Clinical and brain MRI follow-up study of a family with *COL4A1* mutation

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ABSTRACT

Objective: To better delineate the clinical spectrum and the natural history of *COL4A1* mutations, a newly defined genetic cause of small vessel disease including the brain and retina.

Methods: Clinical and brain MRI follow-up study of a family with *COL4A1* mutation.

Results: During a 7-year period, two affected members died from intracranial hemorrhage. Four other members had a *COL4A1* mutation (age ranges 25 to 74 years). None reported stroke or retinal hemorrhage or hematuria and none had dementia according to Diagnostic and Statistical Manual of Mental Disorders–IV criteria. Follow-up brain MRI showed grade 3 diffuse leukoencephalopathy in three out of four patients. All had dilated perivascular spaces and three out of four had silent microbleeds mainly in the deep white matter. MRI signal abnormalities did not change in severity, number, or location between baseline and follow-up imaging.

Conclusions: *COL4A1* mutation carriers have great diversity in the clinical expression of the disease within the same family. Some affected family members may remain asymptomatic during several years of follow-up and have no evidence of progression of vascular changes on brain MRI.

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GLOSSARY

FSE = T2 fast spin echo; **GE** = gradient echo; **PVS** = perivascular spaces; **TE** = echo time; **TI** = inversion time; **TR** = repetition time.

COL4A1, a gene that encodes type IV collagen alpha 1 chain, has recently been identified in families with a variable phenotype of infantile hemiparesis related to perinatal stroke, congenital porencephaly, intracranial hemorrhage during adulthood, and retinal arteriolar tortuosity.[1-6] Type IV collagen, an essential component of all basement membrane including vascular basement membrane, is a triple-helical molecule composed of three alpha chains.[1,2,7] Six different alpha chains belong to the family of type IV collagen molecules, which can form three different networks. Mutations in alpha 3 (*COL4A3*), alpha 4 (*COL4A4*), and alpha 5 (*COL4A5*) cause Alport syndrome, which is characterized by glomerulonephropathy with deafness and eye defects.[8] Mutations in both alpha 5 (*COL4A5*) and alpha 6 (*COL4A6*) cause allelic variants of Alport syndrome.[8,9] To date no mutation in *COL4A2* has been described in humans. In mice, *Col4a1* mutants have structural alterations of basement membranes in many tissues including the brain, the eye, and the kidney.[1,2,7,10] Because of the specific alterations of vascular basement membrane in the brain, mutant *Col4a1* mice have frequent perinatal hemorrhages, and may develop intracranial hemorrhages during adulthood.[1,2] In addition, they have frequent microhemorrhages predominantly in the deep cerebral structures.[2] There are so far no reported neuropathologic data on *COL4A1* mutation related vascular alterations in humans. Brain CT and MRI abnormalities associated with *COL4A1* mutations include

diffuse periventricular leukoencephalopathy, microbleeds, dilated perivascular spaces, deep porencephaly, brainstem atrophy related to Wallerian degeneration, corpus callosum atrophy, and fresh deep intracerebral hemorrhages.[3-6] These abnormalities suggest that *COL4A1* mutation is responsible for a diffuse small vessel disease of the brain.

To better delineate the clinical spectrum and the natural history of *COL4A1* mutations, we conducted a clinical and brain MRI follow-up study of six members of a family with a previously reported G562E *COL4A1* mutation.[2]

METHODS After informed consent was obtained, we reexamined six members of a family with a previously reported *COL4A1* mutation who were first examined between 1998 and 1999.[5] Two other family members died before follow-up, one at age 33 of a traumatic subarachnoid hemorrhage with vasospasm and multiple brain infarctions.[2,5] The other was first examined in 1999 at age 36. He had a history of migraine with visual and sensory aura, retinal arteriolar tortuosities, left lower limb atrophy with pyramidal signs, and on brain MRI periventricular leukoencephalopathy with dilated perivascular spaces (figure, D) and a slight right ventricular enlargement. He died at age 40 of a massive anticoagulant related deep intracerebral hemorrhage (figure, E).[2]

All six re-examined individuals had a complete physical examination, a brain MRI, and a fasting blood examination including blood count, serum urea nitrogen, creatinine, sodium, potassium, calcium, chloride, uric acid, liver enzymes, CRP, homocysteine, and anticardiolipids. The systolic and diastolic blood pressure was measured after resting for 10 minutes in a sitting position. As COL4A1 is expressed in the developing glomerular basement membrane and mutant mice have proteinuria,[2] in each participant the urine was evaluated for microscopic hematuria and a 24-hour urine collection was obtained for measurement of total protein, albumin, and creatinine.

MR examinations were performed on a 1.5 T scanner, using the following parameters: T1 SE (repetition time [TR] 560 msec, echo time [TE] 15 msec; sagittal and axial planes), FLAIR (TR 8,000 msec, TE 160 msec, inversion time [TI] 2,100 msec; axial plane), T2 fast spin echo (FSE) (TR 7,000 msec, TE 100 msec; axial plane), T2* gradient echo (GE) (TR 460 msec, TE 15 msec; axial, sagittal, and coronal slices), and three-dimensional time-of-flight sequence (TR 30 msec, TE 2.7 msec, 1.4 mm thickness) on the basal cerebral arteries.

A neuroradiologist (M.B.) blinded to the subject's clinical and genetic status analyzed all MR sequences for the presence and location of infarcts, microbleeds, white matter abnormalities, dilated perivascular spaces, porencephaly, ventricle enlargement, cortical, thalamus, and brainstem atrophy. Microbleeds were defined as rounded areas of marked and homogenous signal loss on GE-MRI not located in sulcal areas to avoid confusion with flow voids from cerebral vessels. Symmetric subcortical hypointense lesions were

considered likely to represent calcifications and were excluded. The presence, location, number, and size of microbleeds were assessed for each of the following locations: basal ganglia, thalamus, subcortical white matter, deep white matter, gray–white matter junction, cortex, cerebellum, and brainstem. Leukoaraiosis was evaluated by T2 FSE and FLAIR sequences and classified into four grades as previously described.[11]

Dilated perivascular spaces (PVS) were defined as homogenous round punctate hypointense lesions on T1SE with corresponding hypointense and hyperintense lesions on FLAIR and T2 FSE sequences, without surrounding hyperintensity. The maximum number, the maximum size, and the location (basal ganglia, thalamus, subcortical and deep white matter, cortex, cerebellum, and brainstem) of dilated PVS were recorded.

RESULTS **Clinical and biologic findings.** Among the six re-examined family members, four had a *COL4A1* mutation (Subjects I-2, II-1, II-4, and III-2, table). Among them two had an infantile hemiparesis with a non-progressive residual hemiparesis with limb atrophy and a slight disability (modified Rankin 1). Two had typical migraine with visual aura. None of the patients had Diagnostic and Statistical Manual of Mental Disorders–IV criteria of dementia based on routine clinical examination. All had, as previously reported, diffuse retinal arteriolar tortuosities on fundus examination. None reported retinal hemorrhage or stroke during follow-up. Blood pressure was normal in two and the other two were treated for moderate arterial hypertension. The results of blood count and the levels of serum electrolytes, calcium, creatinine, urea nitrogen, uric acid, protein, liver enzymes, homocysteine, and CRP were all in the normal range in all four individuals except the oldest one aged 74 who had a urea concentration of 7.3 mmol per liter with a creatinine clearance of 65 mL per minute and a slight hyperhomocysteinemia (15.97 μmol per liter). No family member had abnormal protein or albumin urinary concentration and none had hematuria. Anticardiolipids and lupus anticoagulant were also negative.

The two family members without a *COL4A1* mutation (aged 18 and 23 years) had a normal neurologic, ophthalmologic, biologic, and brain MRI examination but one, a 23-year-old woman, reported attacks of migraine with typical visual aura.

MRI findings (figure). *Leukoencephalopathy.* Among the four family members with *COL4A1* mutation, all except one had grade 3 diffuse leukoencephalopathy involving mainly the periventricular and the deep white matter (figure, A through C). The youngest, age 20, had slight periventricular ab-
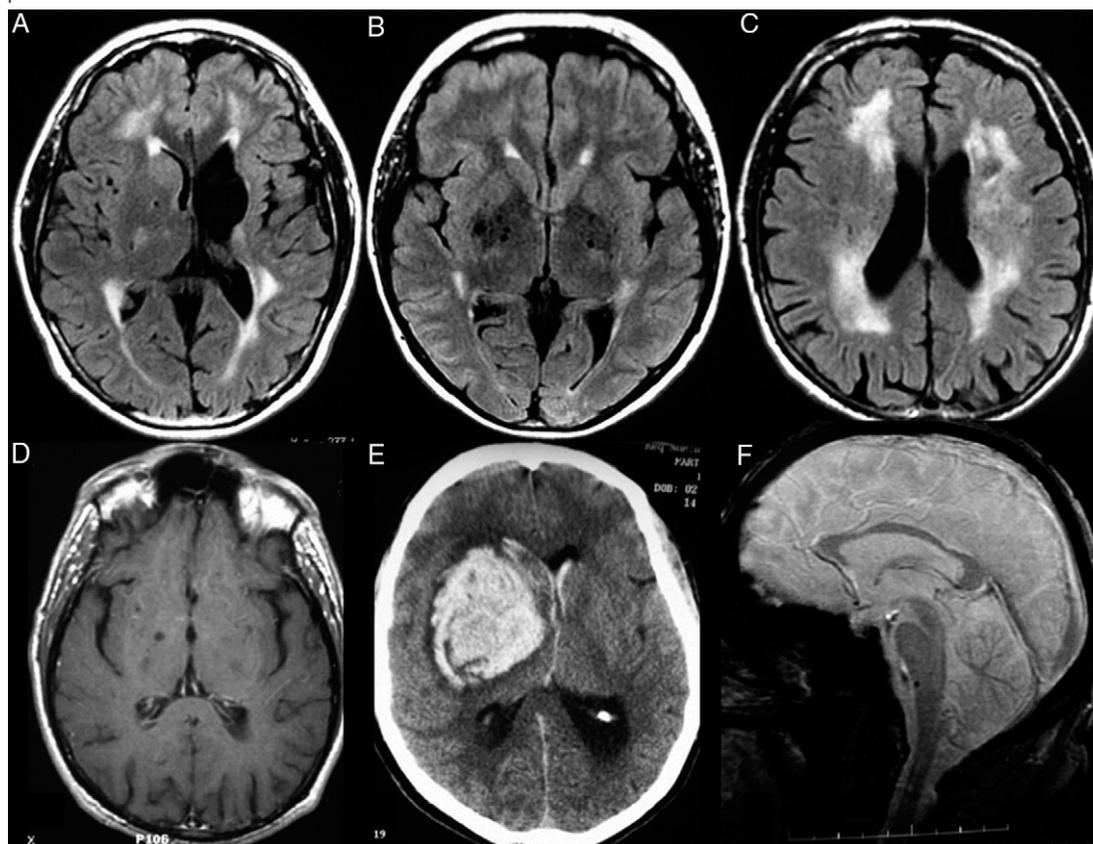
years later. It is also of note that no patient had external capsule, anterior temporal white matter, or cerebellar leukoencephalopathy. Slight pontine white matter FLAIR imaging signal abnormalities were observed in one patient. No family member had brain infarction on FLAIR sequences.

Dilated perivascular spaces. All four members with a *COL4A1* mutation had dilated PVS (figure, B and D). Their number ranged from less than 10 to more than 30. The basal ganglia were involved in all cases. Deep structures were more frequently involved although the oldest patient, age 74, also had a marked number of dilated PVS in the gray–white matter junctions. Most dilated PVS were small (<2 mm) but three patients also had a few large ones (10 to 12 mm diameter) that did not change in number or in size between the first and the follow-up MRI examinations.

Microbleeds. Three out of the four patients with a *COL4A1* mutation had microbleeds on GE TE examination. Microbleeds were all very small (<2 mm diameter) foci of homogenous signal hypointensity. They were few and mainly located in the deep cerebral structures including brainstem and cerebellar white matter. In one patient with infantile hemiparesis, microbleeds were located in the periventricular tissue adjacent to the porencephaly. There was no obvious correlation between the number of microbleeds and age as the oldest family member had a single microbleed in the brainstem (figure, F).

Cerebral and brainstem atrophy. A marked atrophy of brainstem, thalamus, cerebral cortex, and skull ipsilateral to the dilated ventricle was observed in the two patients with infantile hemiparesis. Corpus callosum atrophy was present in two patients, the oldest patient with infantile hemiparesis and his 73-year-old father with no history of infantile hemiparesis.

Three-dimensional time-of-flight sequences on the basal cerebral arteries disclosed no abnormalities.

DISCUSSION We report detailed clinical, biologic, and brain MRI follow-up data of 4 individuals with *COL4A1* mutation. All, including the oldest aged 74, remained clinically asymptomatic during a follow-up period of 7 years. None had stroke or retinal hemorrhage in contrast to two affected family members who died of traumatic or anticoagulant related intracranial hemorrhage as previously reported.[2,5] These data show that *COL4A1* mutation carriers within the same family have a great diversity in the clinical expression of the disease. In addition, by comparison to the

first examination, follow-up brain MRI did not disclose new white matter signal changes or new microbleeds. However, this study has some limitations as the use of semi-quantitative score may not detect slight progression of white matter changes. In addition, no standardized and repeated neuropsychological testing was performed to detect subclinical cognitive decline.

Disabling and recurrent spontaneous intracranial hemorrhages have been reported in two unrelated young patients with two different *COL4A1* mutations (G1236R and G805R).[6,12] Whether different *COL4A1* mutations may explain this phenotypic variation remains to be analyzed in large cohorts of patients. Interestingly *Col4a1* mutant mice have phenotypic variability for kidney and eye defects depending on their genetic background or on the type of mutations.[2,7,10] In mice, eye defects associated with *Col4a1* mutations in the highly conserved glycine residue consist of anterior segment dysgenesis optic nerve hypoplasia, retinal arteriolar silvering with alteration of retinal vessel endothelial cells and pericytes, and retinal arteriolar tortuosities. All patients with *COL4A1* mutation in our family had on fundus examination retinal arteriolar tortuosities that remained asymptomatic during follow-up but none had cataract or glaucoma. Although type IV collagen alpha chain is also an essential component of developing glomerular basement membrane, no individuals in our family had proteinuria, recurrent hematuria, or renal failure.

Gene–environment interaction also plays a major role in the expression of *COL4A1* mutation since surgical delivery prevents severe hemorrhage in mutant *Col4a1* pups and as head trauma, use of anticoagulant, and physical exercises have been reported as precipitating factors for intracranial hemorrhage in patients with *COL4A1* mutation.[2,12] Whether age is an additional risk factor for the phenotypic expression of *COL4A1* mutation is uncertain since the oldest affected family member in our study, aged 74, remained asymptomatic during follow-up.

As in other genetic small vessel diseases of the brain, we found on T2* GE-MRI in our patients a high frequency of microbleeds located mainly in the deep white matter. In cerebral amyloid angiopathy the number of new silent microbleeds has been correlated with a greater risk of intracranial hemorrhage.[13,14] In patients with *COL4A1* mutation, the number of silent microbleeds may also be a risk factor for intracranial hemorrhage since the reported patients with a severe phenotype of recurrent and disabling intracranial hemorrhages

had multiple silent and newly appearing microbleeds whereas affected family members without stroke in the present study had few microbleeds and no new ones on follow-up.[6,12] It is noteworthy that the two patients who died from intracranial hemorrhage in this study did not have T2*GE on baseline MRI.

COL4A1 mutation is frequently associated with leukoencephalopathy.[5,6,12] In our study, it involved mainly the periventricular and deep white matter. Although leukoencephalopathy in small vessel disease of the brain is considered to be due to ischemia, no patient with *COL4A1* mutation has yet been reported with a symptomatic ischemic stroke.

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