inheritance in SFD. In this family, two brothers, 41 and 37 years of age, were diagnosed as being affected, whereas both parents, two sisters, and all children appeared to be unaffected at the time of diagnosis (François 1958). However, it has to be stressed that the father had died at the age of 32 years, that the oldest child was only 17 years old at the time of examination, and that no clinical information on preceding generations was given. Therefore, it appears that the conclusions drawn in this report should be regarded as speculative. In the same publication, François had cited Sorsby's description of two consanguineous children, 8 and 12 years of age at the onset of "an exudative type of macular dystrophy" (Sorsby 1940, p. 478). It is noteworthy that in this early article Sorsby already had described the Kempster family but had not compared the fundus findings in the two children with those in the Kempsters. Although he included the Kempster family in his classical description of SFD 9 years later, there is no mention of the two consanguineous children (Sorsby et al. 1949). Consequently, the available data on the two sibs give no conclusive evidence that the clinical features were indeed compatible with SFD, and, in addition, they seem to be too vague to establish autosomal recessive inheritance. Taken together, our data on the Finnish SFD family, as well as the rather questionable reports of the two autosomal recessive SFD pedigrees that, unfortunately, are not available for reinvestigation, do not support the occurrence of an autosomal recessive mode of inheritance in this disorder. In the absence of clearly documented cases of autosomal recessive SFD, we propose that this disorder constitutes a homogeneous, autosomal dominantly inherited entity.

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