Variants of Anterior Segment Dysgenesis and Cerebral Involvement in a Large Family With a Novel *COL4A1* Mutation

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• PURPOSE: To investigate the diverse ocular manifestations and identify the causative mutation in a large family with autosomal dominant anterior segment dysgenesis accompanied in some individuals by cerebral vascular disease.

• DESIGN: Retrospective observational case series and laboratory investigation.

• METHODS: Forty-five family members from 4 generations underwent ophthalmic examination. Molecular genetic investigation included analysis with single nucleotide polymorphism (SNP) markers and DNA sequencing. Whole exome sequencing was performed in 1 individual.

• RESULTS: A broad range of ocular manifestations was observed. Typical cases presented with corneal clouding, anterior synechiae, and iris hypoplasia. Posterior embryotoxon, corectopia, and early cataract development were also seen. One obligate carrier and several other family members had minor ocular anomalies, thus confounding the scoring of affected and unaffected individuals. Cerebral hemorrhages had occurred in 4 individuals, in 3 at birth or during the first year of life. Seven patients with corneal clouding were considered "definitely affected" for linkage studies. Haplotype mapping revealed that they shared a 14 cM region in the terminal part of chromosome 13q that included the locus for *COL4A1*. The affected family members were heterozygous for a novel *COL4A1* sequence variant c.4881C > G (p.Asn1627Lys) predicted to be damaging and not found among 185 local blood donors. Exome sequencing showed that this variant was the only one in the candidate region not found in dbSNP.

• CONCLUSION: Among the family members shown to carry the novel *COL4A1* mutation, heterogenous presentations of anterior segment dysgenesis was seen. Testing family members for this mutation also made a definite diagnosis possible in patients with a clinical presentation difficult to classify. In families where anterior segment dysgenesis occurs together with cerebral hemorrhages, genetic analysis of *COL4A1* should be considered. (Am J Ophthalmol 2013;155:946–953.

ANTERIOR SEGMENT DYSGENESIS IS A TERM USED TO denote the presence of developmental anomalies that affect one or several of the structures of the anterior part of the eye. Included are the Axenfeld-Rieger group of disorders (Axenfeld-Rieger anomaly, iris hypoplasia, and iridogoniodysgenesis),[1] Peters anomaly, and aniridia.[2] Anomalies affecting other organs are found in Axenfeld-Rieger syndrome, iridogoniodysgenesis syndrome,[1] and Peters Plus disease.[3] There is a considerable overlap between the different entities, and their presentation may vary widely between affected family members.[4,5] Anterior segment dysgenesis may also be part of several multisystem disorders, such as Alagille syndrome,[6] Wolf-Hirschhorn syndrome,[7] SHORT syndrome,[8] and Abruzzo-Erikson syndrome.[9]

Mutations in more than 12 genes have been associated with anterior segment dysgenesis.[10] Most single gene disorders show autosomal dominant inheritance. In mice, dysfunction of the collagen IV alpha gene (*Col4a1*) was found to be associated with severe congenital anomalies in the eye, brain, and kidney.[11,12] In humans, *COL4A1* mutations were first shown to cause autosomal dominant porencephaly, cerebral hemorrhages, and microangiopathy including retinal arteriolar tortuosity.[12] Later, the HANAC syndrome was described, consisting of hereditary angiopathy, nephropathy (renal cysts, hematuria), aneurysms, and muscle cramps.[13] Recently, mutations in *COL4A1*/*Col4a1* have been associated with an autosomal dominant form of muscle-eye-brain disease (MEB) and Walker-Warburg Syndrome (WWS), both in humans and in mice.[14] Anterior segment dysgenesis associated with mutations in *COL4A1* has been reported in 2 French families.[15,16] In the affected individuals,

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congenital cataract was the most frequent finding, followed by iris anomalies (hypoplasia and corectopia), microcornea with peripheral corneal opacities, and elevated intraocular pressure.

Type IV collagens are basement membrane proteins that are widely expressed. COL4A1 and COL4A2 are the most abundant ones.[17] COL4A1 is not required for the formation of basement membranes, but is essential for their structural integrity and function.[18] In the eye, presence of COL4A1 has been demonstrated in the basement membranes of the conjunctiva, corneal epithelium, corneal endothelium, trabecular meshwork, Schlemm canal, and choroid body, as well as in the lens, retinal inner limiting membrane, Bruch membrane, and vascular basement membranes.[19] Misfolded COL4A1 may thus disrupt the integrity of basement membranes in most parts of the eye. In addition, at least in mice, it may also contribute to cataract development.[20]

In the present report we describe the findings in a large family with heterogenous presentation of anterior segment dysgenesis accompanied in some individuals by cerebral vascular disease. The family was first reported in 1981.[21] Ocular anomalies were seen in a large proportion of family members. Examination with single nucleotide polymorphism (SNP) markers in a subset of 7 definitely affected individuals revealed that they shared a 14 cM region in the terminal part of chromosome 13q encompassing the *COL4A1* locus. DNA sequencing revealed a novel sequence variant in *COL4A1*, c.4881C>G, likely to be the cause of the disorder.

METHODS

• **FAMILY STUDY:** Family members were invited to participate and signed a letter of informed consent. This retrospective study consisted of a review of hospital records including data from routine clinical chemistry analyses and computed tomography (CT) and magnetic resonance imaging (MRI) scans, a general medical and ophthalmologic examination, and a genetic laboratory investigation. The ophthalmologic examination included Snellen visual acuity measurements, slit-lamp examination, gonioscopy, Goldmann tonometry, indirect ophthalmoscopy, and anterior segment and fundus photography. The purpose and the procedures of the study were explained to all participants, and all signed a letter of informed consent. The study adhered to the tenets of the Declaration of Helsinki, and the study as well as the letter of consent were approved prospectively by the Regional Committee for Medical and Health Research Ethics, Western Norway (IRB#00001872) (ref. 2010/1552). Approval included permission to obtain clinical, radiological, and laboratory data, and to perform the genetic analysis.

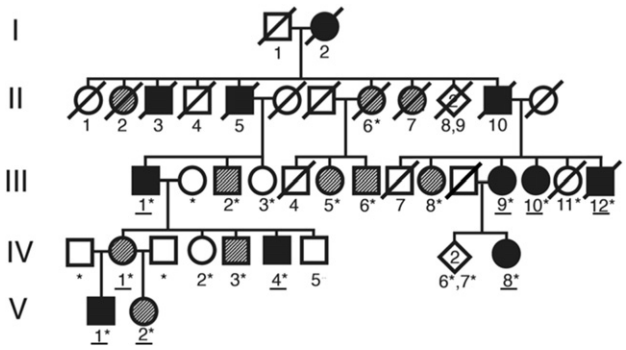

FIGURE 1. Partial pedigree showing individuals with anterior segment dysgenesis. Filled symbols indicate individuals with corneal opacities. Shaded symbols indicate those with minor ocular anomalies of uncertain importance. Individuals examined as part of the present study are marked with an asterisk. Data for other individuals are from Odland.[21] Underlined numbers indicate *COL4A1* mutation carriers.

• **LINKAGE STUDIES WITH SNP MARKERS:** Genomic DNA was isolated from whole blood using the nucleic acid extractor model 341 (Applied Biosystems [ABI], Foster City, California, USA). A genome-wide SNP scan was performed in a subset of definitely affected individuals using the Affymetrix 250K chip (Affymetrix, Santa Clara, California, USA) and search for regions of shared identity by descent was performed using the PLINK software.[22]

• **DNA SEQUENCING AND MUTATION DETECTION:** DNA was amplified by polymerase chain reaction (PCR) using standard procedures. After PCR amplification, the PCR products were treated with SAP/exonuclease I (Amersham, Chalfont St. Giles, United Kingdom) and sequenced using the PRISM BigDye Terminator kit (ver1.1) and an ABI 3730 Genetic Analyzer (ABI). DNA sequences were analyzed by SeqScape software (ABI). DNA from 185 healthy local blood donors was used as controls for the sequence variants identified.

• **EXOME SEQUENCING:** Whole exome capture using Roche-Nimblegen's SeqCap EZ Exome v2 and sequencing on the Illumina HiSeq was performed as described previously[23] to a median coverage of 78×. Variant analysis was restricted to the chromosome 13 linkage interval.

RESULTS

• **OCULAR ABNORMALITIES:** Forty-five individuals were included in the study. A partial pedigree is shown in Figure 1. The ocular malformations and their severity varied considerably between the family members examined. Corneal clouding was present in 7 individuals (Table 1, Figure 2). Most had bilateral involvement, but

TABLE 1. Overview of Ocular and Neurologic Signs and Symptoms in Individuals[a] From a Family With Anterior Segment Dysgenesis and Neurologic Disease

	Corneal Clouding		Embryotoxon Posterior		Anterior Synechiae		Iris Hypoplasia		Cataract <45 y						
Patient	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	Fundus Anomalies	Spastic Hemiparesis/ Paraparesis	Cognitive Impairment	Epilepsy	Cerebellar Signs
III-1	−	+	+	+	−	+	+	+	−	−	+	−	−	−	−
III-9	+	+	−	+	+	+	(+)	(+)	+	+	−	(+)	−	−	+
III-10	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+
III-12	+	+	+	+	−	−	+	+	−	−	−	−	−	−	−
IV-4	−	+	+	−	−	+	+	+	−	−	−	−	(+)	−	−
IV-8	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
V-1	+	+	+	+	−	−	+	+	−	−	−	+	(+)	+	−
IV-1	−	−	−	−	−	−	(+)	(+)	−	−	−	−	−	−	+
V-2	−	−	(+)	(+)	−	−	−	−	−	−	−	+	+	+	−
II-6	−	−	(+)	(+)	−	−	−	−	−	−	−	−	−	−	−
III-2	−	−	(+)	(+)	−	−	−	−	−	−	−	−	−	−	−
III-5	−	−	−	−	−	−	−	−	−	−	(+)	−	−	−	−
III-6	−	−	−	−	−	−	−	−	−	−	(+)	−	−	−	−
III-8	−	−	(+)	(+)	−	−	(+)	(+)	−	−	−	−	−	−	−
IV-3	−	−	−	−	−	−	−	−	−	−	(+)	−	−	−	−

− = absent; + = present; (+) = minor changes.

[a]Three groups of family members are listed. In the first group (III-1 to V-1) all have corneal clouding and were, therefore, classified as definitely affected. In the next group, IV-1 and V2 had clear corneas with minimal ocular anomalies. However, both had neurological disease. In the third group, II-6 to IV-3 all had minor ocular anomalies, but no neurological disease. One individual, III-11, is not included in the table. She had no ocular anomalies and a neurologic disease different from that seen in other individuals.

in 2 mainly the left eye was affected (Figure 2). The clouding was located in the periphery of the cornea, except in III-1, IV-4, and V-1. In III-1 and IV-4, both peripheral and central clouding was seen in one eye, while the opposite cornea was clear except for a posterior embryotoxon (Figure 2). Photographs taken of individual IV-4 at the age of 9 and 31 years (Figure 2) indicate that corneal clouding may progress over time. The most severe involvement was seen in V-1, where both corneas were opaque at birth (Figure 2). He underwent penetrating keratoplasties between the age of 5 and 7 years, twice in the right eye and once in the left. Extensive clouding of the transplants occurred within the first year after surgery, and both corneas became completely opaque, resulting in a visual acuity of light perception in both eyes.

Posterior embryotoxon is a consistent feature of the Axenfeld-Rieger group of anomalies. An irregular Schwalbe line was seen in one or both eyes of all individuals with corneal clouding. Anterior synechiae extending to the Schwalbe line were present in 5 of these individuals (Table 1). Individuals II-6, III-2, III-8, and V-2 had clear corneas with a small posterior embryotoxon in the temporal part of both eyes. Angle anomalies were infrequent, although in the most severely affected individuals corneal opacities or anterior synechiae prevented detailed examination. None of the family members had elevated intraocular pressure.

Iris hypoplasia was observed in 9 individuals. In 6 of these, the iris appeared dark, with coarse stromal fibers. In III-8, III-9, and IV-1 a slight circumferential or sectorial involvement was seen, resulting in visualization of all or part of the sphincter pupillae muscle. Mild corectopia was seen in III-8, III-9, III-10, and IV-8. A slightly irregular pupil was also seen in III-5, but the iris color was unremarkable and the sphincter pupillae muscle was not visible. Polycoria was not detected in any family members.

Three individuals had cataract surgery before the age of 45 years. Fundus abnormalities were seen in III-1, III-5, III-6, and IV-3. They consisted of irregular vessels around the optic disc (III-1) (Figure 3), unilateral increased excavation of the optic disc (III-5), preretinal fibrosis (III-6), and a narrow avascular zone of the macula (IV-3). Visual acuity was normal in all eyes except those with corneal opacities, where it ranged from 0.8 to light perception.

• **SYSTEMIC ABNORMALITIES:** A slight dysphonia was present in III-9 and III-12. Individual III-12 also suffered from congenital sensorineural hearing loss and a cardiomyopathy of unknown origin. Supraventricular tachycardia was seen in III-10.

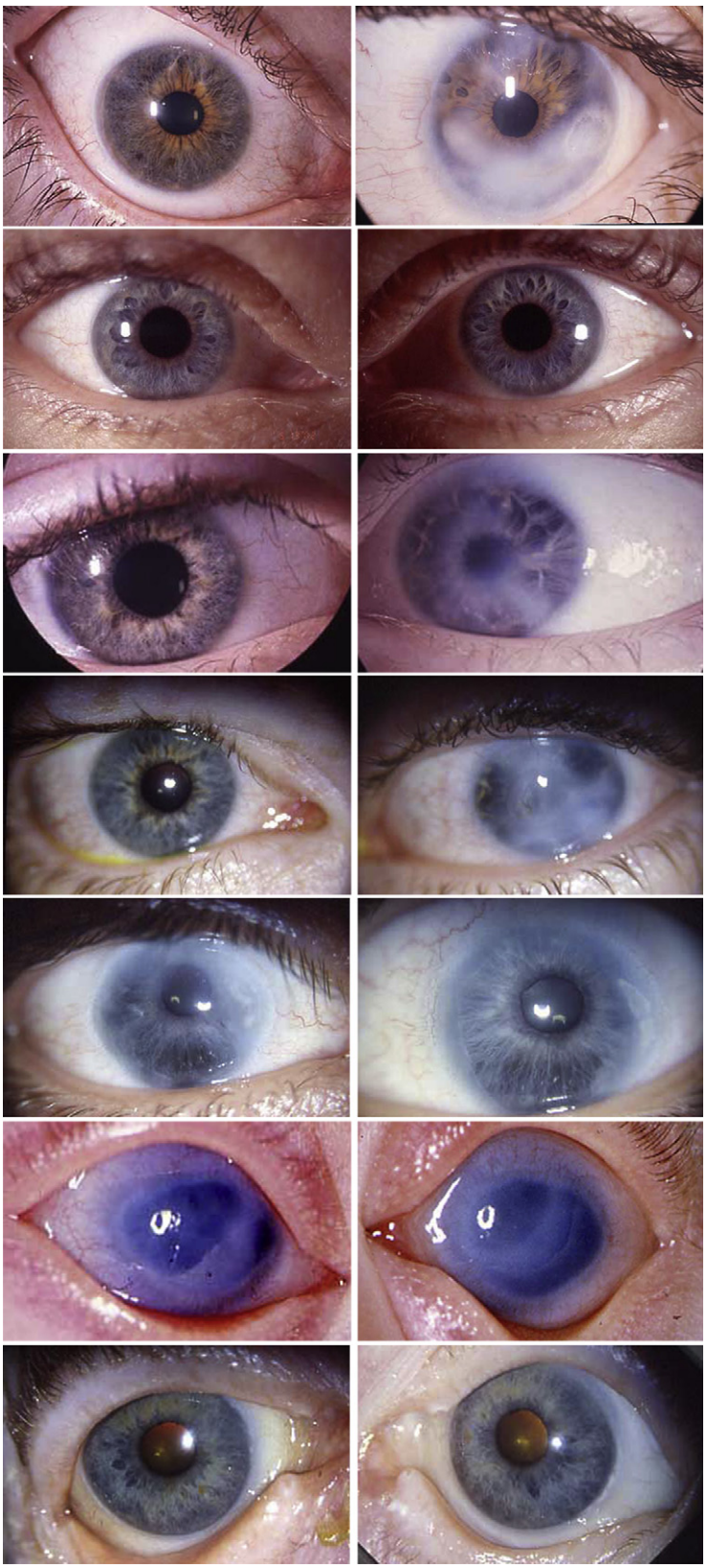

FIGURE 2. Slit-lamp photographs showing the spectrum of anterior segment manifestations seen in the family. (Left column) Right eyes. (Right column) Left eyes. First row: Individual III-1 at 34 years of age. Second row: IV-1 at 39 years of age. Third row: IV-4 at 9 years of age. Fourth row: IV-4 at 31 years of age. Fifth row: IV-8 at 31 years of age. Sixth row: V-1 at 3 months of age. Seventh row: II-6 at 90 years of age. Examples of bilateral corneal clouding are shown for individuals IV-8 (fifth row) and V-1 (sixth row), with

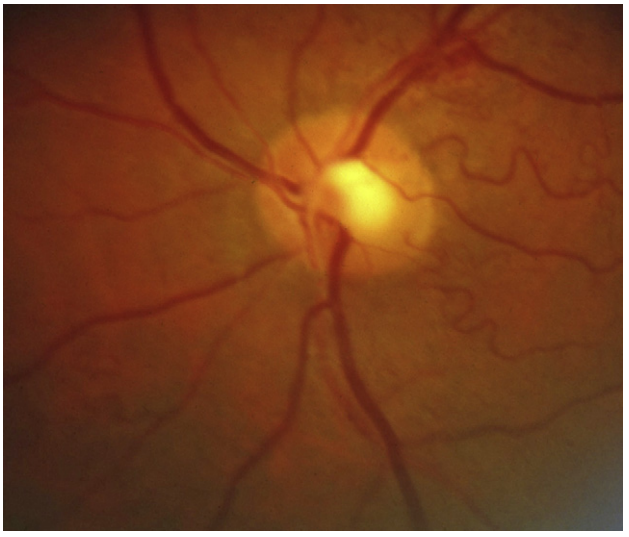

FIGURE 3. Fundus photograph showing retinal arteriolar tortuosity in the left eye of individual III-1, who also suffered from anterior segment dysgenesis.

When this family was first enrolled for studies, cerebral involvement was observed in 4 individuals. Cerebral hemorrhages occurring either at birth or within the first year of life were seen in III-10, V-1, and V-2, causing spastic paraparesis (III-10 and V-1), hemiparesis (V-2), and impaired psychomotor development. Slightly impaired psychomotor development was also present in individual IV-4. During the next decade, signs of cerebral involvement developed in III-9, III-11, and IV-1 (Table 1). Individual III-9 suffered from several intracerebral hemorrhages, IV-1 had an ischemic cerebellar stroke followed by an episode of optic neuritis, and III-11 died from a neurologic disease of unknown origin, classified as encephalomyelitis. Individuals V-1 and V-2 suffered from epilepsy.

Brain CT and/or MRI scans were available for re-examination for 8 individuals. Five of them showed extensive central white matter changes consistent with the presence of leukoencephalopathy (Figure 4), 1 showed mild white matter lesions, and 2 (14 and 19 years old) were normal. Signs of previous hemorrhage, such as deposits of hemosiderin, calcification, and areas of parenchyma loss, were seen in 5 individuals (Figure 4) (Table 2). Contrast series were available for 2 individuals. One showed no enhancement. The other (III-11) showed extensive contrast enhancement, predominantly in the brain stem and basal ganglia.

• **GENETIC ANALYSIS:** From the clinical examination, 7 individuals were scored as definitely affected by the same disorder based on the presence of corneal clouding (Figure 1). Analysis of a genome-wide scan with SNP markers in these individuals showed that they shared a 14 cM area at the terminal part of chromosome 13q ranging from SNP markers at 110, 173, 324-115, 045, 259 (GRCh37/hg19). This region included the *COL4A1* locus now known to be associated with anterior segment dysgenesis. DNA sequencing revealed a novel sequence variant, c.4881C>G, present in these 7 individuals considered certainly affected (Figure 5), but not in a panel of 185 blood donors (370 chromosomes). The same sequence variant was also present in IV-1 and V-2, but not in any of the other sampled family members (Figure 1).

To rule out the possibility of another causal variant present in the linkage region, whole exome sequencing was performed on a sample from Patient III-1. The 2 main candidate genes in the region, *COL4A1* and *COL4A2*, were highly covered (mean 78× coverage, all 100 exons were covered at >10× mean coverage). The c.4881C>G mutation was the only exonic variant in the 14 cM linkage interval not present in dbSNP (Supplemental Table, available at AJO.com, contains a complete list of all variants discovered in the region).

DISCUSSION

IN THE PRESENT STUDY WE HAVE IDENTIFIED A NOVEL PUTATIVELY functional mutation in *COL4A1* in a large family with anterior segment dysgenesis as the prominent ocular presentation. We show that the same *COL4A1* mutation is associated with a wide spectrum of ocular anomalies among mutation carriers, ranging from eyes with minor anomalies (as in IV-1) to severe corneal opacity, anterior synechiae, and iris hypoplasia (as in V-1). Most of the mutation carriers also have cerebral involvement with great variation in clinical presentation.

Congenital or early onset of cataract has previously been observed in patients with neurologic disease associated with mutations in *COL4A1*.[24–26] In the 2 families reported with anterior segment dysgenesis in association with *COL4A1* mutations,[15,16] congenital cataract occurred in all 7 patients described. Among these 7 patients the following anomalies were also observed: corneal opacities in 2, iridocorneal synechiae in 2, iris anomalies in 6 (hypoplasia, corectopia), microcornea in 6, high intraocular pressure in 3, glaucoma in 1, myopia in 3, and retinal detachment and macular hemorrhages each in 1 patient. The anomalies observed among the mutation carriers examined in our family were different

particularly severe involvement in the latter. Unilateral corneal clouding is seen in III-1 (first row) and IV-4 (third row), with posterior embryotoxon in the eye with clear cornea. In individual IV-4 progression of corneal clouding over time can be seen (Right, third and fourth row). Slight iris hypoplasia is present in IV-1, who is a carrier of the COL4A1 sequence variant (second row). Posterior embryotoxon is present in II-6, who is not a carrier of the *COL4A1* mutation (seventh row).

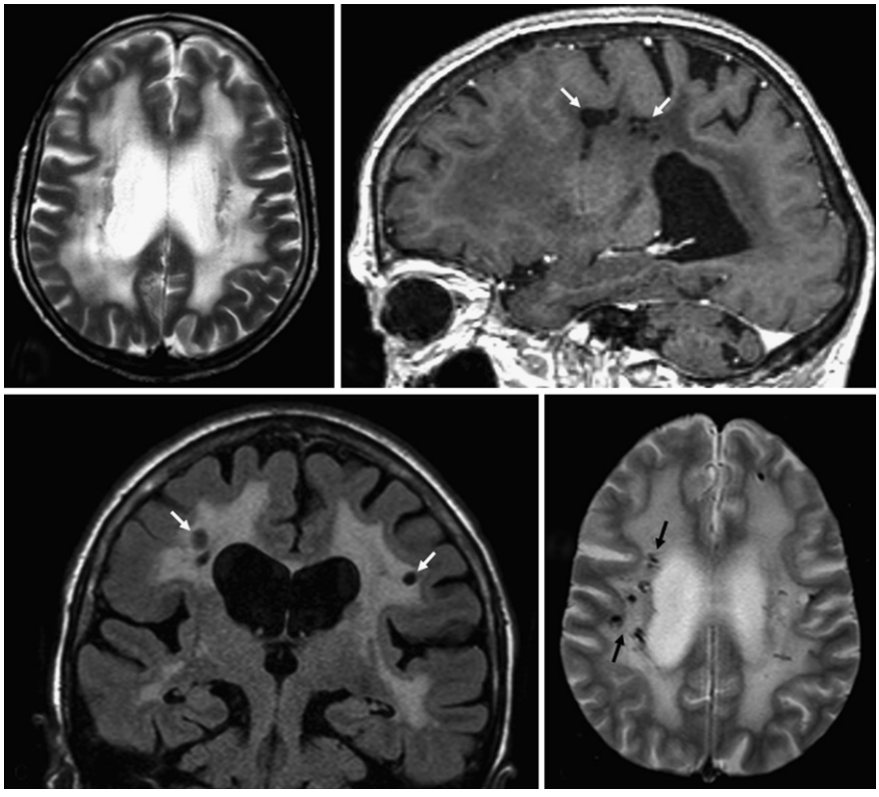

FIGURE 4. Brain magnetic resonance imaging of an individual with anterior segment dysgenesis and neurologic disease. (Top left) T2-weighted image shows extensive high signal changes in white matter. (Top right) T1-weighted image and (Bottom left) flair shows multiple fluid-filled cavities in white matter (arrows). (Bottom right) Gradient echo sequence shows hemosiderin deposits (arrows), confirming that these cavities most likely have a hemorrhagic origin.

TABLE 2. Overview of Neuroradiologic Features in Individuals From a Family With Anterior Segment Dysgenesis and Neurologic Disease

Patient	Leukoencephalopathy	Intracerebral Hemorrhages	Calcifications	Wide Ventricles	Cerebellar Atrophy	Cerebellar Infarct	Contrast Enhancement
III-1	+	−	−	−	−	−	ND
III-9	+	+	−	+	(+)	−	ND
III-10	+	+	+	−	+	−	ND
IV-8	+	−	−	−	−	−	ND
V-1	−	+	+	+	−	−	ND
IV-1	(+)	(+)	−	−	−	+	−
V-2	−	−	−	+[a]	−	−	ND
III-11	+	+	−	−	−	−	+

− = absent; + = present; (+) = minor changes; ND = not done.

[a]Porencephalic cysts. Small porencephalic cysts secondary to intracerebral hemorrhages were also seen in III-9 and V-1.

in that corneal clouding was the most prominent feature, none had elevated intraocular pressure, and only 3 developed cataract that required surgery before the age of 45 years.

The ocular features that were first described in patients with *COL4A1* mutations were retinal arteriolar tortuosity with prominent enlargement of perivascular spaces and cataract.[12] In some patients, primarily those with HANAC, retinal arteriolar tortuosity is accompanied by retinal hemorrhages.[13] Retinal arteriolar tortuosity was seen in only 1 of our patients (III-1) and was quite similar to that previously described in other individuals with *COL4A1* mutation. The other fundus anomalies observed in our family are likely to be normal variants since they occurred in individuals with wild-type *COL4A1* alleles.

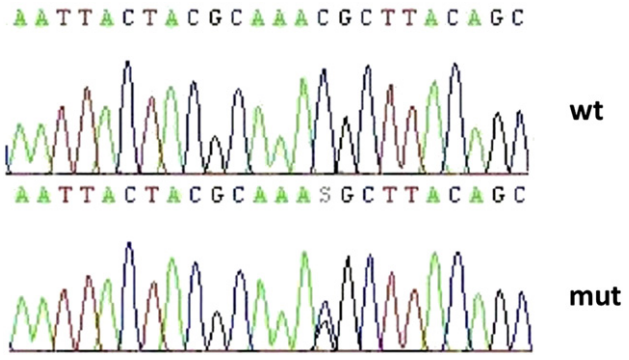

FIGURE 5. Mutation analysis of *COL4A1* in normal individuals and individuals with anterior segment dysgenesis. Partial sequence chromatograms show the DNA sequence of a normal person (Top) and the DNA sequence of an affected individual heterozygous for the *COL4A1* c.4881C>G mutation (Bottom).

Some family members, including 1 obligate carrier (IV-1), had minor anomalies that can be seen in anterior segment dysgenesis but may also represent normal variants. Such anomalies include posterior embryotoxon, variations in the shape of the iris, and occasional goniosynechiae.[27,28] This group of individuals posed a particular challenge when scoring individuals as affected or unaffected. To identify the mutation associated with the anterior segment dysgenesis, the genetic analysis was therefore restricted to those considered definitely affected (ie, those with corneal clouding). Testing additional family members for the *COL4A1* mutation makes a definite diagnosis possible in those with a clinical presentation difficult to classify.

Cerebral involvement in individuals with *COL4A1* mutations is the result of impaired function of the basement membrane mainly in small cerebral vessels leading to cerebral microangiopathy, aneurysms, cerebral hemorrhages, porencephaly, and leukoencephalopathy. Familial porencephaly usually presents in infancy.[29] It can be caused by an antenatal or perinatal parenchymal hemorrhage.[30,31] The neurologic manifestations in individuals III-10, V-1, and V-2 are typical for *COL4A1*-associated neurologic disease presenting early in life. Affected individuals may also suffer from cerebral microbleeds, leukoencephalopathy, symptomatic intracranial hemorrhage, and ischemic stroke in later life, as seen among others in individuals III-9 and III-10. Thus, the neurologic manifestations seen in our family are similar to those previously reported in individuals with *COL4A1* mutations.

Although not systematically examined, creatinine values were available for 8 of the 9 individuals carrying the *COL4A1* mutation. None had clinical signs of renal involvement, but slightly elevated levels of creatinine in 2 individuals (113 and 114 μmol/L, respectively, reference range 60-105 μmol/L) suggest that they could be at risk for developing kidney disease. The nephropathy associated with *COL4A1* mutations may include renal cysts, hematuria, and mild renal failure.[13]

Alpha chains of type IV collagens consist of an N-terminal 7S domain, a triple helical collagenous domain containing the Gly-Xaa-Yaa repeat amino acid sequence, and a C-terminal noncollagenous domain (NC1). When type IV collagen is assembled, COL4A1 first participates in formation of a protomer (ie, a trimer of $\alpha1\alpha1\alpha2$ chains). The folding of the protomer starts in the NC1 part of the molecule.[32] The protomers then assemble to form dimers through their NC1 domains and later tetramers through their 7S domains. The normal asparagine in amino acid position 1627 is located in the fifth segment, predicted to form the hexamer interface during assembly of 2 protomers.[33] This region is highly conserved from mammals to birds and reptiles. The position 1627 corresponds to one of the contact points where the 2 protomers will bind to each other. We therefore hypothesize that dimerization of the protomers could be disturbed by substitution of this amino acid.

In humans, most of the disease-causing *COL4A1* mutations are heterozygous missense mutations that affect one of the numerous glycines within the collagenous region of the protein. All mutations associated with the HANAC syndrome result in changes within the collagenous domain positioned N-terminal to other mutations described in *COL4A1*. It has therefore been suggested that there could be a correlation between this phenotype and the location of the mutation within *COL4A1*.[13] In the 2 French families presenting predominantly with anterior segment dysgenesis, the mutations in *COL4A1*, c.2159G>A (p.Gly720Asp) and c.2263G>A (p.Gly755Arg),[16] also alter the collagenous domain of the protein. The effect of the mutation identified in the present study is an amino acid substitution in the NC1 region of the protein. Three other mutations that involve the NC1 have been reported previously.[30,31,34] All individuals with these mutations had antenatal onset of brain parenchymal hemorrhage, as had possibly only 3 in our family. Except for retinopathy of prematurity observed in 2 individuals, no ocular anomalies were reported in the 3 NC1 families. Thus, further studies are necessary to elucidate a possible influence of genotype on the ocular phenotype.

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SUPPLEMENTAL TABLE. Variants Detected in the Linkage Interval by Whole Exome Sequencing (Grch37/Hg19) in an Individual With Anterior Segment Dysgenesis

Change	Gene Information
Synonymous SNV	IRS2:NM_003749:exon1:c.C2487T:p.P829P,
Nonsynonymous SNV	**COL4A1:NM_001845:exon51:c.C4881G:p.N1627K,**
Synonymous SNV	COL4A1:NM_001845:exon51:c.C4800T:p.S1600S,
Synonymous SNV	COL4A1:NM_001845:exon49:c.C4470T:p.A1490A,
Nonsynonymous SNV	COL4A1:NM_001845:exon45:c.A4002C:p.Q1334H,
Synonymous SNV	COL4A1:NM_001845:exon37:c.A3189T:p.R1063R,
Synonymous SNV	COL4A1:NM_001845:exon37:c.G3183A:p.G1061G
Synonymous SNV	COL4A1:NM_001845:exon29:c.G2130A:p.P710P,
Nonsynonymous SNV	COL4A1:NM_001845:exon25:c.A1663C:p.T555P,
Synonymous SNV	COL4A1:NM_001845:exon7:c.T432A:p.A144A,
Synonymous SNV	COL4A2:NM_001846:exon5:c.G297A:p.T99T,
Synonymous SNV	COL4A2:NM_001846:exon17:c.C1008T:p.P336P,
Synonymous SNV	COL4A2:NM_001846:exon22:c.G1488A:p.P496P,
Nonsynonymous SNV	COL4A2:NM_001846:exon22:c.G1550A:p.R517K,
Nonsynonymous SNV	COL4A2:NM_001846:exon28:c.C2152T:p.P718S,
Synonymous SNV	COL4A2:NM_001846:exon41:c.T3804A:p.P1268P,
Synonymous SNV	COL4A2:NM_001846:exon41:c.T3807C:p.G1269G,
Synonymous SNV	COL4A2:NM_001846:exon43:c.T4083C:p.T1361T,
Synonymous SNV	COL4A2:NM_001846:exon45:c.C4290T:p.F1430F,
Synonymous SNV	COL4A2:NM_001846:exon46:c.A4515G:p.P1505P,
Synonymous SNV	RAB20:NM_017817:exon2:c.T324C:p.F108F,
Synonymous SNV	CARS2:NM_024537:exon12:c.T1239C:p.D413D,
Synonymous SNV	CARS2:NM_024537:exon8:c.A852G:p.E284E,
Nonsynonymous SNV	ING1:NM_005537:exon1:c.T374G:p.L125R,
Synonymous SNV	C13orf16:NM_152324:exon3:c.C66T:p.D22D,
Synonymous SNV	ATP11A:NM_015205:exon11:c.G942A:p.L314L,ATP11A:NM_032189:exon11:c.G942A:p.L314L,
Nonsynonymous SNV	ATP11A:NM_015205:exon11:c.A949G:p.M317V,ATP11A:NM_032189:exon11:c.A949G:p.M317V,
Synonymous SNV	ATP11A:NM_015205:exon19:c.G2238C:p.L746L,ATP11A:NM_032189:exon19:c.G2238C:p.L746L,
Synonymous SNV	ATP11A:NM_015205:exon27:c.G3138A:p.S1046S,ATP11A:NM_032189:exon27:c.G3138A:p.S1046S,
Synonymous SNV	ATP11A:NM_032189:exon29:c.T3330C:p.N1110N,
Nonsynonymous SNV	MCF2L:NM_001112732:exon8:c.G873T:p.Q291H,
Synonymous SNV	MCF2L:NM_001112732:exon15:c.C1764T:p.G588G,MCF2L:NM_024979:exon15:c.C1758T:p.G586G,
Synonymous SNV	MCF2L:NM_001112732:exon22:c.T2415A:p.S805S,MCF2L:NM_024979:exon22:c.G2409A:p.Q803Q,
Synonymous SNV	F10:NM_000504:exon7:c.C792T:p.T264T,
Synonymous SNV	PROZ:NM_003891:exon5:c.A447G:p.T149T,
Synonymous SNV	LAMP1:NM_005561:exon4:c.C556A:p.R186R,
Synonymous SNV	ATP4B:NM_000705:exon1:c.A106C:p.R36R,
Synonymous SNV	GRK1:NM_002929:exon1:c.A588G:p.L196L,
Synonymous SNV	GAS6:NM_001143945:exon5:c.G444C:p.L148L,GAS6:NM_000820:exon11:c.G1263C:p.L421L,GAS6:NM_001143946:exon3:c.G366C:p.L122L,
Synonymous SNV	RASA3:NM_007368:exon14:c.T1326C:p.T442T

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SUPPLEMENTAL TABLE. Variants Detected in the Linkage Interval by Whole Exome Sequencing (Grch37/Hg19) in an Individual With Anterior Segment Dysgenesis (*Continued*)

Chr	Pos 1	Pos 2	Ref	Alt	State	Qual	DP	dbSNP
13	110435914	110435914	G	A	hom	81	12	rs12853546
13	**110804728**	**110804728**	**G**	**C**	**het**	**200**	**37**	
13	110804809	110804809	G	A	het	81	25	rs650724
13	110813709	110813709	G	A	het	98	42	rs1133219
13	110818598	110818598	T	G	Hom	222	178	rs3742207
13	110827574	110827574	T	A	Het	167	67	rs874203
13	110827580	110827580	C	T	Het	152	71	rs874204
13	110833702	110833702	C	T	Het	135	41	rs16975492
13	110839550	110839550	T	G	Hom	222	92	rs536174
13	110864225	110864225	A	T	Het	225	126	rs532625
13	111077197	111077197	G	A	Hom	222	109	rs4238272
13	111098226	111098226	C	T	Het	189	96	rs4103
13	111111173	111111173	G	A	Het	181	61	rs7990214
13	111111235	111111235	G	A	Het	168	38	rs7990383
13	111121620	111121620	C	T	Het	218	171	rs9583500
13	111154058	111154058	T	A	Hom	222	40	rs439831
13	111154061	111154061	T	C	Hom	222	40	rs409858
13	111155773	111155773	T	C	Hom	222	56	rs438758
13	111156499	111156499	C	T	Hom	217	75	rs4771683
13	111158874	111158874	A	G	Hom	222	31	rs445348
13	111176393	111176393	A	G	Hom	222	36	rs419244
13	111298392	111298392	A	G	Het	109	69	rs436462
13	111319754	111319754	T	C	Het	150	122	rs4628819
13	111368164	111368164	T	G	Hom	124	28	rs7338333
13	111980537	111980537	C	T	Het	222	141	rs1359428
13	113479813	113479813	G	A	Hom	222	68	rs9549564
13	113479820	113479820	A	G	Hom	222	66	rs368865
13	113508839	113508839	G	C	Hom	179	13	rs9549573
13	113527967	113527967	G	A	Het	220	322	rs1320525
13	113536132	113536132	T	C	Het	176	43	rs1290177
13	113720476	113720476	C	T	Het	106	26	rs2297192
13	113733009	113733009	C	T	Het	158	22	rs35155188
13	113741590	113741590	G	A	Het	82	22	rs9604022
13	113801737	113801737	C	T	Hom	222	90	rs5960
13	113818900	113818900	A	G	Hom	222	50	rs564081
13	113965176	113965176	C	A	Hom	222	53	rs9577503
13	114312354	114312354	T	G	Hom	166	24	rs9285616
13	114322289	114322289	A	G	Hom	208	34	rs9796035
13	114531565	114531565	C	G	Het	225	104	rs8191975
13	114780764	114780764	A	G	Het	57	27	rs2274717

Alt = alternative allele (nonreference allele); DP = read depth at the position; het = heterozygous; hom = homozygous for alternative allele; Qual = quality; Ref = reference allele; SNV = single nucleotide variant.

Bold font indicates COL4A1 c.C4881G mutation, the only variant not found in dbSNP.