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Mutational Hot Spot Potential of a Novel Base Pair Mutation of the *CSPG2* Gene in a Family With Wagner Syndrome

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Abstract

Objective—To report a 3-generation white family clinically diagnosed variably with Wagner, Stickler, and Jansen syndromes and screened for sequence variants in the *COL2A1* and *CSPG2* genes. Wagner syndrome is an autosomal dominant vitreoretinopathy with a predisposition to retinal detachment and cataracts. It has significant phenotypic overlap with allelic Jansen syndrome and ocular Stickler syndrome type 1. Sticker syndrome type 1 maps to chromosome 12q13.11-q13.2, with associated *COL2A1* gene mutations. Wagner syndrome maps to chromosome 5q13-q14 and is associated with mutations in *CSPG2* encoding versican, a proteoglycan present in human vitreous.

Methods—Genomic DNA samples derived from venous blood were collected from all family members. Complete sequencing of *COL2A1* was performed on a proband. Primers for polymerase chain reaction and sequencing were designed to cover all exon and intronexon boundaries. Direct sequencing of *CSPG2* was performed on all family member samples.

Results—No detectable *COL2A1* mutations were noted, making the diagnosis of ocular Stickler syndrome highly unlikely for this family. A unique base pair substitution (c.9265+1G>T) in intron 8 of the *CSPG2* gene cosegregating with disease status was identified. This mutation occurred in a highly conserved previously reported splice site with a similar base pair substitution(G>A). Direct sequencing of this splice site mutation in 107 unrelated external controls revealed no variants, supporting the rarity of this base pair change and its causation in Wagner syndrome. This novel base pair substitution is thought to cause the deletion of exon 8 and formation of a truncated protein product.

Conclusion—Mutation screening of *CSPG2* in autosomal dominant vitreoretinopathy families is important for accurate diagnosis.

Clinical Relevance—This study underscores the importance of obtaining extensive pedigree information and comparative ophthalmologic clinical information, as the phenotypic findings may vary greatly among independent family members. The study also affirms the paradigm shift from diagnosis assignment based on eponyms to that based on gene mutation type.

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Additional Contributions: We thank Claxton Baer, MD, for bringing this family to our attention. We are thankful to all patient subjects who participated in this study.

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Wagner First Described the ophthalmic clinical features of his eponymous syndrome (OMIM 14200) with a 16-member Swiss kindred in 1938.[1] The ophthalmic features consist of an optically empty vitreous with avascular vitreous strands and veils, moderate myopia, presenile cataracts, and retinal degeneration with atrophy. In 1995, clinical reexamination of the expanded original Wagner pedigree was performed, incorporating an additional 44 relatives.[2] An optically empty vitreous with avascular strands or fibrillary condensation, chorioretinal atrophy, and cataracts were a consistent finding in all affected individuals. By age 45 years, 87% of syndromic individuals had electrophysiologic testing abnormalities, and 55% had tractional retinal detachments.[2]

Families with Wagner syndrome demonstrate an autosomal dominant inheritance pattern with near complete penetrance.[3] The prevalence estimate of Wagner syndrome is less than 1:1 000 000.[3] The condition was mapped to chromosome 5q, and in 2005, a mutation of the chondroitin sulfate proteoglycan 2 gene (*CSPG2*) encoding for the protein versican was found to cosegregate with the disease in a Japanese pedigree.[4] Versican is a large proteoglycan found in many tissues and is a major constituent of the extracellular matrix of human vitreous.[4,5] Since 2005, several groups have identified similar mutations of the *CSPG2* gene in families with Wagner syndrome.[4–7]

Two ocular-only syndromes share clinical and allelic features with Wagner syndrome. Jansen syndrome has a predominance of retinal detachments.[4,8] Jansen syndrome maps to the same chromosome 5q region as Wagner syndrome.[9] Erosive vitreoretinopathy syndrome (OMIM 143200) is a vitreoretinal degeneration first reported in 1994.[10] Affected individuals also have night blindness, visual field defects, and chorioretinal atrophy. Erosive vitreoretinopathy syndrome is also allelic with Wagner syndrome.[7]

Ophthalmic features associated with Stickler syndrome are similar to those found in Wagner syndrome. Stickler syndrome involves myopia, presenile cataract, vitreous degeneration, radial perivascular retinal degeneration, and tractional retinal detachments.[11] The nonocular features of mid-face hypoplasia, cleft palate, bifid uvula, hearing loss, and skeletal abnormalities help to differentiate these 2 syndromes.[12] Vitreous phenotypes can help to distinguish subtypes of Stickler syndrome and Wagner syndrome. Stickler syndrome demonstrates a membranous anterior vitreous (type 1 vitreous) or a fibrillar or beaded vitreous (type 2 vitreous.)[11] The vitreous phenotype in Wagner syndrome is described as having an optically empty vitreous with avascular veils or a fibrillary condensation.[5] A variant of Stickler syndrome devoid of systemic findings, the so-called ocular-only Stickler syndrome, apart from the vitreous phenotypes can be particularly difficult to distinguish from Wagner syndrome.[13,14]

Stickler syndrome is genetically distinct from Wagner syndrome. Three forms of autosomal dominant Stickler syndrome are each associated with an extracellular matrix collagen gene.[12] Type I (OMIM 108300) is associated with *COL2A1*, while type II (OMIM 604841) is associated with *COL11A1*.[12] Both types have ocular and systemic manifestations. The vitreous phenotype is a distinguishing feature between type I and type II. Type I Stickler syndrome is associated with retrolental membranous vitreous while type II Stickler syndrome is described as having a fibrillar or beaded vitreous phenotype. Type III (OMIM 184840) is associated with *COL11A2* and involves only systemic manifestations.[12] The ocular-only variant of Stickler syndrome is a subgroup of type I (*COL2A1*) and shares its membranous vitreous phenotype.[13]

In this report, we clinically and genetically characterize a family with autosomal dominant vitreoretinal degeneration demonstrating a wide ocular phenotypic spectrum. Affected individuals of this family had tentative initial clinical diagnoses of Stickler syndrome and

Wagner syndrome/Jansen syndrome as well as retinitis pigmentosa. This study underscores the importance of obtaining consolidated family history and clinical data as well as seeking genetic testing in the setting of highly variable clinical presentations of a mutual disorder.

METHODS

STUDY SUBJECTS

The study family was identified after a referral of 2 different family members from a community retinal specialist (Claxton Baer, MD) to the Duke University Eye Center. Consenting family members were recruited under an approved human subject research institutional review board protocol for the clinical and molecular analysis of genetic eye disorders to include molecular genetic testing.

All participating family members were offered full ophthalmic examinations. In addition to the standard ophthalmic history, health histories included questions regarding hearing loss, previous repair of hard or soft cleft palate, other midline defects, skeletal or joint abnormalities, and early-onset arthritis. The clinical evaluation included assessment tests of Early Treatment Diabetic Retinopathy Study visual acuity (Snellen equivalent) and intraocular pressure, slit lamp inspection of the anterior segment, and indirect ophthalmoscopy to inspect the fundus.[15,16] Ancillary tests included fundus photographs, axial length measurements, keratometry measurements, and ocular coherence tomography. Goldman visual field tests were performed in affected individuals.

Venous blood was collected from participating family members. Genomic DNA was extracted from venous blood using Auto Pure LS DNA extractor and PUREGENE reagents (Gentra Systems Inc, Minneapolis, Minnesota) and stored.

GENE SCREENING

COL2A1—Genomic DNA from 1 affected individual was screened for sequence variations of the *COL2A1* gene by a commercial laboratory (Matrix DNA Diagnostics, New Orleans, Louisiana). All 54 exons, including intronexon boundaries, were examined. The resulting sequence was compared with DNA of normal controls and available published sequences.

CSPG2—Primers for polymerase chain reaction and sequencing were designed to cover coding and untranslated gene regions, including intronexon boundaries, using the ExonPrimer and Primer3 programs (Helmholtz Center Munich, Munich, Germany) (http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html). Primers were selected to produce amplification product sizes not to exceed 600 base pairs (bp) for optimal sequence output and reading. Large exons or untranslated gene regions were covered with overlapping amplicons, with a minimal 50 bp of overlapped sequence. Table 1 displays the optimized primer sequences used in this study.

Polymerase Chain Reaction and Sequence Analysis—Genomic DNA of 2 affected and 2 unaffected family members was initially screened. The DNA of remaining family members was screened if any sequence variants followed the affection status. Polymerase chain reactions were performed and amplicons were visualized by agarose gel electrophoresis by standard procedures. Polymerase chain reaction amplicon purification was conducted using the Quickstep 2 SOPE Resin (Edge BioSystems, Gaithersburg, Maryland). BigDye Terminator 3.1 (Applied Biosystems Inc, Foster City, California) was used to perform sequencing reactions, and ABI3730 robotics (Applied Bio-systems Inc) was used to process the DNA fragments. Base pair calls were made using the Sequencher 4.8 software (Gene Codes, Ann Arbor, Michigan). Sequences of affected and unaffected

individuals were aligned to a known reference genomic sequence (UCSC Genome Browser, http://genome.ucsc.edu) and compared for sequence variation.

RESULTS

CLINICAL FEATURES

The study family consisted of 3 generations with 6 affected and 3 unaffected participating individuals (Figure 1). The 6 affected individuals provided blood samples for genomic DNA isolation and underwent complete clinical evaluations. Of the 3 unaffected individuals, 2 provided blood samples and underwent clinical examinations (individuals 2 and 9), and 1 provided a blood sample and ophthalmic history but was unavailable for clinical inspection (individual 7).

The participant demographic and clinical examination information is summarized in Table 2. None of the affected family members had historical or systemic clinical features of the Stickler syndromes. The funduscopic examinations revealed significant phenotypic variation and are described next.

INDIVIDUAL 8 (PROBAND)

The proband was a 16-year-old boy who presented with complaints of acute onset of floaters in his right eye. Tentative diagnoses of Wagner/Jansen syndrome and Stickler syndrome had been made in the past based on his family history of retinal detachments. He had no history of cataract or retinal detachment. His distance visual acuity was 20/20 OD and 20/32 OS by Early Treatment Diabetic Retinopathy Study testing. On examination, his lenses were clear bilaterally. He had an optically empty vitreous, with preretinal vitreous condensation in the mid-periphery and avascular vitreous sheets in the far periphery bilaterally. There was severe retinal traction in both eyes, with nasal dragging of the arcades and fovea as seen in Figure 2A. The optic nerve was inverted nasally in the left eye and was detected in the corresponding ocular coherence tomography (Figure 2B). No chorioretinal atrophy was noted; however, both eyes had multiple areas of peripheral hyperpigmentation, lattice degeneration, and cystic tufts. Multiple round retinal holes and a localized tractional detachment were identified in the periphery of the left eye. The detachment and holes were successfully treated with encircling scleral buckle, pars plana vitrectomy, gas tamponade, and laser. Bilateral Goldman visual field testing demonstrated an enlarged blind spot in the right eye and a paracentral scotoma in the left.

INDIVIDUAL 4

The proband's mother was 37 years of age at the time of examination and had a history of bilateral presenile cataracts requiring surgical extraction at 18 years of age for the left eye and 30 years of age for the right eye. She had a long-standing history of a large exotropia. She had no history of retinal detachment. Her Snellen distance visual acuity was 20/20 OD and 20/25 OS. A composite fundus photograph of her right eye is shown in Figure 3A. There was an optically empty vitreous except for a vascular vitreous sheets in the midperiphery and far periphery. Both the temporal and nasal retinal vascular arcades were straightened, suggesting moderate retinal traction. There was a focal area of chorioretinal atrophy and pigmentary changes. There were also perivascular pigmentary changes. Visual field testing demonstrated ring scotomata of both eyes (Figure 3B).

INDIVIDUAL 1

The proband's maternal grandfather was 60 years of age at the time of examination. He had primary open-angle glaucoma requiring tube shunt placement in the right eye, with subsequent development of a pupillary membrane. He had bilateral presenile cataracts with a

history of surgical extraction at approximately 30 years of age. He had no history of retinal detachment. His distance visual acuity was hand motions OD and 20/25 OS. The view of the right fundus was obscured by anterior ocular media opacification. His vitreous cavity on the left was optically empty except for an avascular vitreous membrane inferiorly, as demonstrated in Figure 4. There were retinal pigmentary changes underlying the area of the vitreous membrane. A few patchy areas of chorioretinal atrophy could also be seen. Goldman visual field testing of the left eye revealed a paracentral scotoma.

INDIVIDUAL 6

The proband's maternal aunt was 40 years of age, with an ocular history significant for a chronic retinal detachment of the right eye requiring 3 surgical repairs starting at the age of 5 years. She had a significant cataract of the left eye requiring surgical extraction at 25 years of age. She had an initial diagnosis of retinitis pig-mentosa. She described slow degradation of left eye visual acuity since childhood. She had a long-standing small-angle right exotropia. Her visual acuity was no light perception OD and 20/400 OS. There was no view of the fundus of the right eye because of a dense cataract. The left eye vitreous cavity was optically empty except for avascular vitreous sheets in the far periphery. The left fundus had notable diffuse chorioretinal atrophy with dense pigment in the midperiphery to far retinal periphery (Figure 5A). The left eye retinal vascular arcades were straightened, suggesting mild retinal traction. The corresponding Goldman visual field test of the left eye showed severe constriction with a few scattered small islands (Figure 5B).

INDIVIDUAL 10

The proband's maternal cousin (and son to individual 6) was 16 years of age at the time of evaluation. He had a history of a retinal detachment in the right eye at a young age, leading to a poor visual outcome. He had not had cataract surgery. He described slow worsening visual acuity of his left eye. His distance visual acuity was no light perception OD and 20/160 OS. There was a dense cataract in the right eye, obscuring the view of the fundus. The left vitreous cavity was optically empty except for a vascular preretinal membranes in the far periphery. The left fundus demonstrated findings similar to that of his mother, with diffuse retinal pigmentary changes, patchy chorioretinal atrophy, and straightened vascular arcades (Figure 6A). The corresponding left visual field showed moderate constriction (Figure 6B).

INDIVIDUAL 11

A second maternal cousin and daughter to individual 6 had a history of a retinal detachment in the right eye at the age of 8 years. She was treated for a macula-off retinal detachment at Duke University Eye Center. The detachment originated from a posterior retinal break and was associated with rings of dense vitreous bands at the equator. The retina was successfully reattached with vitrectomy, scleral buckling, and silicone oil tamponade with subsequent oil removal. She had a moderate-angle exotropia. Her distance visual acuity was 20/200 OD and 20/50 OS. She had a significant posterior subcapsular cataract of the right eye that had developed after retinal detachment repair. The vitreous cavity of the left eye was optically empty except for a subtle midperipheral a vascular vitreous membrane (Figure 7A). Retinal pigmentary changes were conspicuously absent except for mild peripapillary pigmentation. There was straightening of the vascular arcades. She had a blonde fundus with subtle inferior chorioretinal atrophy, which corresponded to early superior scotomatous changes with visual field testing (Figure 7B).

MOLECULAR GENETIC EVALUATION SCREENING OF CANDIDATE GENES

Proband (individual 8) genomic DNA was commercially screened for sequence variation of the *COL2A1* gene. No sequence changes were identified with comparison to internal and external control DNA and to the published sequence for the *COL2A1* gene.

Initial sequence screening of the *CSPG2* gene uncovered 17 single-nucleotide polymorphic (SNP) variations: 10 exonic SNPs, 2 untranslated gene region SNPs, 4 in-tronic SNPs, and 1 SNP at a splice site (Table 3). Only the splice site SNP cosegregated with affection status in the initial 4 DNA samples screened. The splice site sequence change was a single base pair substitution of a guanine for a thymine at the 5′ end of intron 8 at position c.9265+1G>T (Figure 8). For confirmation, all family member DNA was sequenced at the mutation site. The mutation cosegregated with all affected individuals (n=6). The splice site mutation did not appear with sequence screening of the DNA of the 3 unaffected family members or in 107 external control DNA samples.

COMMENT

We have identified a novel mutation with a single base pair substitution of a guanine for a thymine at the 5′ end of intron 8 at position c.9265+1G>T. This mutation co-segregates with the disease state in our study family with clinical manifestations of Wagner syndrome. This splice site has previously been associated with Wagner syndrome. In 2006, Kloeckener-Gruissem et al[5] identified a guanine to adenine substitution at the 9265+1 position in the original Swiss family described by Wagner. With messenger RNA transcript analysis, this group found a21-bp deletion causing an in-frame deletion of 7 amino acids. This was likely caused by the disruption of the usual splice site sequence, allowing activation of a subsequent cryptic splice site, although none was identified in that study. In 2007, Meredith et al[6] reported a father-daughter pair with the same c.9265+1G>A mutation. We believe that our mutation c.9265+1G>T leads to the same 21-bp deletion described by Kloeckener-Gruissem et al. We were unable to confirm this with transcript analysis, however, because the cell lines for the family samples were not viable. No control DNA samples had sequence variants at this site, which strongly suggests that this is a highly conserved splice site consensus sequence and that the base substitution does not represent a common polymorphism.

The wide ocular phenotypic spectrum demonstrated in this relatively small family is notable. In the original Wagner syndrome report, the term *situs inversus* was used to describe the nasal displacement of the temporal arcades presumably due to tractional effects.[1] To our knowledge, the current report is the first to describe tractional forces leading to the inversion of the papilla in Wagner syndrome. Figure 2B is an ocular coherence tomography photograph showing the “inverted papilla” of individual 8 (proband) and also demonstrating nasal foveal dragging. This is in contrast to the proband’s mother (individual 4) with a temporally displaced fovea (Figure 3A). She had a large-angle exotropia on examination, which likely represents a positive angle kappa. A large angle kappa is an infrequent feature of Wagner syndrome.[10]

Retinal detachments were not thought to be a significant finding in Wagner syndrome until the reexamination of the expanded original family in 1995.[2] In that study, rhegmatogenous retinal detachments were rare (4%), while tractional retinal detachments were a common finding after the age of 45 years (55%). A majority of the affected individuals in our pedigree sustained retinal detachments, suggesting that this is a more common feature than the rate previously reported. More interestingly, the average age of retinal detachment occurrence and detection in our family was 9.5 years. This finding refutes retinal detachment

as a middle-age issue and has significant implications for how patients with Wagner syndrome should be monitored clinically.

Our studied pedigree contains a mother-son pair with unusual fundus features that are not necessarily associated with age. The diffuse retinal atrophy and pigmentary changes seen in individuals 6 and 10 could readily be diagnosed as a distinct disease entity if they had not been evaluated as members of a larger kindred. This clinical phenotype closely resembles erosive vitreoretinopathy syndrome with diffuse chorioretinal degeneration and constriction of the visual fields.[7] This report, as well as that by Meredith et al, demonstrates that a single kindred can have members with the more traditional Wagner syndrome phenotype features along with individuals with an erosive vitreoretinopathy syndrome phenotype, all caused by a single *CSPG2* mutation.[10] As a progressive degeneration, the severity of the clinical presentation cannot be fully explained as late changes since both individuals are relatively young. In our kindred, the oldest affected family member (individual 1) had the mildest phenotype and had an initial diagnosis of retinitis pigmentosa.

Given the broad phenotypic variation seen in Wagner syndrome, as is evident in the family we present herein, making a definitive diagnosis clinically can be extremely challenging. This may be especially difficult in families with limited family member availability because of adoption or estrangement or in questions of paternity. Affected individuals from this kindred were given different diagnoses that changed over time with disease progression. Confirming a molecular diagnosis is helpful in determining a clinical care plan and prognosis and also allows the patient and family to seek same-condition larger-family support networks.

Acknowledgments

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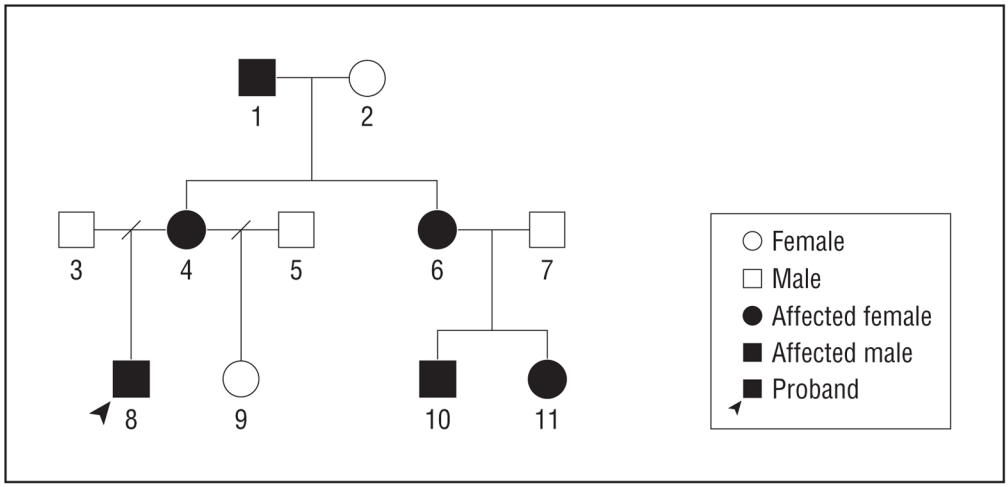

Figure 1.
Study family pedigree. The study family consisted of 11 individuals in 3 generations with 6 affected and 3 unaffected participants.

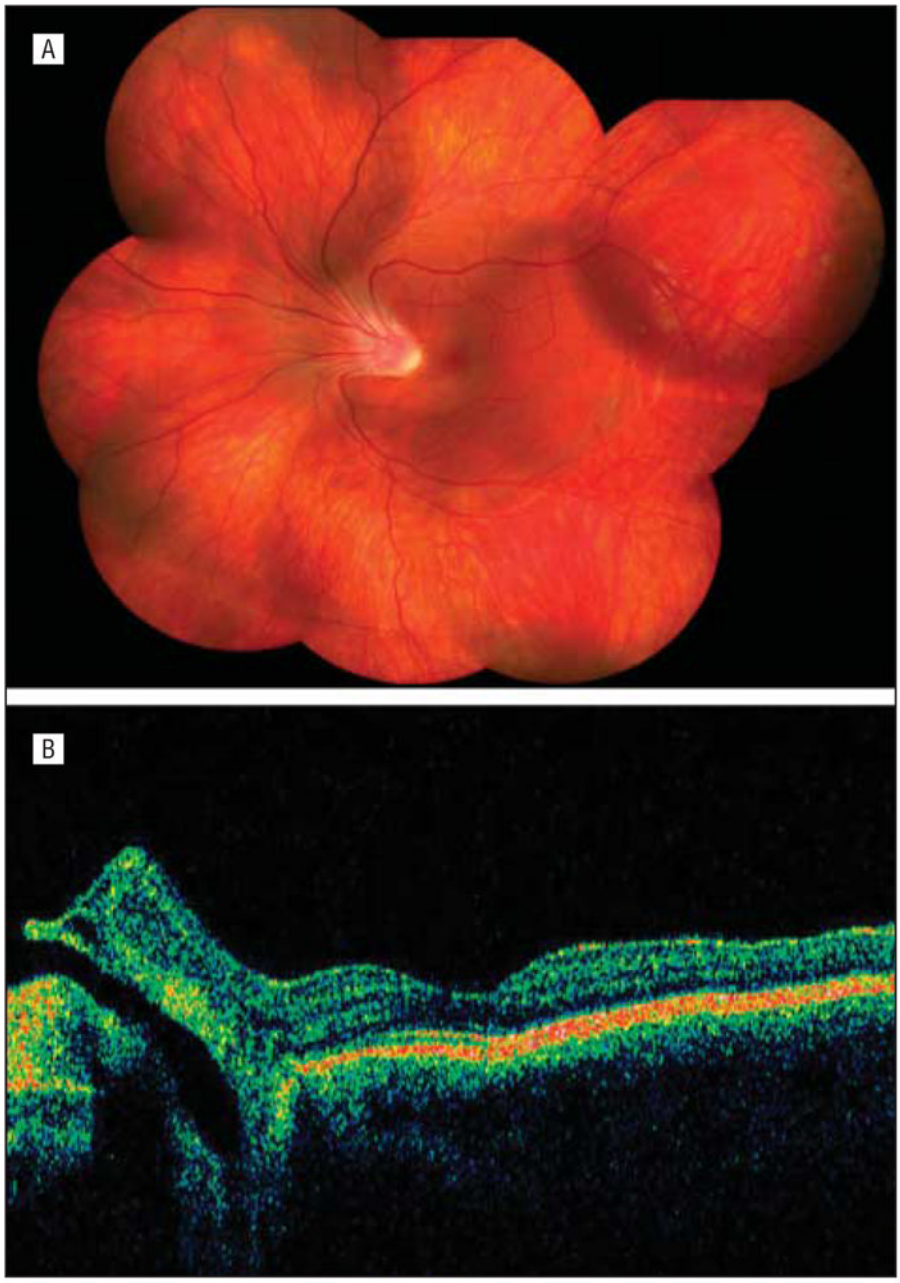

Figure 2.
Individual 8. A, Composite fundus photograph of the left eye shows an optically empty vitreous with preretinal vitreous condensation in the midperiphery, avascular vitreous sheets in the far periphery, and nasal dragging of the arcades and fovea. B, An optical coherence tomogram of the left eye shows the optic nerve inverted nasally.

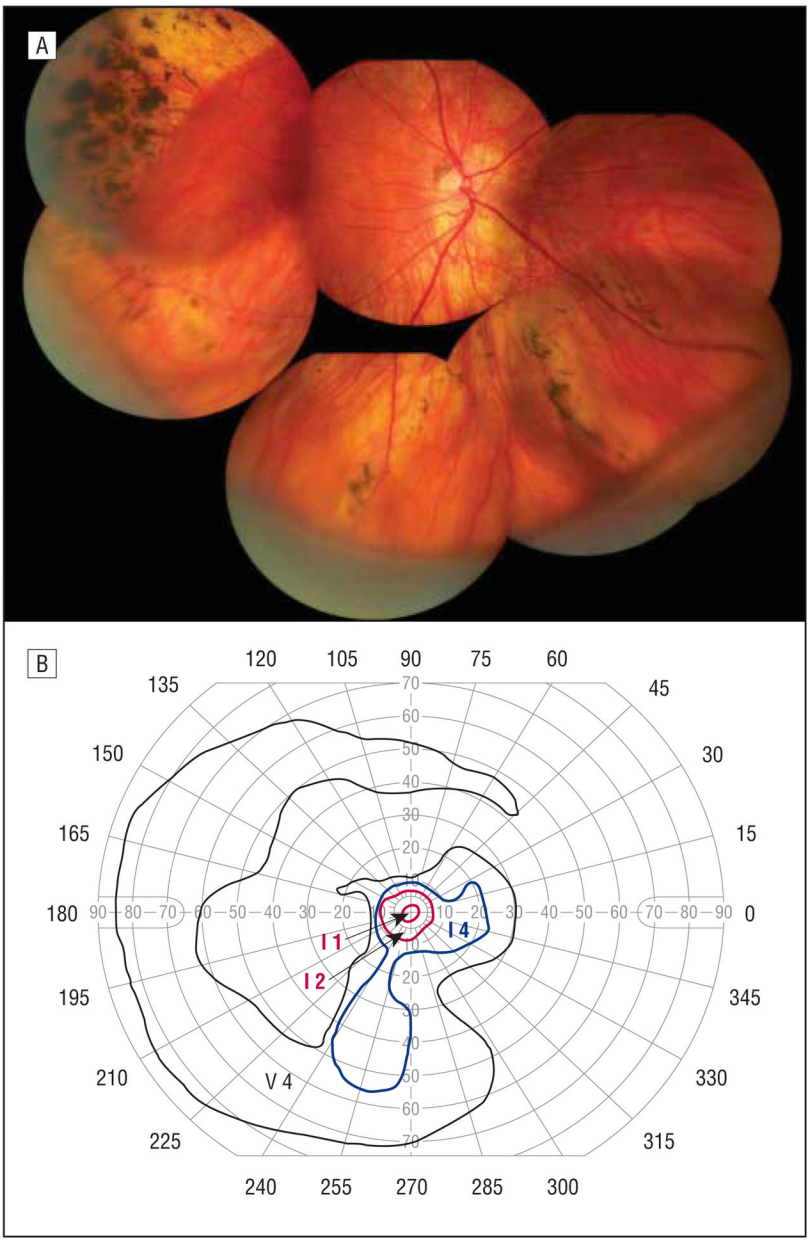

Figure 3.
Individual 4. A, Composite fundus photograph of the right eye shows an optically empty vitreous except for avascular vitreous sheets in the midperiphery and far periphery, straightening of the temporal and nasal retinal vascular arcades, an area of chorioretinal atrophy, and perivascular pigmentary changes. B, Goldmann visual field test of the left eye shows a ring scotoma.

Figure 4.
Individual 1. Composite fundus photograph of the left eye shows an optically empty vitreous except for an avascular vitreous membrane inferiorly with underlying retinal pigmentary changes and patchy areas of chorioretinal atrophy.

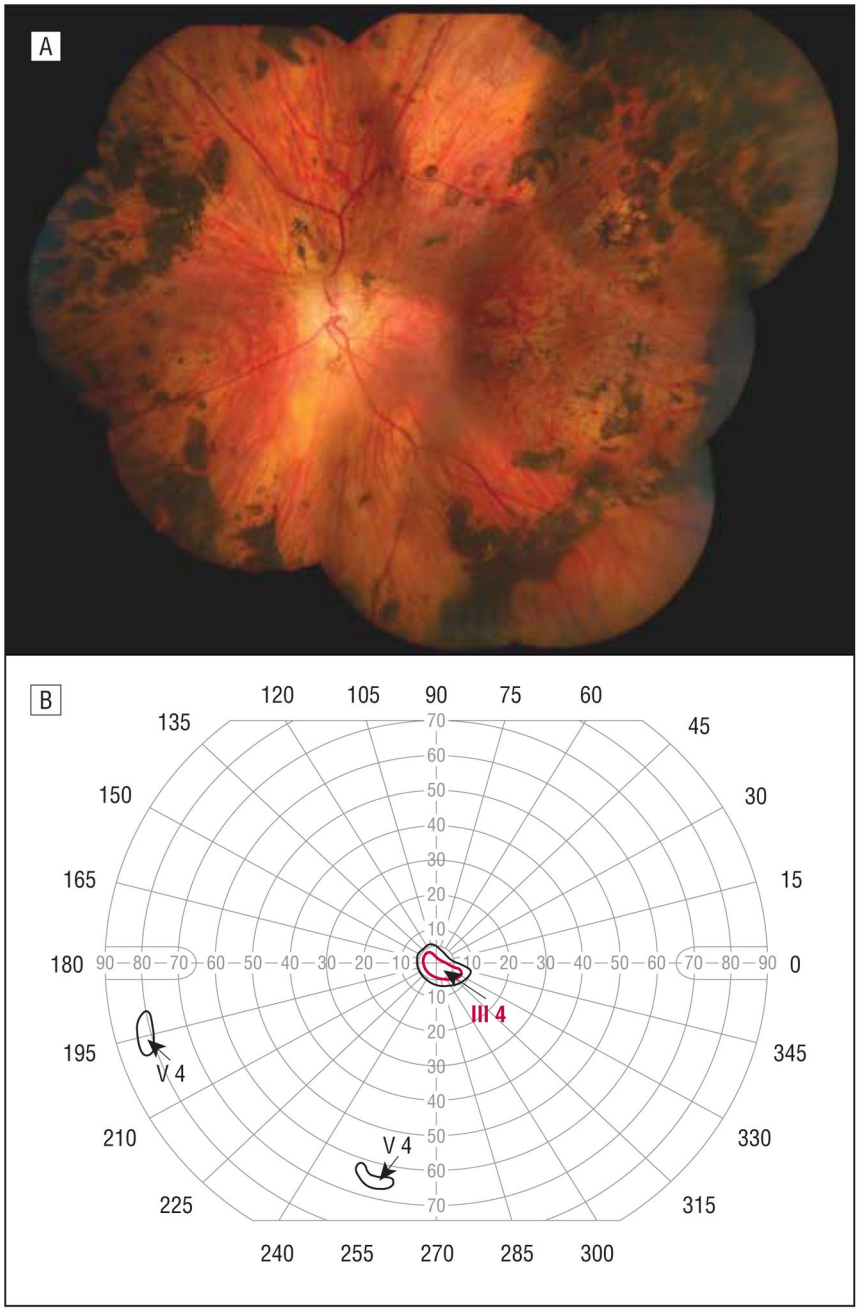

Figure 5.

Individual 6. A, Composite fundus photograph of the left eye shows an optically empty vitreous cavity except for avascular vitreous sheets in the far periphery, diffuse chorioretinal atrophy with dense pigment in the midperiphery to far retinal periphery, and straightening of the vascular arcades. B, Goldmann visual field test of the left eye shows severe constriction with a few scattered small islands.

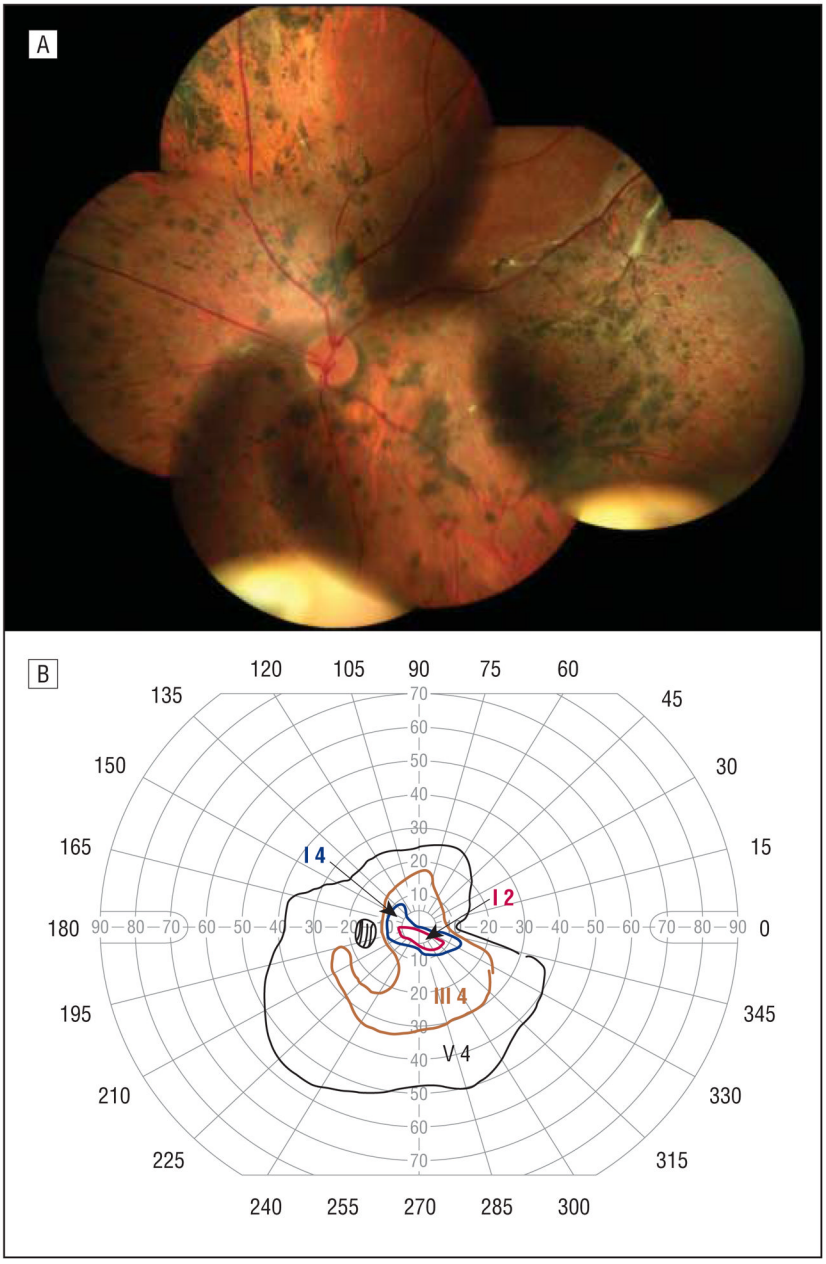

Figure 6.
Individual 10. A, Composite fundus photograph of the left eye shows an optically empty vitreous except for avascular preretinal membranes in the far periphery, diffuse retinal pigmentary changes, patchy chorioretinal atrophy, and straightened vascular arcades. B, Goldmann visual field test of the left eye shows moderate constriction.

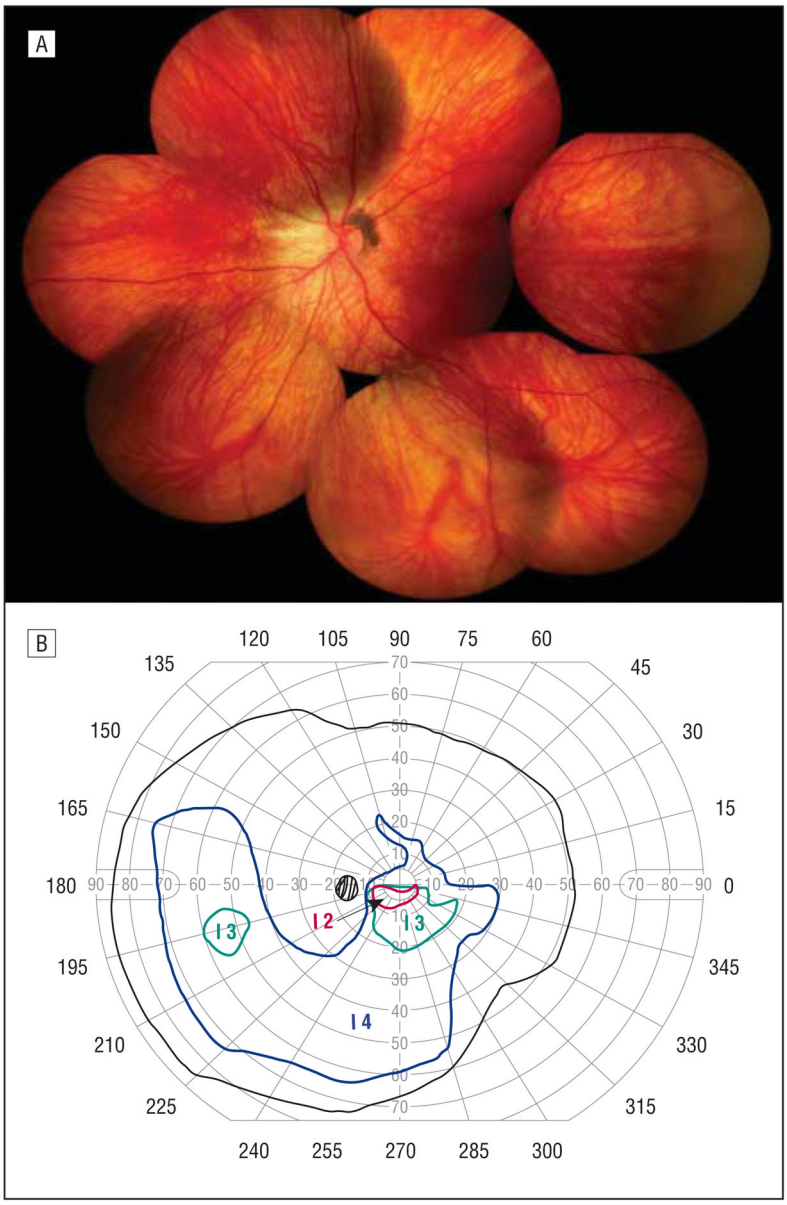

Figure 7.
Individual 11. A, Composite fundus photograph of the left eye shows an optically empty vitreous except for a subtle midperipheral avascular vitreous membrane, mild peripapillary pigmentation, straightening of the vascular arcades, and subtle inferior chorioretinal atrophy. B, Goldmann visual field test of the left eye shows early superior scotomatous changes that correspond to the inferior chorioretinal atrophy seen on fundus photography.

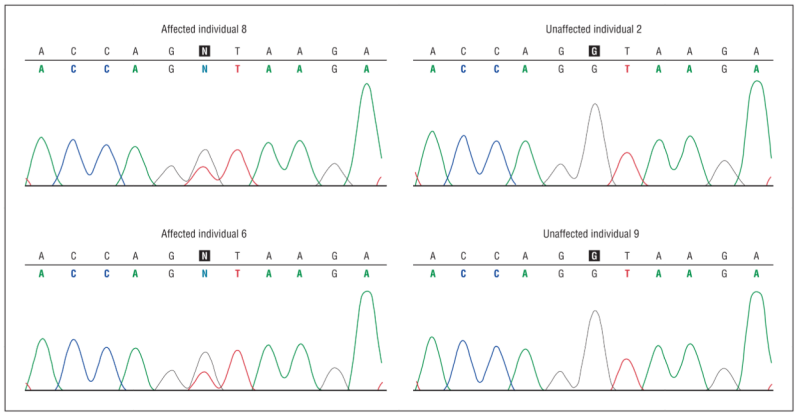

Figure 8.

Sequence chromatogram of *CSPG2* splice site mutation in intron 8 *CSPG2* splice donor mutation (c.9265+1G>A) from 4 individuals (2 affected, 2 unaffected) who were initially screened. The *N* in the sequence depicts the heterozygous mutation present. DNA analysis of all other affected family members showed cosegregation with this mutation. One hundred seven control DNA samples were negative for this mutation.

Table 1

Primers for Polymerase Chain Reaction and Sequencing of *CSPG2*

Exon	Forward Primer	Reverse Primer	Approximate Size, Base Pairs
1	GTGAATGAACcctcctc	TTTCTTAACTTCGCCTTAC	517
2	ACCCTTATTACATA-CAATGC	ATCAATCTTTTATTC-CAGTG	270
3	CAAACTATTATAAAGGC-TGC	CAAACAACAGTTACTTCT-GAG	562
4/5	CTAAGTTGATACATT-GCTCC	ACAAGCACGAAGACTTG	569
6.1	AAAGTATTACAT-GCTCCTCC	TGTGGTTTTAACCATAT-CAC	578
6.2	CATTGATGTTCAACTAGCC	AGTAAACCTAACTAAA-GATACTCTG	581
7.1	TCCCTACAAAATACTCAGG	ATTTTCTTTTCAGTTTTGG	657
7.2	CCTTTGGTAACATCTATGG	GTATCTGTTCTCATCTC-TGG	605
7.3	AGAATTGTTTCCT-TATTCTG	AATAAGAGTAAATTCATC-TGC	613
7.4	TACAGAACCTTCAGCCTC	AGTTTCTG-GAGAAATAGTCG	610
7.5	TTAGTACCTTCTGTTC-CATC	AATAATCCT-GAGCCTAAATC	599
7.6	TTTACATCATCTTT-GAGTCC	TCTAATAGCAGCATTTGG	658
8.1 UTR	GGAATTTGTCTTGG-TAGTTC	TCGAAAAGTATGTATCAT-CAG	635
8.2 UTR	TTTGCTGATGTATTTCTG	aaaattGAGGCCATATC	584
8.2 UTR	GCTCTAGAACAGAT-TATAAAGG	TGGATCTGTTTCTTCAC-TAC	644
8.1	GATTTGAATCCTTCTTT-GTG	CTCATGGAATGGGACTG	633
8.2	TTACATGGAAGCCTGAG	GTGATTATCCTGCTAGT-GTC	598
8.3	AATTACAGAAGGCTCTGG	CCCTGCTCCATAAAGAC	597
8.4	TACATTAGAAAATTTGGGG	CTACTGGGTCCTTCTGAG	582
8.5	AGAAGAAACGGTAAT-GATG	TTCAGTAAATGTCTGT-GAATC	592
8.6	ATTGAAAGTGAAACAA-CATC	CCACATTTTCCATTCTG	595
8.7	AAGTGGAACAAAT-CAATAAC	ATCCATCAGCAGTAACAG	621
8.8	GAAGTGGATATTGTT-GATTC	TCAGTTAAAGAAAGGTT-GAC	596
8.9	TGGAATGCAAACA-GATATAG	AGTAGCAATACTTAG-ATAGGG	580
8.10	AAACATGCTGGTCCTTC	AAACATCTTGATT-GCTTCTC	582
8.11	GAAGATGATGG-TAAACCTG	AATAATATGAAACTCTC-CATTG	680
9	CTAAATTGCTATGG-TAAAGC	TTGAAATCTCTGATT-GATTC	322
10	aatttaactggctgtcttg	GAAATGTGAATTTC-CACTG	318
11	GACCAAATTTTATGAAT-CAG	ttaagtaattcacagatgagg	433
12	AATCCAGCCAGTAAAGAG	agtctaatgcaccctctg	287
13	GAAATTGTTGAGTC-TATTCC	GTCTCAAACAAT-GAATTTG	342
14	TTGGTAC-CATAAAGAAAGAG	CACACAATATTACTT-GCTCC	387
15.1	CTAGACACCTTCATTT-TACG	AATCATCTTATTTA-CATGGC	576
15.2	TTTAGTTTTCTATTTGCCTC	TATGAAATGCATTGATCG	593
15.3	AGGCCTGAATGGAG-GACTTT	ACAGGAAGAAATGCCCA-CAC	587
15.4	TCCTCACACAATTTG-GAATCA	TCCTTCTAAGCCAAAG-GAGGT	475

Abbreviation: UTR, untranslated region.

Table 2

Subject Demographics and Ocular Clinical Features

Subject/ Sex/Age, y	Eye	Best-Corrected VA (Snellen Equivalent)	Recent Refractive Error Spherical Equivalent,[a] D	Past Refractive Error (Presurgical)	Axial Length, mm	Lens Status	Retinal Detachment Age, y	Surgery
1/M/60	OD[b]	HM	−0.875	NA	NA	ACIOL pupillary membrane	NA	CE/IOL tube shunt
	OS	20/25	0.25	NA	23.44	ACIOL	NA	CE/IOL
2/F/59.5	OD	20/20	0.50	+0.25+0.50×126	NA	NSC	NA	None
	OS	20/20	0.625	+0.25+0.75×40	NA	NSC	NA	None
4/F/37	OD	20/20	−2.00	NA	24.69	PCIOL	NA	CE/IOL
	OS	20/25	−0.25	NA	24.46	PCIOL	NA	CE/IOL
6/F/40	OD[b]	NLP	NA	NA	NA	Dense NSC	5	SB/PPV
	OS	20/400	Plano	NA	22.38	PCIOL	NA	None
8/M/16	OD	20/20	NA	(age 14 y) −1.00+1.00×90	24.56	Clear	NA	None
	OS[c]	20/32	−1.00	(age 14 y) −1.00+2.00×90	25.07	Clear	16	SB/PPV
9/F/8	OD	20/20	Plano	Plano	NA	Clear	NA	None
	OS	20/20	Plano	Plano	NA	Clear	NA	None
10/M/16	OD[b]	NLP	NA	(age 4 y) + 2.50 sph	NA	Dense NSC	9	SB/PPV
	OS	20/160	−0.25	(age 4 y) + 2.50 sph	22.18	Clear	NA	None
11/F/11	OD	20/200	−3.25	(age 2.5 y) 1.25+3.00×90	23.62	PSC	8	SB/PPV
	OS	20/50	0.75	(age 2.5 y) −1.50+4.25×95	22.85	Clear	NA	None

Subject/ Sex/Age, y	Eye	Optically Empty Vitreous	Vitreal Avascular Membranes	Retinal Traction	Chorioretinal Atrophy	Retinal Pigmentary Changes	Ocular Alignment	Visual Field
1/M/60	OD[b]	NA	NA	NA	NA	NA	Ortho	NA
	OS	Yes	Yes	None	Mild	Mild	Ortho	Paracentral scotoma
2/F/59.5	OD	None	None	None	None	None	NA	NA
	OS	None	None	None	None	None	NA	NA
4/F/37	OD	Yes	Yes	Moderate	Moderate	Moderate	XT	Ring scotoma
	OS	Yes	Yes	Moderate	Moderate	Moderate	XT	Ring scotoma
6/F/40	OD[b]	NA	NA	NA	NA	NA	XT	NA
	OS	Yes	Yes	Mild	Severe	Severe	XT	Severe constriction
8/M/16	OD	Yes	Yes	Severe	None	None	Ortho	Enlarged blind spot

Subject/ Sex/Age, y	Eye	Optically Empty Vitreous	Vitreal Avascular Membranes	Retinal Traction	Chorioretinal Atrophy	Retinal Pigmentary Changes	Ocular Alignment	Visual Field
	OS[c]	Yes	Yes	Severe	None	None	Ortho	Paracentral scotoma
9/F/8	OD	None	None	None	None	None	NA	NA
	OS	None	None	None	None	None	NA	NA
10/M/16	OD[b]	NA	NA	NA	NA	NA	Ortho	NA
	OS	Yes	Yes	Moderate	Severe	Severe	Ortho	Moderate constriction
11/F/11	OD	NA[d]	NA[d]	None	Moderate	None	XT	Nasal scotoma
	OS	Yes	Yes	None	Moderate	None	XT	Superior scotoma

Abbreviations: ACIOL, anterior chamber intraocular lens; CE/IOL, cataract extraction/intraocular lens; D, diopters; HM, hand motions; NA, not available; NLP, no light perception; NSC, nuclear sclerotic cataract; Ortho, orthophoria; PCIOL, posterior chamber intraocular lens; PPV, pars plana vitrectomy; PSC, posterior subcapsular cataract; SB, scleral buckle; sph, sphere; XT, exotropia; VA, visual acuity.

[a]Spherical equivalent=sphere+cylinder/2.

[b]No view of fundus because of opacity of anterior ocular media.

[c]Patient examined both previtrectomy and postvitrectomy.

[d]Unable to determine secondary to postvitrectomy status.

Table 3

Sequence Variants Identified in *CSPG2*

Location	SNP	Position	Function	Heterozygous Sequence Variant Present
Exon 3	rs12332199	c.348T>C	Coding synonymous	U2 and U9
Exon 5	rs4470745	c.645A>G	Coding synonymous	U2
Exon 8	rs309559	c.4547A>G	Coding nonsynonymous	U2
Exon 8	rs188703	c.5477G>A	Coding nonsynonymous	U2
Exon 8	rs309557	c.5808T>C	Coding synonymous	U2
Exon 8	rs160279	c.6723A>G	Coding synonymous	U2
Exon 8	rs160278	c.6902T>A	Coding nonsynonymous	U2
Exon 8	rs160277	c.8809G>T	Coding nonsynonymous	U2
Exon 8	rs16900532	c.9003C>A	Coding nonsynonymous	U2
Exon 14	rs308365	c.9882C>T	Coding synonymous	A6 (T/T), A8 (T/T), U2 (T/T), U9 (T/T)
UTR 8	Novel	c.4004–1284C>G	UTR	U9
UTR 8	rs309560	c.4004–687C>G	UTR	U2
Intron 8	Novel	c.9265+1G>T	Splice site	A6 and A8
Intron 9	rs695103	c.9379+7T>C	Intronic	U9
Intron 10	rs6873404	c.9494–63T>A	Intronic	U2
Intron 13	Novel	c.9880+70G>A	Intronic	U9
Intron 13	Novel	c.9880+73T>C	Intronic	A6 (C/C)

Abbreviations: A6, affected individual 6; A8, affected individual 8; U2, unaffected individual 2; U9, unaffected individual 9; UTR, untranslated region.