**COL4A1 Mutation in Axenfeld–Rieger Anomaly with Leukoencephalopathy and Stroke**

Igor Sibon, MD, PhD, 1 Isabelle Coupry, PhD, 2 Patrice Menegon, MD, 3 Jean-Pierre Bouchet, MD, 4 Philippe Gorry, MD, PhD, 2, 5 Ingrid Burgelin, LT, 6 Patrick Calvas, MD, PhD, 6 Isabelle Origianac, MS, 7 Vincent Dousset, MD, PhD, 3 Didier Lacombe, MD, 2, 5 Jean-Marc Orgogozo, MD, 1 Benoit Arveiler, PharmD, PhD, 2, 5 and Cyril Goizet, MD, PhD1, 2, 5

**Objective:** Several hereditary ischemic small-vessel diseases of the brain have been reported during the last decade. Some of them have ophthalmological, mainly retinal, manifestations. Herein, we report on a family affected by vascular leukoencephalopathy and variable abnormalities of the anterior chamber of the eye.

**Methods:** After the occurrence of a small, deep infarct associated with white matter lesions in a patient with a medical history of congenital cataract and amblyopia, we conducted clinical and neuroradiological investigations in 10 of her relatives.

**Results:** Diffuse leukoencephalopathy associated with ocular malformations of the Axenfeld–Rieger type was observed in five individuals. Familial genetic analyses led to the identification of a novel missense mutation in the *COL4A1* gene, p.G720D, which cosegregates with the disease.

**Interpretation:** Our data corroborate previous observations demonstrating the role of *COL4A1* in cerebral microangiopathy and expand the phenotypic spectrum associated with mutations in this gene. We delineate a novel association between the Axenfeld–Rieger anomaly and leukoencephalopathy and stroke.


Several hereditary disorders that affect small blood vessels of the brain recently have been individualized: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (MIM125310), caused by mutations in the gene *NOTCH3* located on chromosome 19p13; cerebro-retinal vasculopathy (CRV); hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS); hereditary vascular retinopathy (HVR) (CRV, HERNS, and HVR are linked to the same locus on chromosome 3p21) (MIM192315); cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) (MIM600142); Fabry’s disease (MIM301500); and familial variants of cerebral amyloid angiopathies. More recently, the role of mutations in the gene *COL4A1* encoding collagen IVA1 has been highlighted as a cause of cerebral microangiopathy (MIM175780) in several reports of animal and human studies.

Ocular manifestations are common in most of these conditions. Funduscopic examination and retinal angiograms may show different types of lesions associated with cerebral small-vessel diseases. For example, retinal infarcts, with vascular occlusions, microaneurysms, and capillary leakage, were described in patients with CRV/HERNS; nerve fiber loss, cotton-wool spots, sheathed arteries, and tortuous arteries were reported in patients with CADASIL; and retinal arteriolar tortuosity with prominent enlargement of perivascular spaces was found in patients with mutations in the gene *COL4A1*.

Other ophthalmological anomalies appear to be extremely rare and have been reported in few cases. Involvement of the anterior chamber of the eye was described in the context of cerebral small-artery disease. A
cataract was noted in three patients with hereditary porencephaly and adult stroke related to a mutation in COL4A1.9

We describe here an autosomal dominant syndrome related to a novel COL4A1 mutation, and characterized by cerebral vasculopathy and variable congenital defects of the anterior segment of the eye that belong to the clinical spectrum of Axenfeld–Rieger anomaly (ARA). This term is used for a variety of overlapping phenotypes, including anomalies of the anterior chamber angle and aqueous drainage structures (iridogoniodysgenesis), iris hypoplasia, eccentric pupil, iris tears, and iridocorneal tissue adhesions traversing the anterior chamber.12,13 ARA appears to be genetically heterogeneous. It has been associated with mutations in three genes: PITX2 (on chromosome 4q25), FOXC1 (also named FKHL7) (6p25), and PAX6 (11p13).12 An additional locus has been identified on chromosome 13q14.14

Subjects and Methods

Subjects

Eleven members of a four-generation family gave informed consent and underwent systematic ophthalmological examination. In addition, the proband (Case III.2) underwent cerebral angiography; cerebral magnetic resonance angiography; cervical and transcranial ultrasound examination; transsthoracic and transesophageal echocardiography; Holter electrocardiography; arterial lower limb and renal Doppler echography; hematological, clotting, biochemical, and immunological laboratory analysis of serum and cerebrospinal fluid; skin biopsy to study cutaneous vessels; muscle biopsy to study the mitochondrial respiratory chain; measurement of sensory- and visual-evoked potentials; and electromyography.

Magnetic Resonance Imaging

Neuroimaging was performed in nine subjects (all subjects except Subjects I.1 and I.2) at 1 to 1.5 Tesla. T1-weighted images were obtained from axial and/or sagittal planes, and T2-weighted images or fluid-attenuated inversion recovery (FLAIR) images were obtained from axial planes. In addition, diffusion-weighted images and T2-weighted gradient-echo images were obtained in the axial plane in Case III.2. A neuroradiologist (P.M.) blinded to the clinical status of subjects reviewed the magnetic resonance images for evidence of anomalies.

Genetic Studies

After informed consent was obtained, blood samples were collected from 10 examined subjects (all subjects except Subject I.1). Genomic DNA was extracted from peripheral blood cells following standard procedures. Microsatellite markers were selected from published data and used for genetic linkage analyses directed toward three loci associated with hereditary vasculopathies: three microsatellite markers (D19S841, D19S226, and D19S199) were used for testing the CADA-SIL locus on 19p13 (NOTCH3 gene)15; four markers (D3S3685, D3S3582, D3S1289, and D3S3616) were used for testing the CRV/HERNS/HVR locus on 3p21; and three markers (D13S1315, D13S148, and D13S261) were used for testing the COL4A1 gene locus on chromosome 13q34. We also performed linkage on the four loci known to be associated with ARA. The markers used for testing these loci were as follows: D6S1600, D6S344, 27919-TG, and 27919-GT (FOXC1) on 6p25; D4S2301, D4S2945, D4S193, and D4S2940 on 4q25 (PITX2); D13S1293, D13S218, and D13S263 on locus 13q14; and Z23802/D11S1322, Z66772, and Z67040 on 11p13 (PAX6). In addition, direct sequencing of the entire coding region and intron-exon boundaries of PITX2 and COL4A1, as well as indirect analysis by denaturing high-performance liquid chromatography of the coding region and intron-exon boundaries of PAX6, was performed in the proband. The designed primer sequences and conditions used for amplification, denaturing high-performance liquid chromatography, and sequencing are available on request.

Results

Case Reports

The main clinical and MRI features of five study cases are summarized in the Table.

Case III.2. The proband, a 37-year-old woman, was born after an uneventful pregnancy; delivery was also uneventful. A bilateral congenital cataract with congenital chronic glaucoma, bilateral microcornea, peripheral opacities, and amblyopia of the left eye were diagnosed early (Figs 1A, B). The cataract was surgically treated at age 12 years. She was treated by hypertensive eyedrops since age 23. The patient was hospitalized for sudden right hemiplegia at age 35. Clinical examination showed right hemiplegia with a Babinski sign, without sensory disturbance, and right central facial palsy. The Mini-Mental State Examination (MMSE) score was 27 of 30. Cardiovascular examination findings were normal. Computed tomography showed a diffuse leukoencephalopathy. Brain MRI demonstrated a left small deep infarct of the centrum ovale on diffusion-weighted images (Fig 2A). FLAIR images showed bilateral periventricular diffuse hyperintensities of the white matter (see Fig 2B) but a normal brainstem. T2-weighted gradient-echo images showed microbleeds located mainly in the basal ganglia and the cerebellum (see Fig 2C). Angiography and magnetic resonance angiography demonstrated an asymptomatic aneurysm of the top of the basilar artery. Ophthalmological examination showed decreased visual acuity in both eyes. Fundus examination findings were normal, without retinal hemorrhages or arteriolar tortuosity and with normal veins in both eyes. Visual field of the right eye was normal. All results of serum and cerebrospinal fluid analysis, including anticardiolipin antibodies, lupus anticoagulant, antinuclear factor, creatinine clearance, lysosomal enzyme activity, and very long chain fatty acids, were normal. There was no microalbumin-
uria. The karyotype of lymphocytes was normal (46,XX). Transthoracic and transesophageal echocardiography, cervical and transtural ultrasonography, measurement of evoked potentials, and electromyography all yielded normal findings. Light microscopy of a skin biopsy, including several arterioles, capillaries, and venules, did not disclose vessel wall lesions; no abnormal deposits were stained by periodic acid–Schiff or Congo Red, or shown by antiubiquitin immunohistochemistry. Biopsy for ultrastructural study included only capillaries and some very small venules, and there were no detectable abnormalities. Muscle biopsy findings were also normal, without ragged red fibers or abnormalities of mitochondrial enzymes. A diagnosis of small deep infarct of unknown cause was established. During the 2 years after this stroke, the patient had no additional cerebrovascular events, but a left detachment of the retina occurred. At age 37, examination showed a right spastic hemiparesis with pyramidal signs and central facial palsy. There was no sensory or cerebellar disturbance. The MMSE score was 27 of 30, and she experienced development of severe depression that required antidepressive and anxiolytic drugs.

CASE IV.1. This 8-year-old girl is the proband’s daughter. She was born after an uneventful pregnancy; delivery was normal. No fetal distress was reported. At birth, malformations of the eyes were obvious. Her ophthalmological abnormalities included a bilateral congenital cataract, iris hypoplasia, microcornea (see Fig 1C), and left amblyopia with normal intraocular pressure. Infantile hemiparesis was noted during the neonatal period. Brain MRI performed at age 11 months showed a left paraventricular porencephaly without other anomalies. No further neurological event was reported after this date. At age 8 years, neurological examination showed a right spastic hemiparesis. Blood pressure and cardiovascular examination findings were normal. Visual acuity was low in both eyes. Fundus examination did not show retinal arteriolar tortuosity or any retinal hemorrhages or exudates in either eye. Fluorescein angiography showed no abnormality.

<table>
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<tr>
<th>Features</th>
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MMSE = Mini-Mental State Examination; NA = not available.
arteriolar caliber was normal, and there was no leakage of fluorescein or capillary dropout. Brain MRI showed widespread, asymmetric, periventricular white matter hyperintensities on FLAIR images, associated with poststroke dilatation of the posterior horn of the left lateral ventricle (see Fig 2D). No brainstem lesion was observed.

CASE III.3. This 32-year-old man is the proband’s brother. He had had bilateral amblyopia since birth. Ophthalmological examination showed bilateral high myopia, polycoria on the left eye, and a bilateral congenital cataract. Bilateral juvenile glaucoma was treated by glaucoma drainage implant surgery on the right side and by hypotensive eyedrops on the left one. The patient had no history of neurological manifestations or headache. Neurological examination disclosed generalized brisk tendon reflexes, and the MMSE score was 27 of 30. Blood pressure was normal, and no vascular risk factors were recorded. Fundus examination showed an excavation of the optic disc caused by glaucoma. T2-weighted images of the brain showed a diffuse hyperintensity of the periventricular white matter (see Fig 2E). No brainstem lesion was observed. The first pregnancy of the patient’s wife ended in spontaneous abortion in the third trimester. Later, the couple had one unaffected child with normal ophthalmological findings.

CASE III.4. This woman, sister of the proband, was 29 years old. She also had bilateral amblyopia, predominating in the left eye. Ophthalmological examination showed a microcornea and an unoperated bilateral cataract, with normal intraocular pressure. Neurological examination results and blood pressure (120/80mm Hg) were normal. The MMSE score was 27 of 30. She did not report headache or other neurological symptoms. Brain MRI showed a diffuse periventricular leukoencephalopathy (see Fig 2F). No brainstem lesion was observed. Fundus examination showed no anomalies of retinal vessels.

CASE II.2. This 58-year-old woman is the mother of the proband. She had bilateral high myopia, iridogoniodygenesis, iris hypoplasia, microcornea, and congenital cataract. At age 55, high intraocular pressure was observed (26mm Hg on both sides) requiring treatment using hypotensive eyedrops. She had no history of diabetes or hypertension. She did not report any neurological symptoms or headaches. Neurological examination showed generalized brisk tendon reflexes without sensorimotor deficit and a rest tremor of the head without akinesia or hypertonia. The MMSE score was 28 of 30. Fundus examination was normal without retinal arteriolar tortuosity. Brain T1-weighted images and FLAIR MRI showed a diffuse leukoencephalopathy with two large holes (see Figs 2G, H) compatible

Fig 1. Ophthalmological features of some affected family members. (A, B) Case III.2 (proband): Contact lens is worn on the right eye (A) to correct aphakia caused by congenital cataract surgery (arrow). Note microcornea, corectopia (arrow 1), and peripheral corneal opacity (arrow 2). On the left eye (B), note microcornea, peripheral corneal opacities (arrow 2), and irregular pupil (arrow 3). (C) Case IV.1: Right eye presents only microcornea; the congenital cataract cannot be seen in this photograph. The corectopia is shown by arrow 1.
with ancient asymptomatic small deep infarct or small hemorrhages. No brainstem lesion was observed.

Subjects I.1, I.2, II.3, and II.4. Subjects I.1, I.2, and II.4 had normal neurological findings. Subject II.3 had a 10-year history of hypertension and showed bilateral Babinski signs with generalized brisk tendon reflexes and a moderate right kinetic cerebellar syndrome on examination. Brain MRI showed asymmetric, slight periventricular leukoencephalopathy on T2-weighted images and few small deep infarcts of the basal ganglia in this subject. Brain MRI was normal in Subject II.4.

Subjects I.1 and I.2 each had a senile cataract surgically treated after age 70 years. Subject I.1 refused to give blood samples for genetic analysis or to undergo cerebral MRI. Neurological examination findings at age 78 years were normal. Because some data could not be obtained, she was considered to be probably healthy.

Genetic Studies
Direct sequencing of the coding region of PITX2 was performed in the proband (Case III.2) and detected no deleterious mutation. Genetic linkage analyses with markers located on chromosomes 19p13 (NOTCH3),
3p21, 6p25 (FOXC1), 4q25 (PITX2), and 13q14 showed that for each of the fully informative markers tested, affected individuals did not share the haplotype transmitted by the obligate affected founder (Case II.2) to the definitely affected offspring. These data strongly suggest that this disorder is not linked to these different loci.

Genetic linkage with markers located on chromosomes 11p13 (PAX6) and 13q34 (COL4A1) showed the transmission of a common haplotype to all definitely affected subjects. Because linkage to these two loci remained possible, we also performed in the proband a genomic DNA search for mutation in the entire coding region and exon-intron boundaries of PAX6 using denaturing high-performance liquid chromatography techniques and of COL4A1 by direct sequencing. No mutation was detected in PAX6. A heterozygous G to A transition at position 2159 (c.2159G>A) was identified in exon 29 of COL4A1 (Fig 3), leading to the replacement of glycine with aspartic acid at position 720 (p.G720D) within the triple-helix domain. This missense mutation cosegregated with the disease in the family (see Fig 3) and was not present in 200 control chromosomes.

**Discussion**

We report on a French Caucasian kindred affected over at least three generations by a white matter disease consistent with cerebral vasculopathy in the context of familial autosomal dominant malformations of the anterior chamber of the eye and missense mutation in the COL4A1 gene. After clinical examination, 5 of the 11 family members were diagnosed as being definitely affected. These five cases had ocular anterior chamber abnormalities of the Axenfeld–Rieger type, of variable severity, and cerebral MRI showed diffuse leukoen-
cerebrovascular complications related to the stress of delivery. ARA has been observed in 11 mouse strains: 10 of these strains also have hemorrhagic stroke and ocular anterior chamber abnormalities have never been described in humans with Col4a1 mutations before now, a polar cataract was reported in the clinical description of three patients with neonatal porencephaly and adult stroke. Conversely, we did not observe retinal vascular tortuosity as Vahedi and colleagues described. Only one case combining cataract and retinal vascular tortuosity has been reported to date. All these data demonstrate a wide variability in the ocular and cerebrovascular phenotypic spectrum and strongly suggest a phenotype–genotype correlation in Col4a1 mutations in humans, as suggested by previous studies in Col4a1-mutated mice. Indeed, several Col4a1-mutated mice have recently been shown to express variable defects in the eye, brain, and kidney, vascular stability, and viability. ARA has been observed in 11 mouse strains: 10 strains carrying different Col4a1 missense mutations, and, recently, in the model Gould and colleagues previously reported, 1 strain carrying a deletion of exon 40. Of particular interest is that mice of four of these strains also have hemorrhagic stroke and noted among all adult subjects affected by the disease (Cases III.2, III.3, III.4, and II.2). Mild-to-moderate mental retardation has been reported in patients with infantile hemiparesis and the COL4A1 mutation; however, no cognitive impairment was described in the absence of neurological symptoms among patients with the COL4A1 mutation. Complementary neuropsychological evaluation and follow-up will be mandatory to clearly identify a cognitive impairment related to the pathology, as has been described in Cadasil.

Type IV collagens are ubiquitous basement membrane proteins, including the vascular basement membrane. Six different α chains belong to the family of the type IV collagen molecules, which can form three distinguishable networks. Collagens IV A1 and A2 are the most abundant type IV collagens and confer vascular stability, as has been described in Cadasil.21 Therefore, it has been suggested that focal disruptions of the vascular basement membrane may predispose to hemorrhage, whereas the swelling of vascular endothelial cells and the increased thickness of the basement membrane may lead to narrowing of vessels and may predispose to ischemic damage. We failed to find any anomaly in our skin biopsy samples. This result may be related to the wide phenotypic variability of the disease or to chance.

The main differences between the family described here and other cases previously described are represented by the presence of ARA. Although the eye’s anterior chamber abnormalities have never been described in humans with Col4a1 mutations before now, a polar cataract was reported in the clinical description of three patients with neonatal porencephaly and adult stroke. Conversely, we did not observe retinal vascular tortuosity as Vahedi and colleagues described. Only one case combining cataract and retinal vascular tortuosity has been reported to date. All these data demonstrate a wide variability in the ocular and cerebrovascular phenotypic spectrum and strongly suggest a phenotype–genotype correlation in Col4a1 mutations in humans, as suggested by previous studies in Col4a1-mutated mice. Indeed, several Col4a1-mutated mice have recently been shown to express variable defects in the eye, brain, and kidney, vascular stability, and viability. ARA has been observed in 11 mouse strains: 10 strains carrying different Col4a1 missense mutations, and, recently, in the model Gould and colleagues previously reported, 1 strain carrying a deletion of exon 40. Of particular interest is that mice of four of these strains also have hemorrhagic stroke and

In reference to previous MRI findings for patients with COL4A1 mutations, our observations confirm that this leukoencephalopathy predominates in the supratentorial posterior periventricular areas and can be observed in subjects with and without focal neurological symptoms. Intracranial hemorrhages after minor or major brain trauma in adults have also been reported previously. Such an event was not observed among the adults of the family described here. However, the case with infantile hemiparesis and porencephaly (Case IV.1) is in accordance with the increased risk, described in humans and mouse models, of cerebrovascular complications related to the stress of delivery. In contrast, a relatively low MMSE score was
small-vessel disease,\textsuperscript{19,20} similar to the cases observed in our study.

Several rare hereditary conditions are known to affect cerebral and retinal vessels, such as CADASIL,\textsuperscript{15,23,24} cerebroretinal hereditary conditions recently linked to 3p21,\textsuperscript{2} and other causes of cerebral vascular leukoencephalopathy, such as Fabry’s disease or CARASIL.\textsuperscript{3,25,26} Our study indicates that careful examination of the anterior segment of the eye, in addition to fundus examination, is recommended in the context of cerebral microangiopathy because it may suggest a possible mutation in \textit{COL4A1}.

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References