Ophthalmological Features Associated With COL4A1 Mutations

Isabelle Coupry, PhD; Igor Sibon, MD, PhD; Bruno Mortemousque, MD; François Rouanet, MD; Manuele Mine, PharmD, PhD; Cyril Goizet, MD, PhD

Objective: To investigate the wide variability of ocular manifestations associated with mutations in the COL4A1 gene that encodes collagen IVα1.

Methods: We clinically evaluated 7 patients from 2 unrelated families in whom ocular features segregated with COL4A1 mutations that were identified by direct sequencing.

Results: The G2159A transition (c.2159G>A) that leads to the missense mutation p.Gly720Asp was identified in family A. An ocular phenotype of variable severity was observed in all affected relatives. The missense mutation c.2263G>A, p.Gly755Arg was identified in family B. One patient from family B also displayed notable ocular features.

Conclusions: The COL4A1 mutations may be associated with various ophthalmologic developmental anomalies of anterior segment dysgenesis type, which are reminiscent of Axenfeld-Rieger anomalies (ARA). Cerebrovascular disorders should be added to the list of signs potentially associated with ARA.

Clinical Relevance: These data suggest that cerebral magnetic resonance imaging may be recommended in the clinical treatment of patients with apparently isolated ARA, even when neurological symptoms or signs are lacking.


A NEW FORM OF HEREDITARY cerebrovascular disorder was recently associated with mutations in the COL4A1 gene that encodes collagen IVα1.1,2 Mutations in COL4A1 were initially associated with cerebral microangiopathy (OMIM 607595) and familial porencephaly (OMIM 175780).3,4 The clinical spectrum of COL4A1 mutations has progressively enlarged in humans as well as mice, with evidence of neonatal and adult intracerebral hemorrhages, aneurysms, ocular manifestations of variable type, and nephropathy.3,5-8

Associated ocular features were retinal arteriolar tortuosity with prominent enlargement of perivascular spaces2 and cataract noted in 3 patients with hereditary porencephaly and adult stroke.3 Our group previously described another family with colonic anterior segment dysgenesis (ASD) and small-vessel disease of the brain.6 Anterior segment dysgenesis represents a clinically and genetically heterogeneous group of disorders.6 The diagnosis of Axenfeld-Rieger anomaly (ARA) refers to a constellation of ocular findings that include anomalies of the anterior chamber angle and aqueous drainage structures (iridogoniodysgenesis), iris hypoplasia, eccentric pupil (corectopia), iris tears (polycoria), and iridocorneal adhesions traversing the anterior chamber. These anomalies are frequently associated with a posterior embryotoxon and confer a high risk of blindness due to glaucoma.9,10

When extraocular developmental abnormalities that affect the teeth, facial bones, and periumbilical skin are associated with ARA, the disorder is named Axenfeld-Rieger or Rieger syndrome (ARS). Patients with ARS may also display hypospadias and, more rarely, hydrocephalus, hearing loss, cardiac and kidney abnormalities, and congenital hip dislocation anomalies. Other syndromic forms of ARA have been rarely described with additional cardiac malformations, sensorineural hearing loss, oculodentodigital dysplasia syndrome, and severe craniosynostosis syndrome.11-14

We present here ocular features associated with COL4A1 mutations in 2 unrelated families.
Table 1. Ophthalmological Features in 2 Families With COL4A1 Mutation

<table>
<thead>
<tr>
<th>Ophthalmological Signs</th>
<th>Patient</th>
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<tbody>
<tr>
<td></td>
<td>Family A</td>
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<tr>
<td></td>
<td>A.I.1</td>
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<tr>
<td>Corneal opacities</td>
<td>−</td>
</tr>
<tr>
<td>Corneal neovascularization</td>
<td>+</td>
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<tr>
<td>Irido-corneal synchiae</td>
<td>+</td>
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<tr>
<td>Congenital cataract</td>
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<td>Iris anomalies</td>
<td>+</td>
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<td>Microcornea</td>
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<td>High IOP</td>
<td>+</td>
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<tr>
<td>Glaucoma</td>
<td>−</td>
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<tr>
<td>Optic nerve morphology</td>
<td>PA</td>
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<tr>
<td>Myopia</td>
<td>+</td>
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<tr>
<td>Retinal detachment</td>
<td>−</td>
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<tr>
<td>Macular hemorrhages</td>
<td>−</td>
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</table>

Abbreviations: E, excavation; ellipses, not interpretable; IOP, intraocular pressure; N, normal; PA, peripapillary atrophy; +, present; −, absent.

REPORT OF A CASE

A detailed description of the personal medical histories and associated neurological phenotypes of family A has been previously published.6

FAMILY A

The main ocular findings observed in this family are summarized in Table 1. The G2159A transition (c.2159G>A) leading to the substitution of a glycine by aspartic acid at position 720 (p.Gly720Asp) was identified by direct sequencing in COL4A1 in all of the affected relatives.6 We have obtained complementary data on the ophthalmological history of these relatives and reexamination of the index case.

Patient A.I.1, the index case, was a 38-year-old woman born after uneventful pregnancy and delivery. A bilateral congenital cataract with iris hypoplasia was discovered early in life. This bilateral cataract was surgically treated at 12 years of age by anterior phakophagia without intraocular lens implantation. Bilateral aphakia and myopia were corrected by contact lens use. Her ophthalmological history indicated a surgically treated right retinal detachment and high intraocular pressure (IOP) treated with hypotensive eye drops since 23 years of age. Later, treatment by latanoprost reduced IOP to 16 mm Hg in both eyes (reference value, <21 mm Hg). At 35 years of age, ophthalmological examination found bilaterally decreased visual acuity predominating in the left eye as a consequence of severe amblyopia. Bilateral microcornea and peripheral corneal opacities were present with corectopia (Figure 1A and B). Fundus examination showed normal optic discs without retinal hemorrhages or arteriolar tortuosity. The visual field appeared normal in the right eye and unavailable in the left, considering the low visual acuity. She had a cerebral small deep infarct at 35 years of age. Neurological examination revealed right spastic hemiparesis and a right central facial palsy. Brain magnetic resonance imaging (MRI) showed diffuse periventricular leukoencephalopathy (Figure 2A).

Patient A.II.1 was the 35-year-old brother of the proband. At birth, malformations of the eyes were obvious including a bilateral congenital cataract, iris hypoplasia, and microcornea (Figure 1C) without congenital glaucoma. Infantile hemiparesis was noted during the neonatal period.6 At 8 years of age, she presented with strabismus and slight amblyopia of the left eye. Fundus examination did not show optic disc anomalies, retinal arteriolar tortuosity, or any retinal hemorrhage or exudates. Findings of IOP measurement were normal. Fluorescein angiography showed no abnormality; the arteriolar caliber was normal, and there was no leakage of fluorescein or capillary dropout. Neurological examination revealed spastic hemiparesis in the right eye with a porencephalic cavity and a diffuse periventricular leukoencephalopathy on MRI in the left eye (Figure 2C).

Patient A.I.1 was the 58-year-old mother of the proband. She had bilateral iridogoniodygenesis with iridocorneal synchiae, iris hypoplasia, microcornea (Figure 1D), congenital cataract surgery at 55 years of age, and high myopia with retinal complications represented by bilateral macular hemorrhages. Fundus examination showed peripapillary atrophy, choroidal atrophy, and scars of macular hemorrhages (Fuchs spots) but no arterial tortuosity. Bilateral high IOP was discovered (26 mm Hg) and efficiently treated with hypotensive eye drops. Findings of neurological examination were normal. A marked periventricular leukoencephalopathy was obvious on brain MRI (Figure 2B).

Patient A.II.2 was the 35-year-old brother of the proband. His major ophthalmological history included bilateral microcornea, high myopia, congenital cataract, and juvenile glaucoma. The cataract was operated on during childhood, with occurrence of bilateral aphakia and severe left amblyopia. At 32 years of age, his IOP was 23 to 24 mm Hg bilaterally; the patient was treated with a combination of 3 hypotensive eye drops in the left eye, and he had undergone glaucoma surgery in the right eye. Ophthalmologic examination showed bilateral microcornea, central and peripheral corneal opacities with corneal neovascularization and iridocorneal...
synechiae, and left corectopia and polycoria (Figure 1, E and F). Optic nerves showed large excavation due to severe glaucoma. Findings of neurological examination were normal. Brain MRI found a diffuse periventricular leukoencephalopathy (Figure 2D). At 35 years of age, progression of central corneal opacities and development of refractory ocular hypertension to medical therapy in the right eye led to unilateral corneal graft.

Figure 1. Ophthalmological features observed in family A. The glance of case A.II.1 is characterized by bilateral microcornea (A) and a postsurgical (congenital cataract) corectopia (B). C. The right eye of case A.III.1 presents microcornea (arrow); the congenital cataract cannot be observed on this photograph. D. The photograph shows the dilated right eye of case A.I.1. It presents microcornea, iris hypoplasia with irregular pupil (arrow). The arrowhead indicates the intraocular lens implant after surgery for congenital cataract. In the right eye of case A.II.2 (E), the microcornea is associated with peripheral corneal opacities and corneal neovascularization (arrow) and triangular pupil (arrowhead) due to iris lesion during the glaucoma surgery. Case A.II.2 also received surgery for congenital cataract without intraocular implant. On the left eye (F), note a corectopia and polycoria with a second small pupil (arrow).

Figure 2. Brain magnetic resonance images obtained in family A. Magnetic resonance imaging fluid-attenuated inversion recovery (A, B, D, and E) and inversion recovery T1-weighted (C) sequences demonstrate a diffuse periventricular leukoencephalopathy in cases A.II.1 (A), A.I.1 (B), A.III.1 (C), A.II.2 (D), and A.II.3 (E). Part C also demonstrates the presence of a porencephaly (arrow) in patient A.III.1.
and glaucoma drainage implant (Molteno implant) surgery. At the time, high IOP (35 mm Hg) in the left eye was found following poor therapeutic observance. Acute retinal detachment in the right eye occurred a few weeks after the corneal graft, which was surgically treated, with a poor final visual outcome.

Patient A.II.3 was the 29-year-old sister of the proband. She had strabismus with severe amblyopia in the left eye, and ophthalmologic examination showed a bilateral microcornea and a bilateral cataract that had not received surgery. Her IOP was normal. Fundus examination revealed normal optic discs and retinal vessels. Findings of neurological examination were normal. Brain MRI showed a diffuse periventricular leukoencephalopathy (Figure 2E).

Patient A.II.3 was the 29-year-old sister of the proband. She had strabismus with severe amblyopia in the left eye, and ophthalmologic examination showed a bilateral microcornea and a bilateral cataract that had not received surgery. Her IOP was normal. Fundus examination revealed normal optic discs and retinal vessels. Findings of neurological examination were normal. Brain MRI showed a diffuse periventricular leukoencephalopathy (Figure 2E).

FAMILY B

The main ocular findings observed in this family are summarized in Table 1. Direct sequencing of COL4A1 in the index case led to identification of a missense mutation c.2263G>A, p.Gly755Arg in exon 30. This mutation cosegregated with the disease in all affected relatives.

Patient B.I.1, the index case, was a 47-year-old woman. She presented with an acute hemiparesis in the right eye at 47 years of age related to spontaneous left lenticular nucleus hemorrhage. Her medical history included migraine headaches and unexplained white matter leukoencephalopathy. Fifteen days later, she experienced an acute left central facial palsy and dysarthria related to a spontaneous contralateral subcortical cerebral hemorrhage. Brain MRI identified an extended periventricular leukoencephalopathy and 2 recent cerebral hemorrhages but neither small deep infarct nor microbleeding (Figure 3, A-C). Ophthalmological examination revealed severe hyperopia and lens opacities without visual impairment. The anterior chamber of the eyes appeared normal, without cornea and iris abnormality. Fundus examination showed no optic disc anomalies, retinal arteriolar tortuositities, retinal hemorrhages, or exudates. Fluorescein angiography was not performed.

Patient B.II.2 was the 10-year-old daughter of the proband. Bilateral congenital cataract, prominent Schwalbe line (posterior embryotoxon) (Figure 4), and relative microcornea (diameter, 11 mm) without congenital glaucoma were observed. Fundus examination showed no optic disc anomalies, retinal arteriolar tortuositities, or any retinal hemorrhages or exudates. Fluorescein angiography was not performed. Migraine headaches without aura were reported by the patient from 8 years of age. Findings of neurological examination were normal. Brain MRI showed periventricular leukoencephalopathy but no small deep infarct, microhemorrhage, macrohemorrhage, or porencephaly were observed (Figure 3D).

![Figure 3](https://example.com/fig3.png)

**Figure 3.** Brain magnetic resonance imaging (MRI) obtained in family B. In the index case (patient B.I.1), brain computed tomographic scans performed at a 15-day interval demonstrated 2 brain hemorrhages in the left lenticular nucleus (A) and the right corona radiata (B). On brain MRI, fluid-attenuated inversion recovery sequencing demonstrated diffuse periventricular leukoencephalopathies in patients B.I.1 (C) and B.II.2 (D).

![Figure 4](https://example.com/fig4.png)

**Figure 4.** Ophthalmological features observed in family B. The image shows isolated prominent Schwalbe line (posterior embryotoxon) without glaucoma observed in patient B.II.2.

We show here that COL4A1 mutations may be associated with various ophthalmologic developmental anoma-
lies of ASD type that are reminiscent of ARA (Table 1). Indeed, ocular features including posterior embryotoxon, microcornea, cornea opacity, and increased IOP, as well as congenital cataracts, fall into the clinical spectrum observed in ARA.

The different ocular anterior chamber anomalies displayed by the affected kindred of families A and B are relevant to the diagnosis of ARA. Additional ocular signs not included in the spectrum of ARA malformations were observed. Microcornea was noted in patients from both families and may consequently be considered characteristic of this familial eye developmental condition. Alternatively, the presence of severe hyperopia observed in the index case of family B (B.I.1) may reflect a fortuitous association considering the high prevalence of hyperopia in general population. The link between ASD and potentially severe myopia found in 3 patients in family A (A.I.1, A.II.1, A.II.2) is more hypothetical. Retinal complications present in the same 3 patients should instead be considered as complications of severe myopia and aphakia. The diagnosis of glaucoma was retained in only 1 patient (A.II.2), as interpretation of visual fields was impossible considering the low visual acuity of patient A.I.1. The IOP was high in another patient (A.II.1), with no alteration of visual fields. There was no evidence of optic nerve hypoplasia in any patient.

Axenfeld-Rieger anomaly is genetically heterogeneous because mutations in 3 genes, PITX2 (on chromosome 4q25), FOXC1 (also named FKHNL7) (6p25), and PAX6 (11p13), have been identified to date. An additional locus has been proposed on chromosome 13q14. Pathogenic alleles of these developmental genes often cause a spectrum of ocular phenotypes that vary in severity. In 2005, Van Agtamel et al described iris/corneal adhesions, buphthalmos, iris defects, corneal opacity, and cataracts in COL4A1 mutant mouse models, suggesting a potential link between ARA and COL4A1 mutations in humans. Very recently, other studies in mice have focused on the role of COL4A1 in abnormal ocular development.

Finally, we previously described family A as the first with inherited syndromic ocular ASD corresponding to ARA of variable severity, caused by mutation in COL4A1. During development of the tissues that compose an anterior eye segment, cells that originate from the surface epithelium or the neuroepithelium need to interact with mesenchymal cells, which predominately originate from the neural crest. This interaction is under the control of a broad range of transcription factors that are active in epithelial or mesenchymal cells, or both. In humans, mutations in PITX2 and FOXC1, 2 genes that encode transcription factors specifically expressed in the mesenchymal cells, result in a broad spectrum of abnormalities during anterior eye development. Most of these phenotypes belong to the broad spectrum of features that are part of ARA/ARS. The PAX6 gene, which codes for a paired domain and pairedlike homeodomain transcription factor, is also critically required for the morphogenesis of mesenchyme-derived tissues in the anterior eye. Patients with heterozygote mutations in PAX6 exhibit the phenotype of aniridia that may variably include iris hypoplasia, corneal opacification, cataract, and foveal dysplasia. The phenotypes associated with PAX6 mutations overlap with those of ARA/ARS. The genetic cause of ARA/ARS in humans was so far solely associated with molecular defects in transcription factors.

However, it has been demonstrated that mutations in basement membrane components may cause ASD. In humans, mutations in laminin-β2 lead to congenital nephrotic syndrome and ASD that differs from ARA. More interestingly, mutant mice that manifest ASD of possible ARA type have also been described. The COL4A1 gene; detailed ophthalmological findings are available (Table 2). The phenotypic presentation of the COL4A1 mutant mouse on the C57BL/6J genetic background is similar to the phenotype of family A, except for the nerve optic hypoplasia only observed in mice. Other mice carrying heterozygous COL4A1 missense mutations showed a very wide spectrum of ophthalmological phenotypes including microphthalmia, buphthalmos, anterior polar opacity with or without cornea-lens adhesion, corneal opacities, lens vacuoles, and total lens opacity.
Members of the type IV collagen family are essential components of all basement membranes and define structural stability as well as tissue-specific functions. Type IV collagen is also crucial for the initial formation of basement membranes during embryonic development. In mice, COL4A1 was detected in the basement membrane underlying the lens pit during early embryonic development and in both the anterior and posterior lens capsules of the lens vesicle both later in development and in newborn and adult mice. Similar abundant expression of COL4A1 was determined in human embryonic and adult lens capsules. The differentiation of mesenchymal cells in the cornea and the formation of an anterior chamber depend on signals controlled by transcription factors (such as PITX2 and FOXC1) that are specifically expressed in the mesenchymal cells and on inductive signals from the lens. Mutations in COL4A1 may disrupt some lenticular signaling in the direction of mesenchymal cells. The link between defects in COL4A1 and in transcription factors that give rise to ARA remains uncertain. A clue may reside in the fact that PITX2 transactivates PLOD1, a procollagen lysyl hydroxylase that catalyzes the formation of hydroxylysine in collagen. Mutations in PLOD1, a downstream target gene for PITX2, are associated with Ehlers-Danlos syndrome type VI. Patients with Ehlers-Danlos syndrome type VI present ocular similarities to ARA/ARS, particularly glaucoma and microcornea. Considering this molecular pathway, the presence of microcornea in association with ARA in our patients reinforces the hypothesis that mutations in ASD-causing transcription factor genes might lead to pathogenesis via extracellular matrix molecules.

In conclusion, the families described here highlight the wide variability of ocular phenotypes related to COL4A1 mutations in humans and suggests phenotype-genotype correlations as established in mutant mouse models. Cerebrovascular disorder, sometimes without clinical consequences (patients A.I.1, A.II.2, A.II.3 and BII.2 had normal results on neurological examination), has to be added to signs potentially associated with ARA. These data suggest that a cerebral MRI may be recommended in the clinical treatment of patients with apparently isolated ARA, even in the absence of neurological clinical manifestations.

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Correspondence: Bruno Mortemousse, MD, Service d’ophtalmologie Hôpital Pellegrin, CHU Bordeaux, 33076 Bordeaux, France (bruno.mortemousse@chu-bordeaux.fr).

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