Autosomal Dominant Rhegmatogenous Retinal Detachment Associated with an Arg453Ter Mutation in the *COL2A1* Gene

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Purpose. To investigate the clinical features and molecular causes of autosomal dominant rhegmatogenous retinal detachment (RRD) in two large families.

Methods. Clinical examination and linkage analysis of both families using markers flanking the *COL2A1* gene associated with Stickler syndrome type 1, the loci for Wagner disease/erosive vitreoretinopathy (5q14.3), high myopia (18p11.31 and 12q21-q23), and nonsyndromic congenital retinal nonattachment (10q21).

Results. Fifteen individuals from family A and 12 individuals from family B showed RRD or retinal tears with minimal (family A) or no (family B) systemic characteristics of Stickler syndrome and no ocular features of Wagner disease or erosive vitreoretinopathy. The RRD cosegregated fully with a chromosomal region harboring the *COL2A1* gene with maximum lod scores of 6.09 (family A) and 4.97 (family B). In family B, an Arg453Ter mutation was identified in exon 30 of the *COL2A1* gene, that was previously described in a patient with classic Stickler syndrome. In family A, DNA sequence analysis revealed no mutation in the coding region and at the splice sites of the *COL2A1* gene.

Conclusions. In two large families with RRD, linkage was found at the *COL2A1* locus. In one of these families an Arg453Ter mutation was identified, which is surprising, because all predominantly ocular Stickler syndrome cases until now have been associated with protein-truncating mutations in exon 2, an exon subject to alternative splicing. In contrast, the Arg453Ter mutation and other protein-truncating mutations in the helical domain of *COL2A1* have been associated until now with classic Stickler syndrome. (*Invest Ophthalmol Vis Sci.* 2003;44:4035–4043) DOI:10.1167/iovs.02-0736

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Supported by grants from the Landelijke Stichting voor Blinden en Slechtzienden (SLG), the Algemene Nederlandse Vereniging ter Voorkoming van Blindheid, the Stichting voor Ooglijders, the Gelderse Blindenvereniging, the Rotterdamse Vereniging Blindenbelangen, the Stichting Blindenhulp, the Stichting Haagsch Oogheelkundig Fonds, the Researchfonds Oogheelkunde, and the Foundation De Drie Lichten in the Netherlands (MAvD, AM).

Submitted for publication July 19, 2002; revised December 20, 2002, and March 14, 2003; accepted April 30, 2003.

Disclosure: **S.L. Go**, None; **A. Maugeri**, None; **J.J.S. Mulder**, None; **M.A. van Driel**, None; **F.P.M. Cremers**, None; **C.B. Hoyng**, None

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Rhegmatogenous retinal detachment (RRD) often is associated with (pathologic) myopia and in most cases leads to visual impairment or blindness if untreated.[1,2] Early diagnosis of RRD and recognition of patients at risk improve the prognosis (see Ref. 3 and the references therein). Nonsyndromic pathologic myopia (−6 D or less) in most cases occurs sporadically, but is also encountered as an autosomal dominant or X-linked trait in families.[4–7] RRD with autosomal dominant inheritance in association with myopia and vitreoretinal degeneration is usually described as a feature of Stickler syndrome or erosive vitreoretinopathy. RRD also has been reported in the original Wagner family, although less frequent.[8]

Stickler syndrome is characterized by such systemic abnormalities as midfacial hypoplasia, midline cleft of the palate, sensorineural hearing loss, early progressive arthropathies, and hypermobility, in combination with ocular abnormalities, such as high myopia, abnormalities of the vitreous structure, paravascular pigmentation, and possibly giant tears causing retinal detachment.[9–11] Mitral valve prolapse also has been reported.[12] These features show intra- and interfamilial variability of expression. Moreover, different types of Stickler syndrome can be distinguished based on the presence or absence of ocular abnormalities, the appearance of the vitreous, and the molecular genetic findings. Type 1 Stickler syndrome is characterized by a membranous vitreous phenotype and is caused by mutations in the *COL2A1* gene.[13–15] Type 2 Stickler syndrome exhibits a different beaded vitreous phenotype and has been associated with *COL11A1* mutations.[15–17] Nonocular Stickler syndrome type 3, with a phenotype displaying characteristic systemic abnormalities such as facial abnormalities, cleft palate, hearing loss, and arthropathies, but without high myopia, vitreoretinal degeneration, or retinal detachments, is caused by mutations in *COL11A2*.[18–20] Evidence of at least a fourth locus for Stickler syndrome has been found, as mutations in the former three known genes were not found in some Stickler families.[17,21]

Wagner disease, on the other hand, is a nonsystemic disorder in which the vitreous is optically empty, and a preretinal membrane is present in the periphery of the retina, sometimes only as a thin white circular line. A progressive complicated cataract appears in most of the patients, chorioretinal atrophy, peripheral pigment foci, and a situs inversus of the optic disc may be present.[22–24] Wagner disease has been mapped to the long arm of chromosome 5 in region 14.3 (5q14.3).[25]

In erosive vitreoretinopathy, progressive thinning of the retinal pigment epithelium resulting in severe degeneration is the major feature. In addition, and in contrast with Wagner disease, a pronounced roped and veiled syneresis of the vitreous body with traction at lesions of the retinal pigment epithelium and frequent development of retinal detachment, both rhegmatogenous and tractional, are observed. As in Wagner disease, no systemic abnormalities are found.[26,27] The disorder maps to the same region as Wagner disease, 5q13-q14,[27] suggesting that erosive vitreoretinopathy and Wagner disease may be allelic disorders.

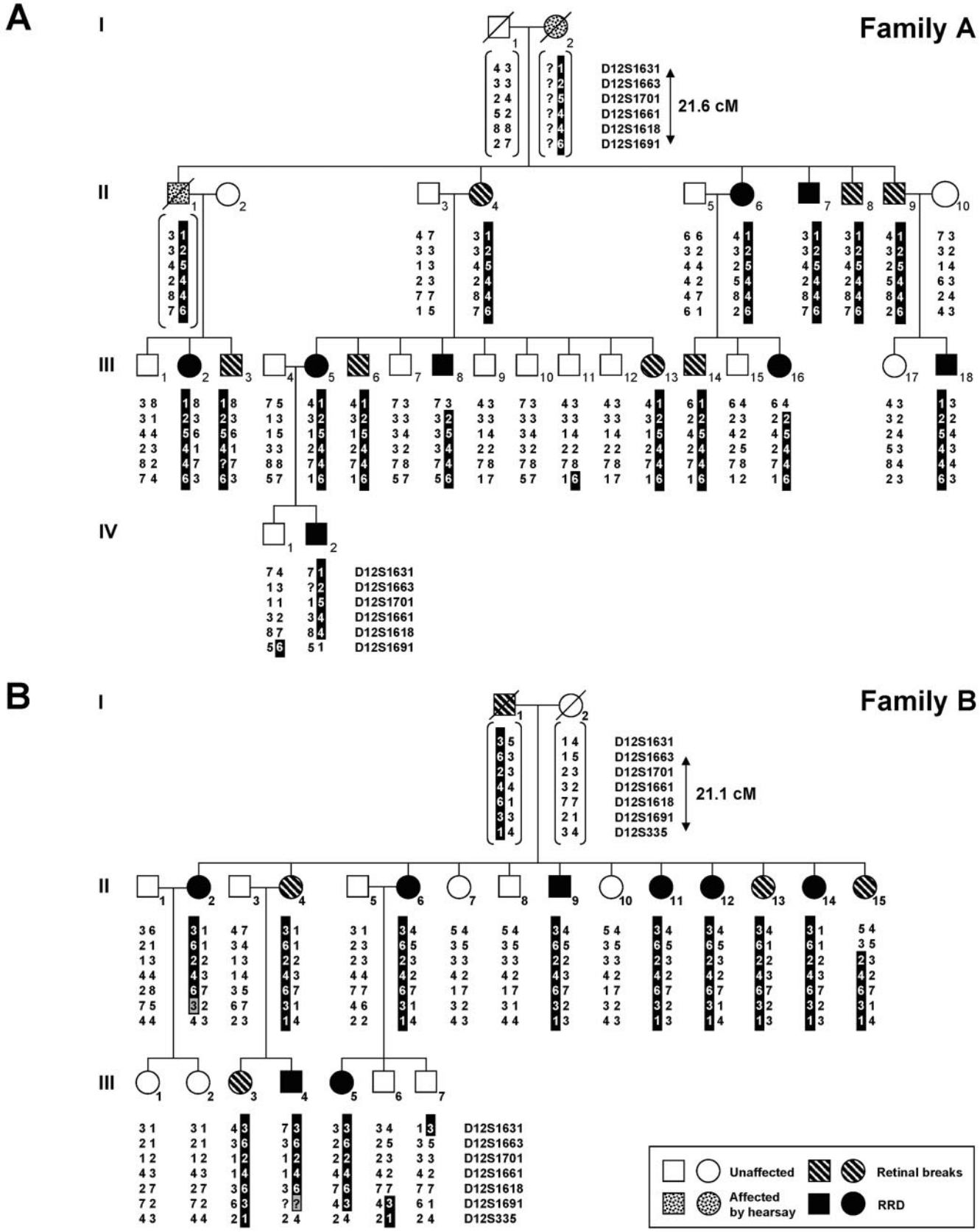

FIGURE 1. Haplotype analysis of families with RRD or retinal breaks with markers encompassing the *COL2A1* gene on the long arm of chromosome 12, region 13.11. The *COL2A1* gene resides between markers D12S1701 and D12S1661. The shared alleles from the at-risk haplotype are shown in *black bars*, marker alleles between brackets are deduced. (**A**) Family A: the boundaries of the critical interval between D12S1631 and D12S1691 are determined by recombination events in affected individuals AIII-8, AIII-16, and AIV-2. (**B**) Family B: the critical interval between D12S1663 and

In this report, we present two large families with autosomal dominant RRD or retinal breaks without or with minimal systemic features, clinically different from Wagner disease, erosive vitreoretinopathy, and typical Stickler syndrome. Both families showed linkage to a genomic region containing the *COL2A1* gene. In one of the families, a stop codon mutation was found in the helical domain of the *COL2A1* gene that had been found earlier in a patient with typical Stickler syndrome.

Patients and Methods

Two unrelated Dutch families of white origin with autosomal dominant RRD were studied. Family A consisted of 28 individuals; family B consisted of 22 (Fig. 1). The study protocol followed the tenets of the Declaration of Helsinki, and informed consent was obtained from each participant or their guardians, after general approval by the Ethics Committee of the University Medical Centre Nijmegen, The Netherlands.

Clinical Examination

An extensive clinical history of all individuals especially regarding ophthalmic, audiologic, cardiologic, and orthopedic disorders, and current symptoms was recorded. Existing ophthalmic records of all participants and, if possible, of the deceased were collected and reviewed regarding age of onset of myopia, structure of the vitreous body, retinal breaks, retinal detachments or other abnormalities of the fundus, biometric measurements, and intraocular pressure. Clinical examination included best corrected visual acuity, slit lamp microscopy, applanation tonography, fundoscopy including fundus photography, and Goldmann three-mirror contact glass examination. These examinations were performed in 27 individuals in family A and 22 individuals in family B by both a highly experienced medical retina specialist (CBH) and by the first author (SLG). Special attention was paid to the vitreous body structure. Axial length measurement and keratometry were performed, using ultrasound. In cases of axial length of 25 mm or longer or in cases with closed pupillae, ultrasound examination of the posterior eye was performed. All individuals with retinal detachments or retinal breaks were considered affected.

Physical examination including assessment of facial, palatal, joint, and heartsound abnormalities was prospectively performed in affected individuals who were willing to cooperate. Existing audiologic, cardiologic, and orthopedic medical records were collected. Facial and palatal photographs were taken. The Beighton score for hypermobility of joints[28] and audiometry were assessed in six members of family A and 10 of family B.

Molecular Genetic Analysis

DNA was extracted from leukocytes from 10 mL of peripheral blood of all individuals, according to a protocol adapted from Miller et al.[29] Linkage analysis was performed with radioactively labeled microsatellite markers. The candidate loci were the two loci for autosomal dominant high myopia on 18p11.31 (MYP2; markers D18S52 [AFM020tf12] and D18S1154 [AFMa056ye1]) and 12q21-q23 (MYP3; markers D12S64 [MFd155a], D12S82 [AFM107xc11] and D12S317 [AFM065ye9]), the Wagner disease/erosive vitreoretinopathy locus on 5q14.3 (markers D5S428 [AFM238xf4] and D5S2094 [AFMa055td9]), the locus for nonsyndromic congenital retinal nonattachment on 10q21 (marker D10S581 [AFM287yf9]), and the genes for Stickler syndrome *COL2A1* on 12q13.11-13.2 and *COL11A1* on 1p21.1. For *COL2A1*, residing between markers D12S1701 and D12S1661, we used the following markers (from pter to qter; genetic distances indicated): D12S1631 (AFMa288wd5) - 5.8 centimorgans (cM) - D12S1663 (AFMb316xd9) - 6.2 cM - D12S1701 (AFM345xf1) - 1.4 cM - D12S1661 (AFMb314yh5) - 4.6 cM - D12S1618 (AFMa224yg1) - 3.6 cM - D12S1691 (AFM312xf5) - 5.3 cM - D12S335 (AFM273vg9).[30] No marker near *COL11A1* was tested, because linkage was found at the *COL2A1* locus. DNA samples were subjected to polymerase chain reaction (PCR) amplification with a standard cycling profile of 30 cycles at 94°C, 55°C and 72°C for 1, 2, and 1 minute(s), respectively, at each step. DNA markers were labeled by the incorporation of $\alpha[^{32}P]$-dCTP, and the products were separated by electrophoresis on a 6.6% denaturing polyacrylamide gel.

Linkage analysis by calculating two-point lod scores was performed using the MLINK routine from LINKAGE (ver. 5.1) software suite (http:www.hgmp.mrc.ac.uk/; provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge, UK).[31–33] Lod scores in both families were calculated with a presumed penetrance rate of 95% and an allele frequency of 0.001.

Mutation analysis of the *COL2A1* gene was performed by direct sequencing (BigDye Terminator on a Prism 377; Applied Biosystems, Foster City, CA). The entire coding region of the gene, comprising 54 exons, was amplified in 38 amplicons. Primer pairs and conditions are available on request. To ascertain mutations that could affect the splicing, at least 42 bp (average 104 bps) of the flanking intronic sequences were amplified. Twenty-one introns (introns 3–6, 9, 13, 20, 21, 24, 25, 30, 32, 35, 40, and 42–48) were entirely amplified. Sequence analysis was performed on both strands of each amplicon using both forward and reverse primers.

To assess the stability of the mutant *COL2A1* messenger RNA (mRNA), Epstein-Barr virus–transformed lymphoblastoid cell lines were established from heparin blood of two affected individuals from family B. Before RNA extraction, half of the cultured cells were incubated for 4 hours with 100 μg/mL cycloheximide. In cells grown with cycloheximide, a protein synthesis inhibitor, the nonsense-mediated mRNA decay process is prevented.[34] After RNA extraction and reverse transcription-PCR (RT-PCR), a fragment of the cDNA encompassing the mutation in exon 30 was amplified using a first set of primers, 5051F (5′-tgcctggtgaaagaggacggac-3′) and 5054R (5′-ggcattccctgaagacctggag-3′), followed by a nested PCR and direct sequencing of the band of interest with primer 5053F (5′-tcaagatggtctggcaggtccc-3′) and the same reverse primer 5054R.

Results

Clinical Examination

The ophthalmic examination was performed in 27 of 28 individuals from family A and in all 22 individuals from family B. One individual (AIII-3) refused prospective clinical examination, but could be considered affected because a retinal break was described in his medical files. Medical files of AI-2 and AII-1 were not available, but these family members were determined by hearsay to be affected. The medical files of individual BI-1 showed a retinal break and that person was thus considered to be affected. BI-2 had no known health problems before her death. The clinical features of all affected individuals (15/28 in family A, 12/22 in family B) and the available information about BI-1 are shown in Table 1. Refractive error comprised the whole scale of mild hypermetropia to high myopia in family A, whereas the scale was limited between no myopia and severe

D12S335 is determined by recombination events in affected individuals BII-15 and BIII-5. Note that marker D12S1691 in individuals BII-2 and BIII-4 are noninformative or not known (*gray bars*). Assuming that individual BIII-6 is not a nonpenetrant, the telomeric boundary is demarcated by marker D12S1691, thereby reducing the critical region to 15.8 cM. The DNA marker order and distances were derived from the Human Genome Browser (April 2002 assembly)[46] and Généthon (www.genethon.fr; provided in the public domain by the French Association against Myopathies, Evry, France).[30] Note the overlap of the critical intervals of families A and B.

TABLE 1. Clinical Features of Affected Individuals from Families A and B

			Vitreous		Retina			Systemic Phenotype§		
Patient	Myopia Grade*	Significant Early Cataracts†	Optical Density	Visible Structure(s) (age in y)	Detachment‡	Age at Onset of RRD (y)	Break (age in y)	Midfacial hypoplasia‖	Hearing Loss (age in y)¶	Articular Symptoms (y)
AII-4		−	Normal	Normal	0		+ (65)	0	−	−
AII-6	3	−	Thin	Strands	1 (postphacogenic uveitis)	60	+ (60)	0	+, Asymmetrical sensorineural low-, mid-, and high-frequency loss with history of a left-side cerebellar cyst operation (76)	−
AII-7		−	Empty	Few posterior condensations	1 (postneovascular glaucoma, possibly as a result of long-term RD)	64	Not found	0	−	−
AII-8	2	−	Empty	Threads, posteriorly: membrane OD, strands OS (64)	0		+ (44)	0	−, Audiologically confirmed (67)	−
AII-9	2	−	Empty	Condensations	0		+ (46)	0	−	−
AIII-2	3	+	Thin		1	35	+ (35)	1	+, Progressive sensorineural low- and midfrequency loss (54)	Incidentally stiff fingers of both hands, pains in left knee after lengthy walks (51)
AIII-3	−1	+	Empty parts	Thick threads ODS (29); PVD or just primary vitreous present (30)#	0		+ (30)	0	−	−
AIII-5	−1	−		Veils and strands	2	45	+ (44)	0	+, Sensorineural mid- and high-frequency loss (52)	Stiffness and pain during unexpected hip movements (49)
AIII-6	0	−	Normal	Threads (49)	0		+ (51)	0	+, Sensorineural loss at 4 kHz with history of noise exposition (51)	−
AIII-8	3	−		Veils	1	29	+ (29)	0	−	−
AIII-13	3	−		Veils (30); threads (35)	0		+ (30)	0	−	−
AIII-14		−		Threads (45)	0		+ (40)	0	−	−
AIII-16	2	−		Veils	1	33	+ (33)	0	−	−
AIII-18	2	−	Empty	Veils (16); veils and membrane (17); collapsed vitreous, fine structured OD, thicker OS (35)	1	16	+ (15)	0	−	−
AIV-2	1	−		Midperipheral white condensations ODS, thick epiretinal fibrosis OS (16); same in OD (17); peroperatively: very thick vitreous, thick gray mass at pars plana (19)	2	16	+ (16)	0	−, Audiologically confirmed (23)	−

TABLE 1 (*continued*). Clinical Features of Affected Individuals from Families A and B

			Vitreous		Retina			Systemic Phenotype§		
Patient	Myopia Grade*	Significant Early Cataracts†	Optical Density	Visible Structure(s) (age in y)	Detachment‡	Age at Onset of RRD (y)	Break (age in y)	Midfacial hypoplasia‖	Hearing Loss (age in y)¶	Articular Symptoms (y)
BI-1	2				0		+(52)		−	
BII-2	3	+	Thin	Threads (11); veils (25)	2	11	+(11)	0	−	−
BII-4	2/3	−	Thin	Retrolental membrane (17); threads and very fine beads (40)	0		+(17)	0	−, Audiologically confirmed (42)	−
BII-6	2	−		Condensations and veils OD (17); fine threads, fine beads (39)	1	17	+(16)	0	−, Audiologically confirmed (42)	−
BII-9	2/3	+		Veils (27)	1	27	Not found	0	−, Audiologically confirmed (35)	−
BII-11	3	+	Very thin OD, thicker OS	Collapsed vitreous (?)	1	27	+(27)	0	−, Audiologically confirmed (31)	−
BII-12	3	−	Dense structure	Retrolental membrane OD (28)	1	22	+(22)	0	−, Audiologically confirmed (30)	−
BII-13	2	+		Retrolental threads (26)	0		+(10)	0	−, Audiologically confirmed (29)	−
BII-14	2	−	Normal	Normal	1	16	+(16)	0	−, Audiologically confirmed (26)	−
BII-15	1	−	Empty	Collapsed vitreous ODS with beaded threads OD (22)	0		+(22)	0	−, Audiologically confirmed but small air bone gap of 7 dB probably related to tubal dysfunction (21)	−
BIII-3	2	−	Very thin	Branched threads, membranelike structure more posteriorly (19)	0		+(19)	0	−, Audiologically confirmed but small air bone gap of 7 dB probably related to tubal dysfunction (24)	−
BIII-4	0	−		Thick veils (9); threads with fine beads and retrolental membrane OS less threads than OD, not PVD (10)	1 (after blunt trauma)	9	+ (9)	1	−, Audiologically confirmed (12)	−
BIII-5	2/3	−	Thin	Veils (6); thick threads OD (9); some retrolental threads ODS (13)	1	6	+ (6)	1	−	−

+, Present; −, absent; RRD, rhegmatogenous retinal detachment; PVD, posterior vitreous detachment.

* Myopia grade prior to RRD or retinal break (using spherical equivalents): −1 mild hypermetropia; 0 emmetropia; 1 mild (0 D to −1.5 D); 2 moderate (−1.5 D to −6.0 D); 3 high myopia (−6.0 D or more).

† Nontraumatic cataracts, at less than 40 years of age.

‡ 0 Absent, 1 unilateral, 2 bilateral.

§ None of the examined patients showed cleft palate or joint hypermobility (Beighton score ≥4).

‖ 0 Absent, 1 mild, 2 moderate, 3 severe.

¶ Hearing loss more than age-related (>P95, ISO7029 040996).

Retrospective data only, examined elsewhere, and examining doctor deceased.

TABLE 2. Lod Scores for Two-Point Linkage Analysis in RRD Families A and B

Locus	Location	Marker	Recombination Fraction							
			0.000	0.010	0.050	0.100	0.200	0.300	0.400	0.500
Family A										
MYP2	18p11.31	D18S52	−11.13	−6.00	−3.16	−1.85	−0.69	−0.22	−0.04	0.00
		D18S1154	−12.01	−7.29	−3.71	−2.03	−0.60	−0.07	0.07	0.00
MYP3	12q21–q23	D12S64	−4.04	−1.17	−0.11	0.35	0.56	0.40	0.14	0.00
		D12S82	−∞	−10.43	−6.67	−4.54	−2.16	−0.93	−0.29	0.00
		D12S317	−8.31	−4.88	−3.01	−1.78	−0.62	−0.17	−0.03	0.00
WGNI/ERVR	5q14.3	D5S428	−9.18	−5.21	−2.54	−1.32	−0.30	0.02	0.07	0.00
		D5S2094	−8.31	−4.33	−2.42	−1.49	−0.63	−0.24	−0.05	0.00
NCRNA	10q21	D10S581	0.86	0.84	0.74	0.63	0.40	0.20	0.05	0.00
COL2A1	12q13.11–q13.2	D12S1631	−5.38	2.00	3.01	3.10	2.63	1.82	0.84	0.00
		D12S1663	4.91	4.82	4.45	3.97	2.97	1.91	0.82	0.00
		D12S1701	5.79	5.69	5.28	4.75	3.63	2.41	1.11	0.00
		D12S1661	6.09	5.98	5.56	5.01	3.83	2.56	1.19	0.00
		D12S1618	5.79	5.69	5.28	4.75	3.63	2.42	1.11	0.00
		D12S1691	−0.81	1.55	2.30	2.46	2.17	1.53	0.69	0.00
Family B										
COL2A1	12q13.11–q13.2	D12S1631	−3.23	1.67	2.28	2.37	2.03	1.39	0.54	0.00
		D12S1663	−1.93	2.89	3.27	3.16	2.56	1.72	0.69	0.00
		D12S1701	2.62	2.58	2.38	2.13	1.59	0.99	0.37	0.00
		D12S1661	1.72	1.69	1.57	1.41	1.07	0.68	0.26	0.00
		D12S1618	4.97	4.89	4.55	4.12	3.17	2.09	0.86	0.00
		D12S1691	0.42	0.47	0.58	0.62	0.54	0.35	0.11	0.00

myopia in family B, with a tendency toward moderate or high myopia. In both families, the myopia was axial-length dependent (mean axial lengths: 24.8 mm [range, 20.2–28.2 mm] in family A; 26.6 mm [range, 24.9–28.7 mm] in family B). There was no specific abnormality of the vitreous body that was found in all affected individuals in both families, especially no consistent vitreal membranes or beaded strands. Only RRDs, or at least retinal breaks, were a consistent ophthalmic finding throughout the families. In family A, 11 RRDs occurred in 8 of the 15 affected family members, with an average age of first onset of RRD of 36 years (range, 16–64 years). Seven of the 12 affected members of family B experienced early RRDs in nine eyes. The average age of onset of RRD in this family was 14 years (range, 7–22 years). Eyes with RRDs showed a tendency to multiple (average, 2; range, 0–7) peripheral holes or horseshoe tears in the temporal superior and inferior quadrants in family A, whereas the periphery of the eyes of the affected in family B mostly revealed round multiple (average, 8; range, 1–28) retinal holes in the temporal superior quadrant. Bilateral RRDs were seen in patients AIII-5, AIII-18, AIV-2, BII-2, and BIII-4.

The history and clinical examination of all examined individuals of both families revealed no systemic abnormalities, except for five persons. Individual AII-6 had a history of surgery for a left-side cerebellar cyst and showed a sensorineural hearing defect in all frequencies of the left ear only. Thresholds at frequencies 0.25, 0.5, 1, 2, 4, and 8 kHz (thresholds more than age-related hearing loss between brackets), respectively, were [50], 20, [50], [70], [100], and [110] dB hearing loss at the age of 76 years. AIII-2, at 54 years of age, had a slightly recessed chin, a symptomatic progressive low- and midfrequency sensorineural hearing loss with thresholds of [25], [42.5], [55], [50], 32.5, and 40 dB, respectively, for both ears (ADS), and symptoms of occasionally stiff fingers of both hands and pains in her left knee after long walks. AIII-5 had thresholds of 15, 17.5, [25], [30], 30, and [67.5] dB hearing loss ADS, but was asymptomatic at 53 years. AIII-6 had a noise-exposition history and at the age of 51 years showed thresholds of 12.5, 17.5, 5, 7.5, [50], and 7.5 dB hearing loss ADS. Finally, individual BIII-4 had a transient flat nose bridge in the first decade of his life, but now has a normal facial appearance. Individuals BII-15 and BIII-3 showed a small air bone gap of 7 dB, probably related to tubal dysfunction at the time. As was true of all other patients from this family, they had a normal symmetrical age-related sensorineural threshold.

DNA Analysis

We excluded the involvement of the MYP2 and MYP3 loci for autosomal dominant high myopia, as well as the loci for Wagner disease/erosive vitreoretinopathy and nonsyndromic congenital retinal nonattachment by linkage analysis in family A (Table 2).

In both families, highly polymorphic DNA markers flanking the *COL2A1* gene showed cosegregation with the disease (Table 2). In family A, the critical region is demarcated by markers D12S1631 and D12S1691 (interval: 21.6 cM, 28 Mb), based on crossovers observed in the affected individuals AIII-8, AIII-16, and AIV-2 (Fig. 1A). The maximum lod score, 6.09, was detected for marker D12S1661 at a recombination fraction (θ) of 0.0. In family B, a linked chromosomal region of 21.1 cM (26 Mb) was delimited by markers D12S1663 and D12S335, based on recombination events observed in affected individuals BII-15 and BIII-5 (Fig. 1B). A maximum lod score of 4.97 was found for marker D12S1618 at $\theta = 0.0$. Assuming that the healthy individual BIII-6 is not a nonpenetrant, the telomeric boundary is demarcated by marker D12S1691, thereby reducing the critical region to 15.8 cM (16 Mb). In family A, analysis of all 54 exons and flanking intronic regions of *COL2A1* failed to identify a mutation in the coding region or at the splicing sites of the gene.

In family B, mutation analysis of the *COL2A1* gene showed a C-to-T transition in exon 30 (previously denoted as exon 28), resulting in a change of codon CGA of Arg453 for a stop codon (Fig. 2A). Ninety-six ethnically matched controls did not show this mutation. Analysis of RNA extracted from lymphoblastoid cells grown with and without cycloheximide from patient BII-6 showed stability of mutant RNA only in cells grown with cycloheximide (Fig. 2B), strongly suggesting that the *COL2A1* mRNA carrying the Arg453Ter mutation is unstable.

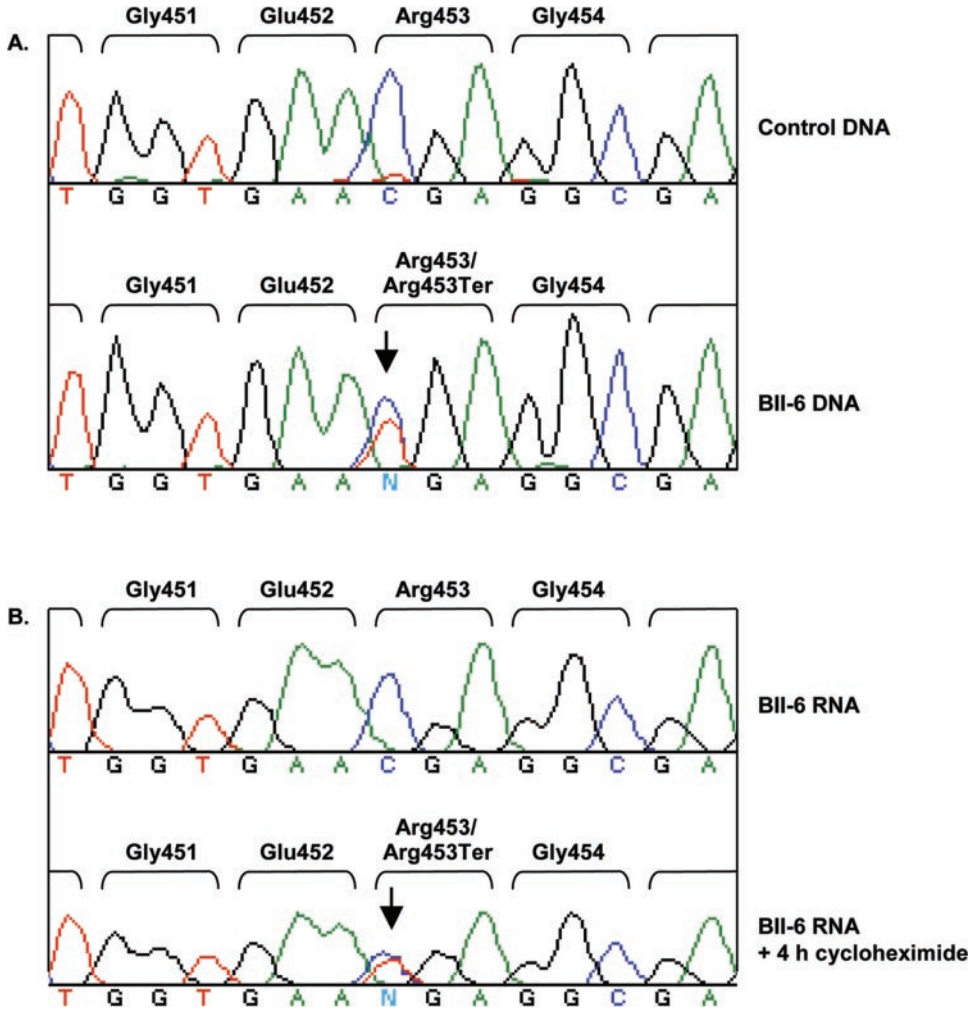

FIGURE 2. *COL2A1* mutation analysis in patient BII-6. (**A**) DNA sequence analysis of part of exon 30 (previously denoted as exon 28) of the *COL2A1* gene. *Top*: sequence of a control individual; *bottom*: sequence of the clinically affected individual BII-6, carrying a heterozygous C-to-T transition resulting in an Arg453Ter mutation. (**B**) RNA analysis of the Arg453Ter mutation in patient BII-6. *Top*: sequence of the cDNA of the patient. Because of mRNA instability, the transcript of the mutant allele was not detectable. *Bottom*: sequence of the patient's cDNA obtained after incubation of lymphoblastoid cells in a medium containing cycloheximide, which prevents nonsense-mediated mRNA decay. Sequence analysis shows presence of transcripts from both the normal and the mutant allele.

DISCUSSION

In this study, we report on a family (B) with autosomal dominant RRD associated with a mutation in the triple helical domain of the *COL2A1* gene. The mutation, Arg453Ter, has been described in a sporadic patient with Stickler syndrome, who had had such classic features as cleft palate, midfacial hypoplasia, sensorineural hearing loss, joint laxity, and joint pains since the second decade, besides high myopia, vitreoretinal degeneration with a typical type I vitreous anomaly (William G. Cole, personal communication, 2002), retinal breaks, and retinal detachment in the first decade.[35] Protein truncating *COL2A1* mutations are commonly found in patients with Stickler syndrome.

The phenotype observed in patients in family B however, was different from the classic Stickler syndrome. In all 12 RRD patients—even in the eldest generation—cleft palate, joint laxity, joint pains or sensorineural hearing loss were absent, whereas these symptoms were already present in the reported 23-year-old patient.[35]

Use of Snead's criteria—that is, a congenital vitreous anomaly (type 1: membranous, or type 2: fibrillar, beaded) and any three of the following: (1) myopia with onset before 6 years of age, (2) RRD or paravascular pigmented lattice degeneration, (3) joint hypermobility with abnormal Beighton score, either with or without radiologic evidence of joint degeneration, (4) audiometric confirmation of sensorineural hearing defect, and (5) midline clefts[15] in this family also indicates that the patients in family B are clinically different from patients with classic Stickler syndrome. First of all, excepting four patients in whom membranelike vitreous abnormalities were found (individuals BII-4, BII-12, BIII-3 and BIII-4), no members of family B revealed a vitreous consistent with a type I or type II Stickler vitreous. In BII-4, the membranelike structure was absent 23 years later, possibly due to degeneration of the membrane.

Furthermore, myopia, if present, was not always present before 6 years of age (data not shown). Joint hypermobility with abnormal Beighton score and midline clefts were not observed, and were not anamnestically present during childhood. Audiometric results were not suggestive of Stickler syndrome, and in both BIII-3 and BII-15 were most probably due to tubal dysfunction at the time of examination. Moreover, although none of the affected individuals from family B had joint pains, according to surveys, 70% of patients with Stickler syndrome have joint pains before 20 years of age.[36]

Family A, in which the underlying genetic defect also cosegregated with the *COL2A1* locus, although no mutation could be detected in the coding region and at the splice sites of the gene, also does not meet the classic criteria nor Snead's criteria for Stickler syndrome. First, the vitreous of all family members does not comprise consistent membrane- or threadlike abnormalities, though a thin vitreous body was present in several family members. In addition, a whole range of refractive errors between mild hypermetropia and high myopia was found in all affected individuals. No cleft palates were found, and though sensorineural hearing loss was found in four indi-

viduals, it was not typical of Stickler syndrome. Two of these hearing defects are explained by noise exposition (AIII-6) and a left-side cerebellar cyst that had been surgically removed (AII-6). AIII-5 had an asymptomatic mid- and high-frequency hearing loss of 10 dB more than age-related hearing loss and only one individual, AIII-2, had a symptomatic, progressive sensorineural hearing defect of 23 dB more than age-related hearing loss. However, the defect in this patient affected the low- and midfrequencies, although in patients with Stickler syndrome, the hearing impairment generally involves the high frequencies and shows no more progression than is associated with normal aging.[11,37] Joint hypermobility was not present during childhood and was not observed in those who had been examined. Except in patient AIII-2 at 51 years of age and patient AIII-5 who had pains in the left hip region during unexpected hip movements at age 49 years, no joint pains were found in patients in family A. Radiography of patient AIII-5 showed a moderate arthrosis of and reduced joint space in her left hip at the age of 52 years.

Previously described families with predominantly ocular Stickler syndrome invariably showed a type I vitreous anomaly, and all had mild to moderate systemic abnormalities, be it that these were present in only approximately half of the examined family members.[38] Also, if we consider each of these families as one unit, the abnormalities found in one family altogether invariably led to a complete Stickler syndrome diagnosis by Snead's criteria. This family diagnosis could not be made in each of our families, when taking into account all clinical abnormalities. There also was no consistent type I vitreous anomaly.

We think that patients in families A and B did not have Wagner disease, because strongly progressive juvenile cataract and inverted papilla, preretinal membranes or peripheral circular lines were not present. Moreover, of the 15 affected members of family A, only 4 showed an optically empty vitreous body and 1 had empty spaces in the vitreous body, whereas in family B only 1 of 12 affected individuals showed an optically empty vitreous body. In contrast, this was invariably present in patients from the original Wagner family.[22]

In conclusion, our results suggest that the patients in families A and B had an atypical form of predominantly ocular Stickler syndrome with RRD as the main clinical feature. An important difference between both families is that retinal breaks and detachments in family B occurred at younger ages, mostly in the second and third decades, whereas in family A they mostly appeared in the fourth and fifth decades and, in a few cases, even later (individuals AII-4, AII-6, AII-7, and AIII-6; Table 1).

Until recently, it seemed that *COL2A1* gene mutations could be associated with type 1 vitreous, whereas C*OL11A1* gene mutations were responsible for type 2 vitreous. Discussion of the role of the vitreous types in predicting the mutated gene, however, was recently published.[39,40] In fact, a Stickler family with a type I vitreous had linkage to *COL11A1*,[39] whereas in two Stickler families with a type II vitreous, *COL11A1* gene mutations were excluded.[21] Earlier posterior vitreoretinal detachment was suggested to have caused these phenotypes, because in two families conversion from vitreous phenotype 2 into 1 was observed.[40] Our data also contradict the hypothesis that all *COL2A1* mutations are associated with a type I vitreous.

The most interesting result of this study, however, is the identification of a *COL2A1* exon-30 protein-truncating mutation (Arg453Ter), previously identified in a patient with classic Stickler syndrome,[35] in a large family with an atypical form of predominantly ocular Stickler syndrome.

Collagen molecules are typically composed of three polypeptide chains (α-chains) that form a triple helix. A characteristic repetitive amino acid sequence, glycine-X-Y, is important for maintaining this helical structure. Three identical α1(II) chains, encoded by the *COL2A1* gene, constitute collagen II, the main collagen in cartilage and vitreous. Moreover, α1(II) chains participate in the formation of collagen V/XI in combination with α1(XI) and α2(XI) chains in the cartilage, and α1(XI) and α2(V) chains in the vitreous.[41]

The *COL2A1* gene is involved in several autosomal dominant disorders.[42] A variety of cartilage disorders, such as achondrogenesis, spondyloepiphyseal dysplasia, and Kniest dysplasia, are caused by missense mutations in *COL2A1*, generally changing one of the glycine residues of the triple helical structure, or by small in-frame deletions.[42] All these mutations probably disrupt normal collagen II and collagen V/XI structure through a dominant negative mechanism.

On the contrary, all *COL2A1* mutations described in patients with Stickler syndrome (Refs. 34, 35 and references therein) with a few exceptions[43,44] lead to a premature termination codon. Some authors demonstrated that mutant mRNAs in patients with Stickler syndrome undergo nonsense-mediated mRNA decay, resulting in *COL2A1* haploinsufficiency.[44,45] Haploinsufficiency of α1(II) chain molecules could affect collagen II production or, more likely, disturbs the stochiometry of V/XI collagen.

The discovery of premature termination mutations in exon 2 of the *COL2A1* gene in all families with predominantly ocular Stickler syndrome[38] led to the speculation that exon 2 null mutations merely give rise to ocular abnormalities, because exon 2 is subject to alternative splicing and is predominantly present in fetal and adult vitreous mRNA, but is absent in mature cartilage mRNA. However, this explanation cannot apply to our families. In fact, no mutations were found in exon 2 of the *COL2A1* gene in either family, whereas the Arg453Ter mutation in family B was located in the *COL2A1* helical domain of the gene.

RNA analysis in a patient in family B suggests that, as in typical Stickler syndrome,[44,45] haploinsufficiency underlies the disease. Although clinical variability in Stickler syndrome is very high, it does not satisfactorily account for the absence of systemic features in as large a family as family B.

As clinical variability in Stickler syndrome can generically be attributed to modifier factors, an intriguing hypothesis in our case would be that a transacting modifier factor is located in the vicinity of the *COL2A1* locus, and that a favorable modifier allele cosegregates in family B with the Arg453Ter *COL2A1* mutation, resulting in the relatively mild phenotype. However, it is worthwhile to note that family B belongs to a relatively closed religious community. It is therefore possible that, more broadly, individuals of this family share a common "favorable" genetic background, due to one or more traits, that can reside everywhere in the genome.

In family A, no *COL2A1* mutation was found in the coding sequence, at the splice sites or in the 21 introns of the gene that have been entirely sequenced. Whether the disease in family A follows a mechanism similar to that in family B remains to be elucidated.

Acknowledgments

The authors thank the patients for their kind cooperation, Hannie Kremer for her help with linkage studies, and Bellinda van den Helm, Saskia D. van der Velde-Visser, Albert L. Aandekerk, and Evert-Jan Steenbergen for expert technical assistance.

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