COL4A1-Related Disease: Raised Creatine Kinase and Cerebral Calcification as Useful Pointers

Davide Tonduti[1] Anna Pichiecchio[2] Roberta La Piana[3] John H. Livingston[4] Daniel A. Doherty[5] Anirban Majumdar[6] Susan Tomkins[7] Manuele Mine[8] Mauro Ceroni[9] Ivana Ricca[9] Umberto Balottin[1,10] Simona Orcesi[1]

[1] Child Neurology and Psychiatry Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy
[2] Neuroradiology Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy
[3] Department of Neuroradiology, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
[4] Department of Paediatric Neurology, Leeds General Infirmary, Leeds, United Kingdom
[5] Department of Paediatrics, University of Washington, Seattle Children's Hospital, Seattle, Washington, United States
[6] Department of Paediatric Neurology, Bristol Children's Hospital, Bristol, United Kingdom
[7] Department of Clinical Genetics, St Michael's Hospital, Bristol, United Kingdom
[8] Laboratoire de Génétique Neurovasculaire, Groupe hospitalier Lariboisière - Fernand Widal, Hôpital Lariboisière, Paris, France
[9] Department of Clinical Neurology, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy
[10] Department of Public Health, Neuroscience, Experimental and Forensic Medicine, Unit of Child Neurology and Psychiatry, University of Pavia, Pavia, Italy

Address for correspondence and reprint requests Davide Tonduti, MD, Child Neurology and Psychiatry Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, via Mondino 2, 27100 Pavia, Italy (e-mail: davidetondu@hotmail.com).

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Abstract

Background Mutations in *COL4A1* are responsible for a spectrum of clinical phenotypes characterized by neurological, ocular, and renal involvement. Neurological features are the most prominent but as such are rather nonspecific.

Case Presentation Here, we report three new cases that, like five patients we previously described, show the novel common finding of raised creatine kinase (CK) concentration.

Conclusion Raised CK concentration, in addition to intracranial calcification, is to be considered another useful pointer to a final diagnosis of *COL4A1*-related disease.

Keywords
- *COL4A1*
- cerebral calcification
- creatine kinase
- cataract

Introduction

Type IV collagen is a fundamental component of the vascular basement membrane; it is a tetrameric protein comprising six homologous chains (α1–α6), each encoded by different genes (*COL4A1-6*).[1] Mutations in *COL4A3-6* cause Alport syndrome, characterized by prominent renal, ocular, and cochlear involvement[2] and in which abnormalities on brain magnetic resonance imaging (MRI) have only rarely been reported.[3] Mutations in *COL4A1* can result in a wide spectrum of clinical features with neurological, ocular, and renal involvement inherited in an autosomal dominant manner.[4] The most common clinical features reported are infantile hemiparesis, congenital porencephaly, and hereditary angiopathy with nephropathy, aneurysm, and cramps (HANAC) syndrome.[5]

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Ocular manifestations include arterial tortuosities, cataract, anterior segment dysgenesis including Axenfeld–Rieger anomaly, microcornea, retinal detachment, and high intraocular pressure.[6] Other features include intracranial aneurysms of the carotid siphon, Raynaud phenomenon, and supraventricular arrhythmia.[1] Recently, Livingston et al[7] reported that in children the simultaneous presence of cerebral calcification and periventricular leukomalacia (PVL) should suggest the possibility of *COL4A1*-related disease when no other cause for the PVL has been identified.

Here, we report three new cases with the novel common finding of raised creatine kinase (CK), reinforcing our previous observation.[7] This feature, in association with the presence of cerebral calcification and periventricular and deep white matter abnormalities, proved to be useful pointers to the diagnosis in these cases.

Case Reports

Patient 1

A 31-year-old woman presented with congenital cataract, mild hematuria, Raynaud phenomenon, and episodic muscular cramps. She was the mother of a previously reported *COL4A1* patient (case 4 in Livingston et al[7]). At the age of 23 years, she experienced a transient ischemic attack. Brain MRI showed a pontine hematoma without an underlying vascular malformation on magnetic resonance angiogram. There were, in addition, multifocal periventricular and deep white matter abnormalities. Focal lesions in the basal ganglia associated with punctate calcification were also evident (►**Fig. 1A**). A schizencephalic cleft was also evident on the right frontomesial hemisphere, with a defect of the right central portion of the callosal body and a narrow fissure, surrounded by dysplastic cortex, that allowed communication between the lateral ventricle and the interhemispheric fissure. A focal heterotopic nodule nearby, lining the roof of the right lateral ventricle, was also present; the dysplastic cortex was also continuous with the underlying basal ganglia. The splenium, the genu, and the left central portion of the body of the corpus callosum were recognizable. Follow-up MRI years later did not demonstrate new abnormalities (►**Fig. 1B–D**).

Work-up for the muscle cramps showed elevated CK (430 U/L; reference range 26 to 140 U/L). Electromyography showed slight and nonspecific signs of muscular impairment (slight reduction of amplitude and duration of motor unit potentials); motor and sensory nerve conduction velocity were normal. Muscle biopsy revealed only mild nonspecific changes.

Following the diagnosis of a *COL4A1* mutation in her son (patient 7 in ►**Table 1**), genetic analysis revealed the same heterozygous mutation in exon 27 (c.1973 G > A) of the *COL4A1* gene. This mutation results in the substitution of aspartic acid for the highly conserved glycine residue at position 658 (p.G658D) within the triple helix domain.

Patient 2

This male is the first son of nonconsanguineous parents. Fetal ultrasound at around 23 weeks' gestation showed intraventricular hemorrhage. He was delivered vaginally at term and had an uneventful perinatal period. In the early life, he was hypertonic and subsequently developed a severe spastic-dystonic tetraplegia. He had no features of muscle disease. Optic coloboma and cataract were present. He developed generalized epileptic seizures in the first year of life which were well controlled by ethosuximide.

Brain MRI at 2 months of age revealed a large porencephalic cyst in the right hemisphere, multifocal white matter abnormalities in the left centrum semiovale, and abnormal basal ganglia. Computed tomography (CT) scan at the same age revealed bilateral periventricular calcification. Renal function tests were repeatedly normal but abdominal ultrasound showed diffuse bilateral hyperechogenicity of the renal pyramids.

CK has been elevated on many occasions, ranging between 600 and 1,200 U/L (reference range 39 to 308 U/L). Muscle CT scan showed atrophy and adipose infiltration in the calves; muscle biopsy revealed only mild nonspecific changes. At the age of 13 years, brainstem auditory evoked potentials and audiograms demonstrated mild sensorineural deafness.

Molecular analysis of the *COL4A1* gene revealed a heterozygous mutation in exon 29 (c.2159 G > A). This mutation results in the amino acid substitution of glycine with aspartic acid at position 720 (p.G720D).

Patient 3

A 48-year-old man, the father of patient 2, was the second son of nonconsanguineous parents. His family history was relevant for migraine in his mother, his sister, one paternal aunt, and one paternal cousin. Pregnancy, delivery, and psychomotor development were normal. In the first year of life, bilateral congenital cataracts were diagnosed. From the age of 10 years, he began to experience intense migraine attacks. In the last decade, the migraine attacks had become more frequent (~1 to 2 per week) and were poorly responsive to the treatment. At the age of 45 years, this patient had a sudden episode of dysarthria lasting ~12 hours. A year later, he presented with a sudden episode of confusion and aphasia, lasting 1 day. Since then, salicylates and antihypertensive drugs have been commenced. He currently demonstrates mild dysarthria and aphasia.

CT and MRI showed diffuse supratentorial white matter abnormalities with low signal on T1- and high signal on T2-weighted images. There were some focal abnormalities in the corona radiata, the basal ganglia, and the thalamus (►**Fig. 2**) associated with punctate calcification in basal ganglia. Multiple bilateral renal cysts were detected by abdominal ultrasound. Sensory evoked potentials and brainstem auditory evoked potentials were normal. The following investigations were also normal: renal function, thrombophilia and autoimmune screen, very long chain fatty acids, cerebroside β-galactosidase and arylsulfatase A enzymatic activity, and *NOTCH3* and *GFAP* molecular analysis. Karyotype analysis showed a pericentric inversion of the Y chromosome. CK level was 227 U/L (reference range 26 to 192 U/L) and has been reported as slightly elevated on several other occasions. Molecular analysis of the *COL4A1* gene showed the same

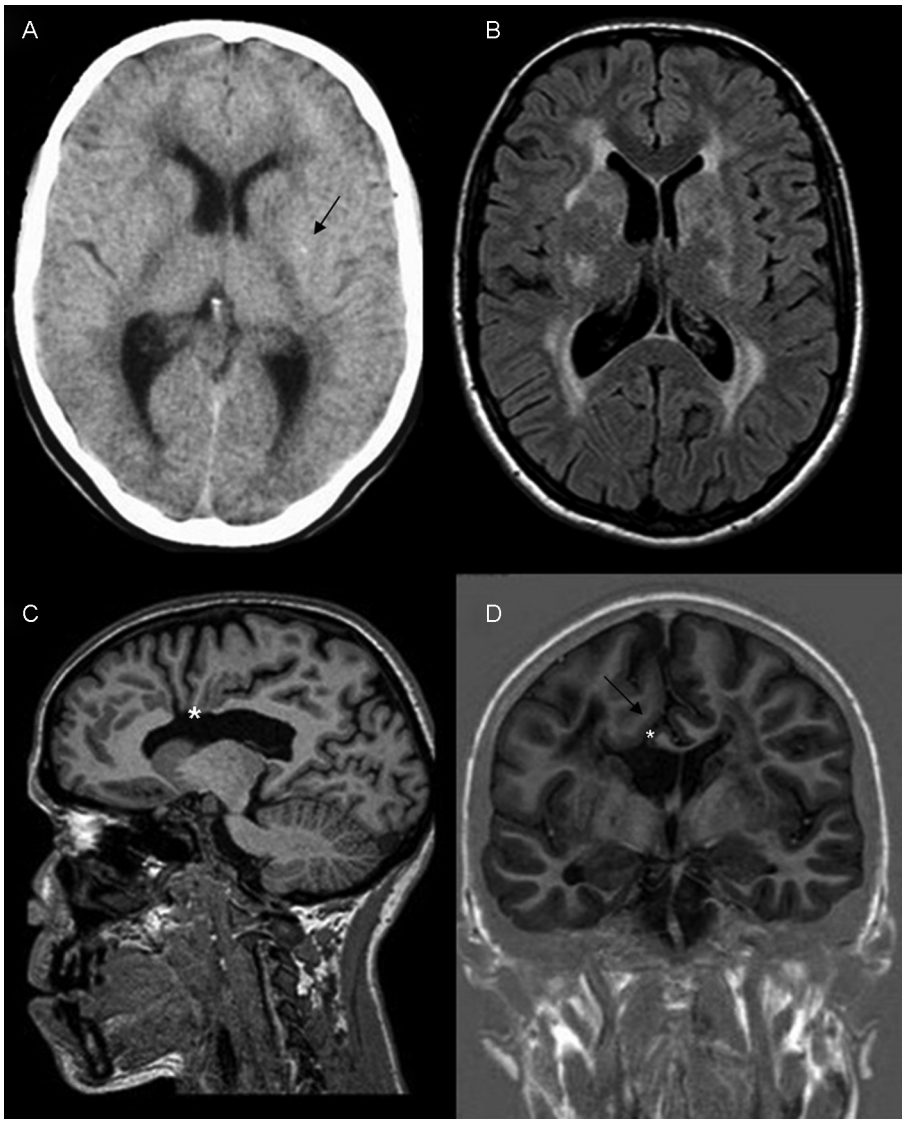

Fig. 1 CT and MRI of patient 1. (A) Axial CT brain image showing a punctate calcification in the left putamen (arrow). (B) Axial T2-weighted MRI scan showing periventricular, deep white matter, and basal ganglia high signal abnormalities. (C) Sagittal T1-weighted magnetic resonance three-dimensional fast-field echo image shows a defect of the right central portion of the callosal trunk (asterisk). (D) Coronal T2-weighted inversion recovery MRI scan: a narrow right mesial frontal schizencephalic cleft lined by dysplastic cortex allows apparent direct communication between the right lateral ventricle and the interhemispheric fissure (arrow). A right central callosal defect is confirmed in the coronal view as well (asterisk). CT, computed tomography; MRI, magnetic resonance imaging.

heterozygous mutation in exon 29 (c.2159 G > A) that was found in his son (patient 2).

Discussion

We have here reported three new cases affected by *COL4A1*-related disorders with the novel finding of raised CK. The clinical details of these patients are shown in ►**Table 1** together with those of patients reported previously in Livingston et al.[7] We thus have a series of eight patients from six families with different phenotypes who nonetheless share the common findings of periventricular and deep white matter abnormalities, raised levels of CK, and cerebral calcification. The neurological picture was variable and nonspecific, and all the patients demonstrated variable extraneurological involvement.

The most intriguing finding in this series of patients with *COL4A1*-related disease is the presence of raised levels of CK. This finding has been reported previously in patients affected by HANAC syndrome and in at least three other patients,[4,8] raising the possibility of an unrecognized effect of *COL4A1* mutations. All the patients reported by Plaisier et al[8] presented with muscle cramps from early childhood and muscle cramps were present in our patients 1 and 7. In patients with severe spastic-dystonic tetraplegia and cognitive impairment, the occurrence of muscle cramps may be difficult to determine. Muscle biopsy in patients 1, 2, and 7 demonstrated only mild nonspecific changes.

Table 1 Main clinical characteristics of the eight *COL4A1*-mutated patients

	Patient 1	Patient 2	Patient 3	Patient 4 (Case 1 in Livingston et al[7])	Patient 5 (Case 2 in Livingston et al[7])	Patient 6 (Case 3 in Livingston et al[7])	Patient 7 (Case 4 in Livingston et al[7])	Patient 8 (Case 5 in Livingston et al[7])	Among patients investigated for the sign or symptom
Sex, age	F, 31 years	M, 14 years	M, 48 years	F, 4 years	F, 4 years	F, 2 years 6 months	M, 10 years	M, 2 years	
Neurological examination	Normal	Severe spastic-dystonic tetraplegia	Mild dysarthria and aphasic disturbances	Severe spastic-dystonic tetraplegia	Spastic tetraplegia	Mild spastic tetraplegia	Mild spastic diplegia	Mild left hemiplegia	From normal to severe spastic-dystonic tetraplegia
WM involvement	+	+	+	+	+	+	+	+	8/8
Intracranial calcification	+	+	+	+	+	+	+	+	8/8
Muscle cramps	+	?	–	?	?	?	+	?	2/3
Raised levels of CK (U/L)	430 (nv 26–140)	200–600 (nv 39–308)	227 (nv 26–192)	1048 (nv 39–308)	927 (nv 20–215)	205 (nv 25–185)	310–1266 (nv 39–308)	260–266 (nv 24–195)	8/8
Congenital cataract	+	+	+	+	+	–	–	–	5/8
Microphthalmia	–	–	–	+	+	–	–	–	2/8
Axenfeld–Rieger anomaly	–	–	–	+	–	–	–	–	1/8
Renal involvement	+	+	+	?	?	–	–	–	3/6
Renal cysts	?	–	+	?	?	–	–	–	1/5
Hematuria	+	–	?	–	?	–	–	–	1/6
Other renal abnormalities	–	+	–	–	?	–	–	–	1/7
Raynauds phenomenon	+	–	–	–	–	–	–	–	1/8
Migraine	–	?	+	?	?	?	–	?	1/3

Abbreviations: CK, creatine kinase; F, female; M, male; ?, not known; nv, normal values; WM, white matter.

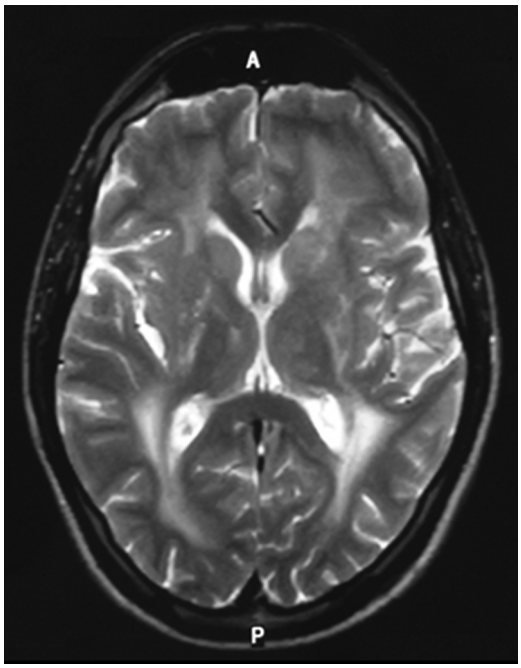

Fig. 2 MRI of patient 3. Axial T2-weighted MRI scan showing diffuse white matter high signal sparing the U fibers. Multiple high signal abnormalities are visible in the basal ganglia and thalami. MRI, magnetic resonance imaging.

The role of collagen IV in muscle cells has still to be clarified. However, Ullrich congenital muscular dystrophy and Bethlem myopathy are associated with *COL6A1-3* mutations.[9] The pathophysiology of these disorders seems to be related to the interaction of collagen VI with the basement membrane collagen IV.[10] Another link between *COL4A1* and muscle disease has recently been suggested by Labelle-Dumais et al[11]: *COL4A1* mutations in mice cause a phenotype resembling muscle–eye–brain disease/Walker Warburg syndrome (MEB/WWS) and these authors also found putative heterozygous mutations in *COL4A1* in two MEB/WWS patients.[11]

In our series, the neuroradiological picture was characterized by periventricular and deep white matter abnormalities of varying severities and associated with focal hyperintense lesions in the basal ganglia. The location and signal characteristics of the leukoencephalopathy are consistent with a small vessel disease resulting in micro- or macrohemorrhages and ischemic damage, secondary to the vascular fragility observed in *COL4A1*-related disorders. Other collagenopathies present arterial fragility as part of their clinical picture, with an increased risk of cerebrovascular disorders such as *COL4A2*-related disorders[5] and also Ehlers–Danlos syndrome (caused by mutations in different genes, including *COL1A1*, *COL3A1*, *COL5A1*, and *COL5A2*).[12] Another interesting finding in our series was the presence, in patient 1, of an open lip schizencephaly which could be the end result of a variety of insults leading to abnormal cortical organization. To date, cortical malformations have not been considered to be part of the phenotype associated with *COL4A1* mutations and, with the exception of the MEB/WWS patients reported by Labelle-Dumais et al,[11] there have been no other reports of cortical malformations. Although it is uncertain whether cortical malformation can be regarded as the part of the *COL4A1*-related disease spectrum, our case suggests that it could be one of the different neuroradiological outcomes, which vary depending on the timing of the ischemic insult.

All the patients in this series demonstrated intracranial calcification on CT. This was characteristically periventricular but could also be in the deep white matter or basal ganglia.[7]

Congenital cataract was present in five patients. Ophthalmological abnormalities have commonly been reported in patients with *COL4A1* mutations.[6] Other collagenopathies may demonstrate ophthalmological abnormalities, such as *COL18A1* mutations causing Knobloch syndrome and *COL2A1*, *COL11A1*,[13]*COL9A1, COL9A2*[14] mutations causing Stickler syndrome. It is of note that *COL9A3* mutations have been described in a patient with a myopathic phenotype and raised CPK.[15] Mutations in *JAM3*,[16] encoding the tight-junction protein, lead to clinical presentations overlapping those of our patients including congenital cataracts, intracerebral hemorrhage, and subependymal calcification. The CK levels in these patients were not reported.

In conclusion, we have reported a total of eight patients with *COL4A1* mutations, three previously unreported, all demonstrating elevated CK levels. In association with intracranial calcification and an autosomal dominant pattern of inheritance, this may be a useful pointer to the diagnosis of *COL4A1*-related disease.

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Note

All authors take full responsibility for the data, the analyses and interpretation, and the conduct of the research. All authors have read and agreed to the content of the manuscript, and included the author list. We have received patient consent forms from the patient in the study.

Contributions of all the authors are as follows:

- Davide Tonduti: study design, data collection and analysis, and manuscript preparation.
- Anna Pichiecchio: neuroimaging evaluation, figure preparation, and manuscript review and corrections.
- Roberta La Piana, John H. Livingston, Daniel A. Doherty, Anirban Majumdar, Mauro Ceroni, Ivana Ricca, and Umberto Balottin: data collection and analysis, and manuscript review and corrections.
- Susan Tomkins andManueleMine: genetic analysis, and manuscript review and corrections.
- Simona Orcesi: study design, data collection and analysis, and manuscript review and corrections.

Conflict of Interest Statement

All authors disclaim any financial or commercial involvement or other conflicts of interest.

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