

# A Novel Mutation in *COL4A1* Gene: A Possible Cause Of Early Postnatal Cerebrovascular Events

# Alice Decio,<sup>1</sup> Davide Tonduti,<sup>1</sup> Anna Pichiecchio,<sup>2</sup> Annalisa Vetro,<sup>3</sup> Roberto Ciccone,<sup>4,5</sup> Ivan Limongelli,<sup>4</sup> Roberto Giorda,<sup>6</sup> Lorella Caffi,<sup>7</sup> Umberto Balottin,<sup>1,8</sup> Orsetta Zuffardi,<sup>5</sup> and Simona Orcesi<sup>8</sup>\*

<sup>1</sup>Child Neurology and Psychiatry Unit, Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy

<sup>2</sup>Department of Neuroradiology, C. Mondino National Neurological Institute, Pavia, Italy

<sup>3</sup>Biotechnology Research Laboratory, IRCCS Policlinico San Matteo, Pavia, Italy

<sup>4</sup>Center for Genetic and Therapeutic Approaches, C. Mondino National Neurological Institute, Pavia, Italy

<sup>5</sup>Department of Molecular Medicine, University of Pavia, Pavia, Italy

<sup>6</sup>Molecular Biology Laboratory, IRCCS Scientific Institute Eugenio Medea, Bosisio Parini, Italy

<sup>7</sup>Child Neurology and Psychiatry Unit, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy

<sup>8</sup>Child Neurology and Psychiatry Unit, C. Mondino National Neurological Institute, Pavia, Italy

Manuscript Received: 22 May 2014; Manuscript Accepted: 14 November 2014

COL4A1 is located in humans on chromosome13q34 and it encodes the alpha 1 chain of type IV collagen, a component of basal membrane. It is expressed mainly in the brain, muscles, kidneys and eyes. Different COL4A1 mutations have been reported in many patients who present a very wide spectrum of clinical symptoms. They typically show a multisystemic phenotype. Here we report on the case of a patient carrying a novel de novo splicing mutation of COL4A1 associated with a distinctive clinical picture characterized by onset in infancy and an unusual evolution of the neuroradiological features. At three months of age, the child was diagnosed with a congenital cataract, while his brain MRI was normal. Over the following years, the patient developed focal epilepsy, mild diplegia, asymptomatic microhematuria, raised creatine kinase levels, MRI white matter abnormalities and brain calcification on CT. During the neuroradiological follow-up the extension and intensity of the brain lesions progressively decreased. The significance of a second variant in COL4A1 carried by the child and inherited from his father remains to be clarified. In conclusion, our patient shows new aspects of this collagenopathy and possibly a COL4A1 compound heterozygosity. 2015 Wiley Periodicals, Inc.

**Key words:** *COL4A1* gene; infants; leukoencephalopathy; cerebral calcification

#### **INTRODUCTION**

*COL4A1* encodes the alpha 1 chain of collagen type IV, a fundamental component of vascular basal membrane. The gene is expressed mainly in the brain, muscles, kidneys and eyes.

Type IV collagen is made from six types of homologous chains indicated as alpha1–6. These chains assemble as heterotrimers.

#### How to Cite this Article:

Decio A, Tonduti D, Pichiecchio A, Vetro A, Ciccone R, Limongelli I, Giorda R, Caffi L, Balottin U, Zuffardi O, Orcesi S. 2015. A novel mutation in *COL4A1* gene: A possible cause of early postnatal cerebrovascular events.

Am J Med Genet Part A. 9999:1-6.

Every tissue containing collagen type IV in its basal membrane has a distinctive expression of alpha chains. Alpha chain heterotrimers associate in dimers and then in tetramers, forming the complex collagen type IV suprastructure [Volonghi et al., 2010].

*COL4A1* is located in humans on chromosome 13q34. Different *COL4A1* mutations have been reported in numerous patients who present a very wide spectrum of clinical symptoms; no clear genotype-phenotype association has been found [Gould et al., 2005; Breedveld et al., 2006; Gould et al., 2006; Van der Knaap

Conflicts of interest: none.

Grant sponsor: Regione Lombardia government; Grant number: DGRS 13465–22/12/2010; Grant sponsor: PRIN 2010–2011; Grant number: 20108WT59Y\_003.

\*Correspondence to:

Simona Orcesi, M.D., Child Neurology and Psychiatry Unit, C. Mondino National Neurological Institute, via Mondino 2, 27100 Pavia, Italy. E-mail: simona.orcesi@mondino.it

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 00 Month 2014 DOI 10.1002/ajmg.a.36907 et al., 2006; Sibon et al., 2007; Vahedi et al., 2007; Alamowitch et al., 2009; Bilguvar et al., 2009; De Vries et al., 2009, Shah et al., 2010; Livingston et al., 2011; Meuwissen et al., 2011; Tonduti et al., 2012; Lemmens et al., 2013].

The presence of a multisystemic phenotype is one of the main characteristics of *COL4A1*-mutated patients [Vahedi and Alamowitch, 2011]. Typically the brain, eyes, kidneys and muscles are variably involved in the disease [Tonduti et al., 2012]. The presence of white matter involvement is typically linked to cerebral hemorrhages and/or small vessel ischemic damage [Van der Knaap et al., 2006; Corlobe et al., 2013]. A specific pattern of intracranial calcification has been associated with mutations in the gene [Livingston et al., 2011; Tonduti et al., 2012].

Here we report on the case of a patient carrying a novel de novo splicing mutation of *COL4A1* associated with a distinctive clinical picture characterized by onset in infancy and an unusual evolution of the neuroradiological features.

## **CLINICAL REPORT**

The child was born after an uneventful pregnancy. The delivery, too, was uneventful and the child's auxological parameters at birth were: weight 3190 g (25–50th centile), length 48 cm (10–25th centile); head circumference was not available. When the child was three months old, his parents started to notice erratic, chaotic eye movements. Ophthalmological examination showed congenital bilateral cataract, without other abnormalities. Brain magnetic resonance imaging (MRI) at this time was normal (Fig. 1). A complete heart assessment, abdominal ultrasound, metabolic examination for plasma and urinary amino acids and urinary organic acids, and screening for galactosemia were all normal. Karyotype analysis was normal. Neurological examination showed microcephaly and nystagmus, otherwise it was normal. The child underwent surgical

implant of artificial lenses and started visual rehabilitation training. The child's motor and language development proceeded normally until the age of two and a half years when he started to present focal epileptic seizures with secondary generalization. An electroencephalogram showed epileptic slow waves in the right frontal hemisphere, spreading during sleep. The patient started antiepileptic therapy, but oxcarbazepine failed to completely control the seizures and focal episodes kept occurring with monthly frequency. On neurological examination, the child showed mild, non-disabling spastic diplegia with good cognitive performance.

A month and a half after the first seizure, a CT scan and a new MRI were performed, which showed (Fig. 2A,B) bilateral confluent periventricular and deep white matter areas of hypersignal intensity (T2 and FLAIR sequences) and a left frontal periventricular cyst. Hemosiderinic deposits were observed in the right frontoparietal cortical-subcortical region (Fig. 2C) and CT scan revealed punctate calcification in the frontal periventricular and basal ganglia areas.

At six years of age, the child presented a paroxysmal episode characterized by painful cramps in his right arm subsequently also involving his right leg. He had a creatine phosphokinase (CPK) level of 5504 U\L (normal values 60–190), which increased to 26.342 U\L the following day. Thereafter, CPK levels were monitored regularly and they were always raised, reaching values higher than 2000 U\L. The neurological examination was unchanged.

The child also presents asymptomatic microhematuria without proteinuria. This has been evident since his first evaluation at the age of 3 years.

During his follow-up, the boy underwent three additional MRI evaluations (at 4, 6, and 8 years of age), which showed progressive attenuation of the white matter areas of signal hyperintensity (Fig. 3).

Molecular analysis of the *COL4A1* gene was performed using a next-generation sequencing panel for small vessel diseases and ischemic strokes on a Genome Analyzer IIx. The analysis identified

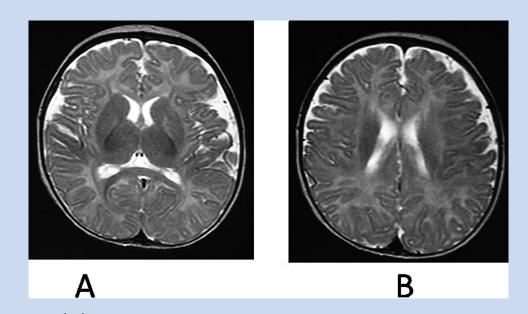


FIG. 1. Axial T2 MR images (A,B) performed at the age of 3 months, showing white matter myelination consistent with the child's age.

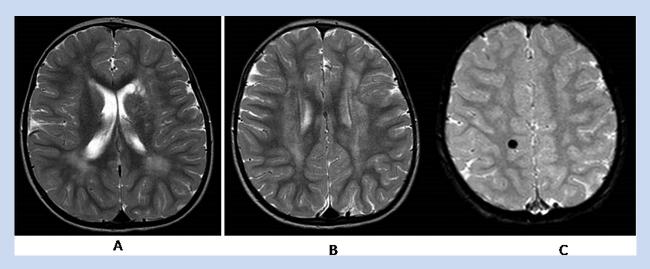


FIG. 2. T2-weighted axial MR images (A,B) and weighted Fast Field Echo (FFE) image (C) performed at the age of two and a half years. The T2 MR images (A,B) show bilateral mainly periventricular and deep white matter hyperintense alterations and a left frontal periventricular cyst. Slight enlargement of the right lateral ventricle is also evident. The axial FFE image (C) shows a small punctate focus of susceptibility, likely due to hemosiderin, in the right corticosubcortical rolandic parasagittal region.

two heterozygous variants affecting the gene, both confirmed by Sanger sequencing (Fig. 4). The first one, c.2458 + 1G>A, was located at the donor splicing site of intron 31. Parental DNA examination showed this novel mutation to be de novo. The second mutation, c.3712C>T, affecting exon 42 of the gene and resulting in a p.Arg1238Cys substitution, was reported in dbSNP138 (rs148801165) with a population frequency well below 1%. This mutation was found to be inherited from the father. Both variants were predicted to be "damaging" by at least one of the in silico prediction tools used (Table I). In particular, the c.2458 + 1G>A mutation was predicted to cause skipping of exon 31 during splicing. Real Time-PCR performed on mRNA from cultured skin fibroblasts of the patient confirmed this hypothesis (Fig. 5).

Our patient's father carried the c.3712C>T mutation. He is a 53-year-old man who has been affected by Raynaud's phenomenon and muscular cramps since he was young; he occasionally presents slightly raised CPK values (highest value 312 U\L- normal values 60–190). A complete ophthalmological assessment revealed absence of retinal arterial tortuosities and completely normal fundus oculi. Renal function was normal and renal and heart ultrasound

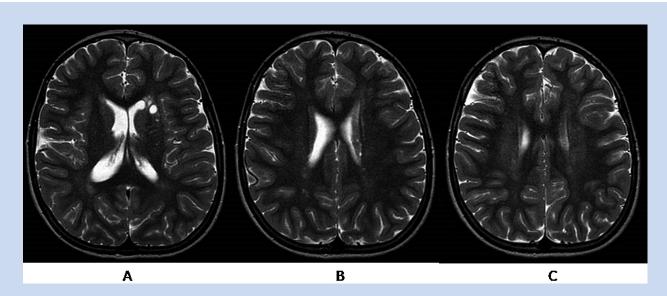


FIG. 3. (A,B,C) T2-weighted axial MRI performed when the patient was 8 years old, showing marked reduction of the previous bilateral periventricular and deep white matter lesions, with persistence of the left frontal periventricular cyst.

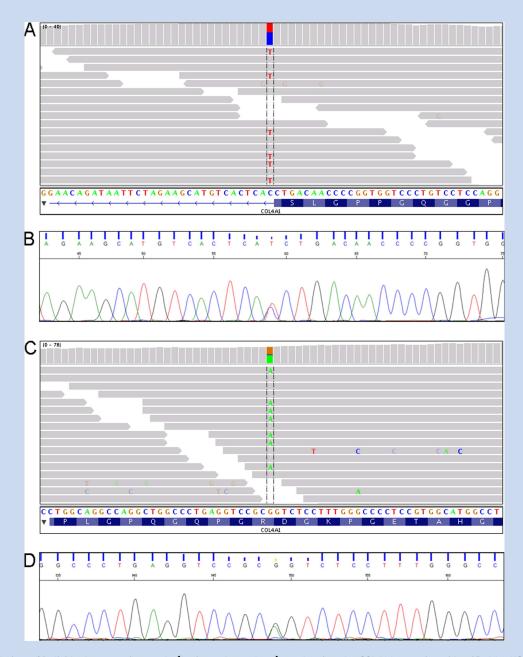


FIG. 4. Detailed view of individual sequencing reads (horizontal gray bars) overlapping the *COL4A1* mutations identified by NGS, as visualized by Integrative Genomics Viewer (IGV) (Thorvaldsdóttir et al., 2013). A coverage plot (vertical gray bars) is displayed for each mutation. Reads are aligned to the reference genome, whose sequence is shown below. The c.2458 + 1G>A mutation is shown in panel (A) together with the corresponding Sanger sequencing confirmation; (B) the inherited c.3712C>T mutation is shown in (C) with (D) its Sanger sequencing confirmation. The medium coverage for the gene was above 200X and sequencing was performed using a 100bp paired-end protocol, as reported elsewhere (Andolfo et al., 2013).

examination did not show abnormalities. Brain MRI did not show lesions due to ischemic or hemorrhagic events, but revealed a dermoid cyst in the superior cerebellar paravermian region. The proband's younger brother is 5 years old and presents asymptomatic microhematuria without proteinuria and moderate language delay. A recent brain MRI was normal, revealing only a mildly dysmorphic corpus callosum; no ischemic nor hemorrhagic lesions were observed. Sanger sequencing showed neither of the two mutations.

### DISCUSSION

We report on the case of a patient carrying two variants in *COL4A1*. One of them, a novel mutation, arose de novo in the proband and is

TABLE I. Details of the Mutations Identified in Our Patient Including the Results of Three in Silico Prediction Tools.

Genomic position	HGVS label	Inheritance	Mutation taster	SIFT	Polyphen2
chr13:110831269C>T	NM_001845.4:c.2458+1G>A	de novo	disease causing	NA	NA
chr13:110822924G>A	NM_001845.4:c.3712C>T	paternal	disease causing	tolerated	probably damaging
NA, not assessed because predictions are active only on missense mutations.					

located at the donor splice site of intron 31 (c.2458 + 1G>A). We demonstrated that this variant, leading to the elimination of an entire exon from the final transcript, causes a significant modification of protein structure and function.

This child's clinical picture was characterized by mild spastic diplegia with abnormal periventricular white matter. As previously reported in other COL4A1-mutated patients, signs of renal, ocular and muscular involvement were evident [Vahedi

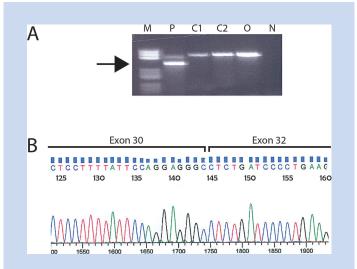


FIG. 5. Skipping of exon 31 in the COL4A1 transcript from proband's fibroblasts. (A) agarose gel analysis of the portion of the COL4A1 transcript spanning exon 31 in the proband (P), two control fibroblast samples (C1 and C2), ovary (O), and water control (N). The shorter fragment amplified only in the proband is indicated by an arrow. DNA Molecular Weight Marker V (Roche) was used as a marker (M). (B) detail of the sequence of the short transcript from the proband showing the junction between exons 30 and 32 and skipping of exon 31. Total RNA was extracted with Trizol (Life Technologies, Monza, Italy); cDNA synthesis was performed with Ready-To-Go You-Prime First strand beads (Amersham, Milan, Italy) and random hexamers; Non-quantitative RT-PCR of the portion of COL4A1 spanning exon 31 was performed in 25 µl reactions, using primers COL4A1-E29\_30-F(5'-CTCCCTTGGAACCTGGAAAC-3') and COL4A1-E32 33-R(5'-GCAGGGCCAGAAGGGAGAG-3') and JumpStart Red ACCUTaq LA DNA polymerase (Sigma). PCR protocol: 1 min at 96 °C; 30 cycles of 30 sec at 94 °C/30 sec at 58 °C/2 min at 68 °C; 5 min at 68 °C final elongation time; PCR products were analyzed on a 2% agarose TAE gel. The shorter fragment amplified only from the proband was excised from the gel and sequenced on an ABI 3500HT (Applied Biosystems).

and Alamowitch, 2011] and CPK levels were persistently augmented [Tonduti et al., 2012]. Some peculiarities were found in our patient: first of all, the neuroradiological abnormalities showed an unusual evolution: they were not present at three months of age, were evident at 30 months, and then improved on subsequent imaging. In the majority of pediatric COL4A1 patients, lesions were documented or supposed to be related to pre- or perinatal damage; in the pediatric setting, clinical onset with stroke has, to date, been reported rarely and only in adolescents [Shah et al., 2010]. No cases with clinical onset in early infancy have been reported: the child here described is the first case in which the vascular insult is hypothesized to have occurred not in the pre-perinatal period but between three and 30 months of age. In fact, at three months of age his MRI was completely normal, consistent with the physiological lack of myelination at birth. We are not able to definitively confirm the absence of cerebral calcification before three months because unfortunately the child did not undergo neonatal cerebral ultrasound or CT. It is also underlined that at 3 months of age he already showed microcephaly. On this basis we can only speculate that vascular events may have occurred between three and 30 months of age, in the absence of significant clinical symptoms, a very unusual finding in infancy. The pattern of neuroradiological abnormalities found at the age of two and a half years was characterized by small cystic changes in the left frontal white matter, which could indicate a vascular insult, associated with bilateral abnormal signal intensity, mainly in the periventricular and deep white matter, compatible with diffuse small vessel disease, and punctate calcification in the frontal periventricular areas and the basal ganglia. Overall, these aspects are similar to what has been previously reported in other COL4A1-mutated subjects [Vahedi et al., 2003; Livingston et al., 2011; Vahedi and Alamowitch 2011]. Moreover, during the neuroradiological follow-up the extension and intensity of the lesions tended to decrease progressively, particularly in the deep white matter. This finding was not previously reported in COL4A1-mutated patients, but in our opinion it could be compatible with the occurrence of vascular insults, considering that the ischemic/ hemorrhagic damage due to vascular events in childhood can be less pronounced than in adulthood and in pediatric patients may improve over time [Kato et al., 2012].

In conclusion, this report of a novel pathogenetic mutation in the *COL4A1* gene provides evidence that brain lesions in *COL4A1*mutated patients can be asymptomatic, and can change and improve over time. We also underline the importance of considering the possibility of *COL4A1* mutations not only in the presence of a pre-perinatal ischemic event, but also in the etiology of early childhood strokes. In this respect, the discovery of a cataract or of raised CPK levels, as described in this patient, is a crucial element evoking *COL4A1* involvement.

Another remarkable aspect of this case is that the child can be considered a compound heterozygote for two *COL4A1* mutations: indeed, our patient carried also a second variant in *COL4A1* in addition to the one already discussed. This second variant was inherited from his father and was of unknown significance. A polymorphism or a hypomorphic variant can be considered improbable because of the low frequency of the allele; this variant, too, was predicted to be "damaging" by at least one of the in silico prediction tools we used. We can therefore hypothesize additive effects of these two mutations: the fact that the father suffers from Raynaud's phenomenon, muscle cramps and slightly raised CPK levels (despite presenting a normal brain MRI and normal renal and ocular phenotype) may suggest that this variant is related to his phenotype and contributes to the unusual picture presented by his son.

#### ACKNOWLEDGMENTS

The Authors would like to thank Dr. O. Manara and Dr. C. Agostinis from Bergamo and Dr. C. Parazzini and Dr. A. Righini from Milan for providing first neuroradiological images of the patient. This work has received funding from the Regione Lombardia government as an Independent Research Project (SVE-LA Project, DGRS 13465–22/12/2010). We also acknowledge PRIN 2010–2011 (20108WT59Y\_003) to O.Z.

#### REFERENCES

- Alamowitch S, Plaisier E, Favrole P, Prost C, Chen Z, Van Agtmael T, Marro B, Ronco P. 2009. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. Neurology 73:1873–1882.
- Andolfo I, Alper SL, De Franceschi L, Auriemma C, Russo R, De Falco L, Vallefuoco F, Esposito MR, Vandorpe DH, Shmukler BE, Narayan R, Montanaro D, D'Armiento M, Vetro A, Limongelli I, Zufferdi O, Glader BE, Schrier SL, Brugnara C, Steward GW, Delaunay J, Iolascon A. 2013. Multiple clinical forms of dehydrated hereditary stomatocytosis arise from mutations in PIEZO1. Blood 121:3925–3912 S1–S12.
- Bilguvar K, DiLuna ML, Bizzarro MJ, Bayri Y, Schneider KC, Lifton RP, Gunel M, Ment LR, Pacifier and Breastfeeding Trial Group. 2009. COL4A1 mutation in preterm intraventricular hemorrhage. J Pediatr 155:743–745.
- Breedveld G, de Coo IF, Lequin MH, Arts WFM, Heutink P, Gould DB, John SWM, Oostra B, Mancini GMS. 2006. Novel mutations in three families confirm a major role of COL4A1 in hereditary porencephaly. J Med Genet 43:490–495.
- Corlobe A, Tournier-Lasserve E, Mine M, Menjot de Champfleur, Carra N, Dalliere C, Ayrignac X, Labauge P, Arquizan C. 2013. *COL4A1* mutation revealed by an isolated brain hemorrhage. Cerebrovasc Dis Basel Switz 35:593–594.
- De Vries LS, Koopman C, Groenendaal F, Van Schooneveld M, Verheijen FW, Verbeek E, Witkamp TD, van der Worp HB, Mancini G. 2009. *COL4A1* mutation in two preterm siblings with antenatal onset of parenchymal hemorrhage. Ann Neurol 65:12–18.

- Gould DB, Phalan FC, Breedveld GJ, van Mil SE, Smith RS, Schimenti JC, Aguglia U, van der Knaap MS, Heutink P, John SWM. 2005. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science 308:1167–1171.
- Gould DB, Phalan FC, van Mil SE, Sundberg JP, Vahedi K, Massin P, Bousser MG, Heutink P, Miner JH, Tournier-Lasserve E, John SWM. 2006. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. N Engl J Med 354:1489–1496.
- Kato T, Okumura A, Tsuji T, Hayashi S, Kito M, Natsume J. 2012. Punctate white matter lesions in a late preterm-born infant with hypoxic ischaemic encephalopathy: Chronological change in magnetic resonance imaging. Dev Med Child Neurol 54:862.
- Lemmens R, Maugeri A, Niessen HWM, Goris A, Tousseyn T, Demaerel P, Corveleyn A, Robberecht W, van der Knaap MS, Thijs VN, Zwijnenburg PJC. 2013. Novel COL4A1 mutations cause cerebral small vessel disease by haploinsufficiency. Hum Mol Genet 22:391–397.
- Livingston J, Doherty D, Orcesi S, Tonduti D, Piechiecchio A, La Piana R, Tournier-Lasserve E, Majumdar A, Tomkins S, Rice G, Kneen R, van der Knaap MS, Crow Y. 2011. COL4A1 mutations associated with a characteristic pattern of intracranial calcification. Neuropediatrics 42:227–233.
- Meuwissen MEC, de Vries LS, Verbeek HA, Lequin MH, Govaert PP, Schot R, Cowan FM, Hennekam R, Rizzu P, Verheijen FW, Wessels MW, Mancini GMS. 2011. Sporadic COL4A1 mutations with extensive prenatal porencephaly resembling hydranencephaly. Neurology 76: 844–846.
- Shah S, Kumar Y, McLean B, Churchill A, Stoodley N, Rankin J, Rizzu P, van der Knaap M, Jardine P. 2010. A dominantly inherited mutation in collagen IV A1 (COL4A1) causing childhood onset stroke without porencephaly. Eur J Paediatr Neurol 14:182–187.
- Sibon I, Coupry I, Menegon P, Bouchet J-P, Gorry P, Burgelin I, Calvas P, Orignac I, Dousset V, Lacombe D, et al. 2007. COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke. Ann Neurol 62:177–184.
- Thorvaldsdóttir H, Robinson JT, Mesirov JP. 2013. Integrative Genomics Viewer (IGV): High-performance genomics data visualization and exploration. Brief Bioinform 14:178–192.
- Tonduti D, Pichiecchio A, La Piana R, Livingston JH, Doherty DA, Majumdar A, Tomkins S, Mine M, Ceroni M, Ricca I, Balottin U, Orcesi S. 2012. COL4A1-related disease: raised creatine kinase and cerebral calcification as useful pointers. Neuropediatrics 43:283–288.
- Vahedi K, Alamowitch S. 2011. Clinical spectrum of type IV collagen (COL4A1) mutations: a novel genetic multisystem disease. Curr Opin Neurol 24:63–68.
- Vahedi K, Massin P, Guichard J-P, Miocque S, Polivka M, Goutières F, Dress D, Chapon F, Ruchoux M-M, Riant F, Joutel A, Gaudric A, Bousser M-G, Tournier-Lasserve E. 2003. Hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy. Neurology 60:57–63.
- Vahedi K, Boukobza M, Massin P, Gould DB, Tournier-Lasserve E, Bousser M-G. 2007. Clinical and brain MRI follow-up study of a family with COL4A1 mutation. Neurology 69:1564–1568.
- Van der Knaap MS, Smit LME, Barkhof F, Pijnenburg YAL, Zweegman S, Niessen HWM, Imhof S, Heutink P. 2006. Neonatal porencephaly and adult stroke related to mutations in collagen IV A1. Ann Neurol 59:504–511.
- Volonghi I, Pezzini A, Del Zotto E, Giossi A, Costa P, Ferrari D, Padovani A. 2010. Role of COL4A1 in basement-membrane integrity and cerebral small-vessel disease. The COL4A1 stroke syndrome. Curr Med Chem 17:1317–1324.