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.

Patient A.I.1 was the 58-year-old mother of the proband. She had bilateral iridogoniodygenesis with iridocorneal synchia, 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 (Fuch’s 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 synchia.
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
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 showed 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). Ophthalmologic 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 tortuosities, 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 tortuosities, 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).

**COMMENT**

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 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 an 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 FKHHL7) (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 C57BL/6J 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 paired-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 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.

### Table 2. Type of Mutation and Ophthalmological and Extraocular Findings in Mice With COL4A1 Mutation

<table>
<thead>
<tr>
<th>Mouse Background</th>
<th>Natural Mutant Mice&lt;sup&gt;10&lt;/sup&gt;</th>
<th>Mouse Lineage&lt;sup&gt;4&lt;/sup&gt;</th>
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<tr>
<td>Mutation in COL4A1</td>
<td>Gly827Trp</td>
<td>Gly1064Asp</td>
</tr>
<tr>
<td>Ophthalmological signs</td>
<td>+ + +</td>
<td>+</td>
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<tr>
<td>Iridocorneal angle dysgenesis</td>
<td>+ + +</td>
<td>+</td>
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<tr>
<td>Corneal opacity</td>
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<td>−</td>
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<tr>
<td>Cataract</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Iridocorneal adhesions</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Iris anomalies</td>
<td>+</td>
<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>Extraocular signs</td>
<td>G</td>
<td>SBS</td>
</tr>
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</table>

Abbreviations: CVD, cerebrovascular disorder; B, buphthalmos; G, glomerulopathy; IOP, intraocular pressure; N, normal; ND, not documented; SBS, small body size; V, vacuolar; ++, severe; +, mild; −, present; −, absent.
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 collagens. 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 Mortemousque, MD, Service d’ophtalmologie Hôpital Pellegrin, CHU Bordeaux, 33076 Bordeaux, France (bruno.mortemousque@chu-bordeaux.fr).

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REFERENCES


