European Journal of Human Genetics (2021) 29:1654–1662
https://doi.org/10.1038/s41431-021-00880-3

ARTICLE

Deletion in *COL4A2* is associated with a three-generation variable phenotype: from fetal to adult manifestations

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Received: 20 September 2020 / Revised: 4 February 2021 / Accepted: 23 March 2021 / Published online: 9 April 2021
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Abstract

Genetic alterations in *COL4A2* are less common than those of *COL4A1* and their fetal phenotype has not been described to date. We describe a three-generation family with an intragenic deletion in *COL4A2* associated with a prenatal diagnosis of recurrent fetal intracerebral hemorrhage (ICH), and a myriad of cerebrovascular manifestations. Exome sequencing, co-segregation analysis, and imaging studies were conducted on eight family members including two fetuses with antenatal ICH. Histopathological evaluation was performed on the terminated fetuses. An intragenic heterozygous pathogenic in-frame deletion; *COL4A2*, c.4151_4168del, (p.Thr1384_Gly1389del) was identified in both fetuses, their father with hemiplegic cerebral palsy (CP), as well as other family members. Postmortem histopathological examination identified microscopic foci of heterotopias and polymicrogyria. The variant segregated in affected individuals demonstrating varying degrees of penetrance and a wide phenotypic spectrum including periventricular venous hemorrhagic infarction causing hemiplegic CP, polymicrogyria, leukoencephalopathy, and lacunar stroke. We present radiographic, pathological, and genetic evidence of prenatal ICH and show, for what we believe to be the first time, a human pathological proof of polymicrogyria and heterotopias in association with a *COL4A2* disease-causing variant, while illustrating the variable phenotype and partial penetrance of this disease. We highlight the importance of genetic analysis in fetal ICH and hemiplegic CP.

Introduction

Perinatal intracerebral hemorrhage (ICH) may be diagnosed antenatally or postnatally, resulting in various brain lesions depending upon the mechanism, vascular anatomy, and timing of the injury [1–4]. Fetal intraventricular hemorrhage (IVH) usually originates in the germinal matrix and may extend to the ventricles by a mechanism similar to that occurring in premature babies [5–7]. When they are extensive, germinal matrix hemorrhages (GMH) can lead to impaired venous drainage in the periventricular white matter (PVWM), causing periventricular venous hemorrhagic infarction (PVHI) that can subsequently evolve into a porencephalic cyst [5, 8, 9]. Diagnosis can be made by ultrasonography during pregnancy or by the appearance of early limb preference during infancy [9–14]. Prenatal GMH may result from acquired causes, such as fetal alloimmune thrombocytopenia or other coagulopathies, thrombophilia, antenatal trauma, or severe maternal disease, although the etiology is often not established [15–17]. Disease-causing variants in the collagen type IV alpha 1 (*COL4A1*) and the

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collagen type IV alpha 2 (*COL4A2*) genes are known genetic causes of antenatal/perinatal-onset [11, 12, 18–24].

COL4A1 and *COL4A2* are abundant components of nearly all basement membranes (BMs), and therefore, as suggested by the evidence to date, both *COL4A1* and *COL4A2* are associated with systemic vascular BM disease resulting in a broad phenotypic spectrum, including neurological, renal, ophthalmological, muscular, and cardiac manifestations, starting in fetal life and extending until adulthood [10–14, 20, 23, 24]. The neurological phenotype associated with *COL4A1* is expanding and includes small-vessel disease (SVD) of varying severity [13, 14, 21, 23, 25–27]. Fetal ICH with subsequent porencephalic cavitation has been described in association with *COL4A1* and may result in destructive changes resembling hydranencephaly at the severe end [1, 11, 19, 28, 29]. Disease-causing variants in *COL4A2* are rarer, and a frequency bias for *COL4A1* over *COL4A2* disease-causing variants has been recognized [30]. Although both sporadic and familial porencephaly are associated with *COL4A2* and are considered to be caused by an antenatal/perinatal insult, they are usually discovered in infancy or childhood, commonly following an uneventful pregnancy and neonatal period [25, 28, 31–34]. To the best of our knowledge, the *COL4A2*-associated phenotype has not been documented in human fetal life. In the present study, we report six members from a three-generation family with a novel *COL4A2* deletion that was identified following the diagnosis of fetal ICH in two consecutive pregnancies.

Methods

We performed exome sequencing (ES) in fetal DNA of two consecutive pregnancies that were terminated because of extensive brain damage due to fetal IVH, their healthy mother, and their father who has hemiplegic CP. Following genetic diagnosis, additional family members were genetically and clinically evaluated.

Participants

The clinical phenotype of the proband fetuses and all the affected family members was obtained from clinical records and by clinical and neurological examinations when possible.

Genetic analysis

Genomic DNA was extracted from peripheral blood according to standard protocols. Fetal DNA was extracted from fetal material taken following the termination of pregnancy (TOP). ES was performed for both fetuses and their parents by Fulgent Diagnostics SEQ on Illumina HiSeq4000 using the Roche Nimblegen Protocol at a sequencing depth of 60X. Analysis of the raw data was performed with the Variantyx diagnostic platform (Variantyx Genomic Intelligence®) [35]. Nonsense, nonsynonymous, canonical splice sites, and indels in coding regions with a minor allelic frequency of <0.01 in public databases (gnomAD, ExAc, and 1000 genomes) were analyzed and interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines [36].

Histopathological analysis

TOP was approved by the institutional committee according to the local law. A postmortem evaluation was performed on both fetuses at 26 weeks' gestation after receiving parental consent. The autopsy procedures followed routine protocol [37]. Gross examination of the brain was performed by coronal sectioning of the cerebral hemispheres and mid-sagittal sectioning of the midbrain and cerebellum. Multiple tissue samples were submitted for histological processing. Four-µm-thick histological sections were prepared and stained with hematoxylin and eosin and examined along with the corresponding gross photographs. Immunohistochemical stains for GFAP, CD68, Collagen IV, CD 31, and CD34, PAS, and Prussian blue (Iron) were carried out. The immunohistochemical stains were carried out with a Ventana Benchmark XT immunostainer.

Results

Proband cases

Clinical findings

The clinical characteristics and pedigree of the study participants are summarized in Table 1 and Fig. 1, respectively. Case III-4 was a female fetus of a 32-year-old healthy mother. The pregnancy was spontaneous and uneventful, with normal prenatal sonographic scans until 25 weeks' gestation, when a routine US examination revealed PVWM echogenicity and bilateral Grade II IVH. There was no history of maternal trauma, infection, or drug abuse. A neurosonographic examination at 26 weeks' gestation showed ventriculomegaly with diffuse involvement of the brain parenchyma consistent with Grade III IVH with PVHI. Maternal serum did not demonstrate any platelet alloantibodies, and platelet genotyping did not reveal any human platelet antigen incompatibility, thus excluding neonatal alloimmune thrombocytopenia. TOP was performed at 26 weeks' gestation.

Table 1 Summary of the clinical manifestations in affected family members.

	Onset	Neurological manifestations	Neuroimaging	Systemic manifestations
I-1	Unknown (m/p perinatal and adult)	Asymptomatic	Hemorrhagic lacunar infarct PVWM T2-hyperintesity Cerebral microbleeds	
II-2	perinatal	Hemiplegic CP	Porencephaly, PMG	High myopia Tilted disc
II-3	Unknown	Asymptomatic	Peri-atrial left T2-weighted hyperintense lesion, PMG	
III-4	Prenatal	Fetal IVH	Grade 3 IVH with PVHI PVWM T2-hyperintesity	Transverse reduction of fingers
III-5	Prenatal	Fetal IVH	Grade 3 IVH with PVHI PVWM T2-hyperintesity PMG and heterotopias	
III-6	Perinatal	Hemiplegic CP	Porencephaly, PVWM T2-hyperintesity	

CP cerebral palsy, *PMG* polymicrogyria, *PVWM* periventricular white matter, *IVH* intraventricular hemorrhage.

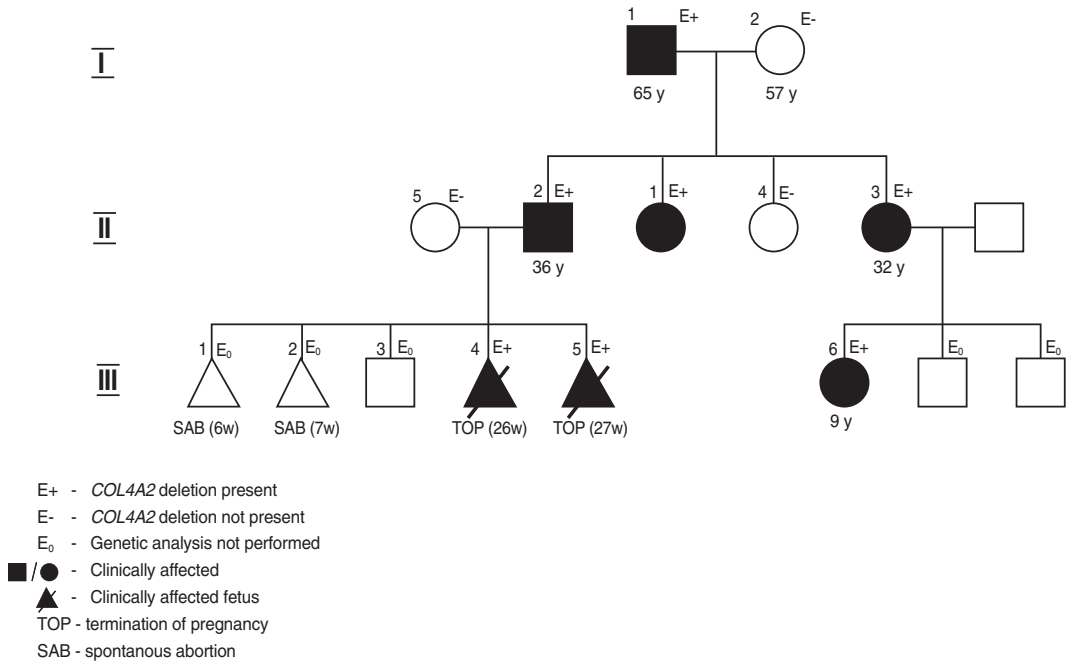

Fig. 1 Pedigree of the family. Co-segregation of the *COL4A2* c.4151_4168del p.(Thr1384_Gly1389del) variant in the family pedigree with various degrees of CVD. Squares represent males, circles represent females, and triangles indicate fetuses. The crossing line denotes the termination of pregnancy. The filled symbols represent clinically affected cases. E (+/−/0) indicates whether genetic analysis was positive for the *COL4A2* variant, negative, or not performed, respectively. The Arabic number is an identifier for each individual, whereas the generation is marked with a roman number.

Case III-5 was a female fetus conceived 15 months later. The prenatal follow-up was normal until 26 weeks' gestation when the mother complained of reduced fetal movements. A repeat transvaginal US examination showed severe bilateral ventriculomegaly with right IVH together with extensive PVWM and caudate nucleus echogenicity, suggestive of deep venous hemorrhagic infractions (Fig. 2ii). A TOP was performed.

Histopathological findings

Postmortem examinations of cases III-4 and III-5 were performed at 26 weeks' gestation and revealed fetal weight adequate for gestational age with normal head circumference. The gross and histological appearance of the internal organs and tissues, including kidneys and muscle, were normal in both autopsies. There was a transverse

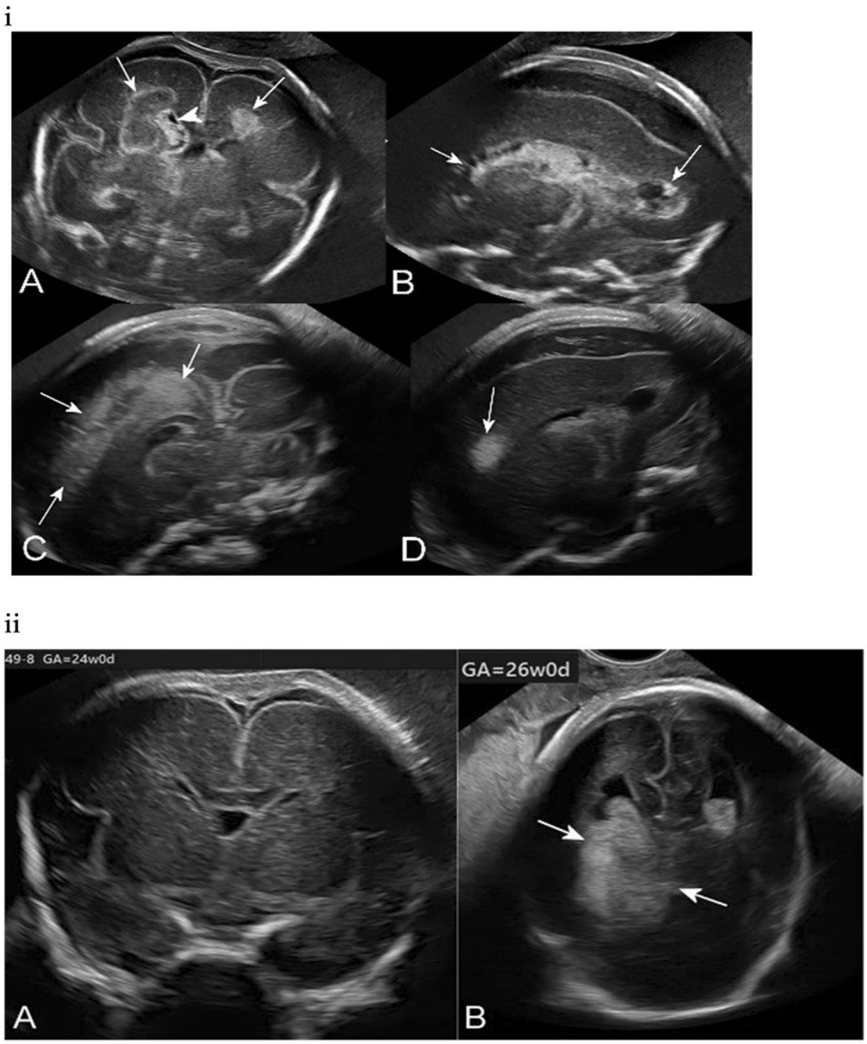

Fig. 2 Fetal ultrasound. Transvaginal neurosonographic examination of case III-4 (i) and III-5 (ii). (i) A modified coronal plane at 25w5d (**A**, **B**) showing echogenicity in the frontal PVWM bilaterally at different stages of evolution (arrows), and of IVH without ventriculomegaly (arrowhead, see also arrows in **B**). Parasagittal planes at 26w1d (**C**, **D**) demonstrating ventriculomegaly and a more extensive PVWM echogenicity (arrows) as well as blood clots within the dilated lateral ventricles. (ii) Transvaginal coronal plane US at 24 weeks' gestation showing normal-appearing lateral ventricles and PVWM (**A**). **B** Transvaginal modified axial plane at 26 weeks' gestation demonstrating bilateral lateral ventricle dilatation with extensive right IVH, and increased echogenicity suggestive of hemorrhagic infarction of the caudate.

reduction of fingers 1, 3, 4, and 5 of the left hand in fetus III-4 (Fig. 3J).

Gross and histological examinations of the brains of both fetuses revealed partially resorbed and digested hemorrhagic content within the lateral ventricles with ependymal destruction as well as extensive damage to the PVWM with areas of focal necrosis, microscopic calcifications, and mineralization of the WM. Numerous microglia and reactive changes in astrocytes in both fetuses were demonstrated by CD68 and GFAP immunostains, respectively. The immunostains for collagen IV in the hemorrhagic PVWM revealed marked irregular thickening of vascular BM (Fig. 3C–E).

Focal cortical dysplasia, focal WM neuroblastic heterotopia associated with marked cortical thinning in the left anterior temporal lobe and 2-microscopic foci of polymicrogyria (PMG) in the left frontal and opercular areas (Fig. 3F–I) were noted in case III-5.

Genetic analysis

Molecular analysis revealed a novel intragenic heterozygous in-frame deletion of 18 base pairs in exon 44 of the *COL4A2* gene; c.4151_4168del, (p.Thr1384_Gly1389del), (ENSG0000134871.19 ENST00000360467.7, ENSE00001468433) in the two fetuses (cases III-4, III-5), inherited from the father (individual II-2). The deletion causes a loss of six amino acids, including two glycine residues within the conserved area of the triple helical collagenous part of the protein. This deletion has not been reported in the population database (gnomAD, https://gnomad.broadinstitute.org) and segregated with other affected family members with various degrees of penetrance (Fig. 1). Therefore, it was defined as likely pathogenic based on ACMG criteria [36]. The variant and phenotype presented in the study was submitted to ClinVar (accession SCV001477492, URL: https://www.ncbi.nlm.nih.gov/clinvar/variation/7105/).

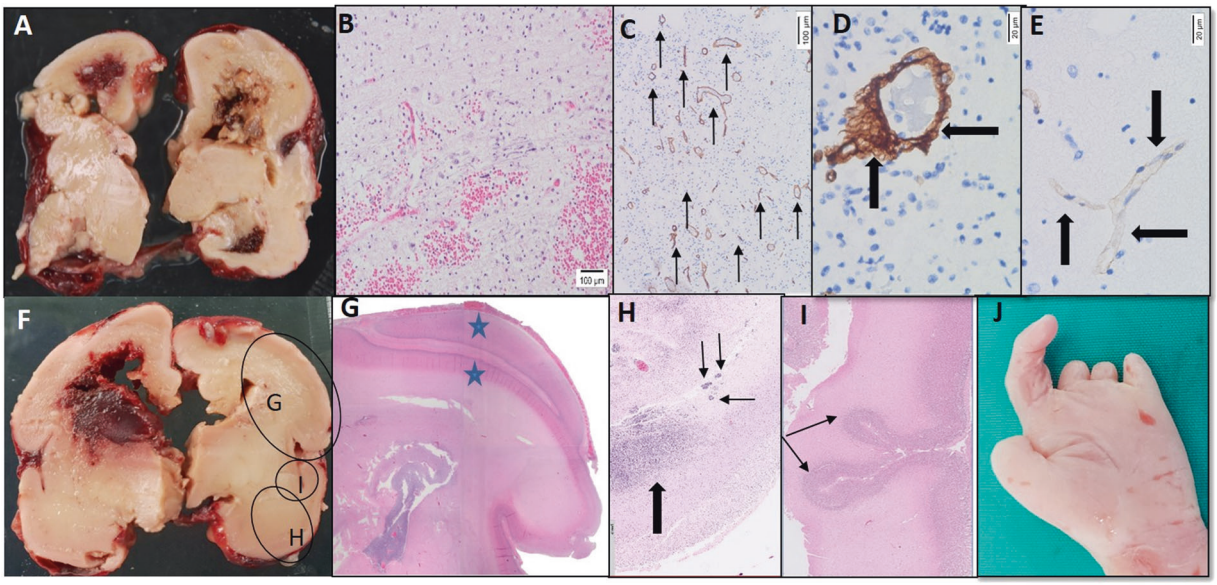

Fig. 3 Histopathological evaluation of fetuses with *COL4A2* intragenic deletion at 26 weeks of gestation. A Gross image of a coronal section of the temporoparietal lobes showing bilateral IVH grade IV. Hemorrhagic contents in lateral ventricles, disintegrating the PVWM. **B** Disintegration of white matter with extravasation of erythrocytes, reactive astrocytes, and early axonal mineralization (H&E). **C** Prominent collagen IV in PVWM BM vasculature (arrows, collagen IV immunostain). **D** Marked irregular thickening of collagen IV in a PVWM-dilated vessel (collagen IV immunostain). **E** Thin delicate collagen IV in BM of a subcortical white matter vessel (collagen IV immunostain). **F** IVH grade IV, right temporoparietal lobe. The circles denote the location of histologic images **G–I**. **G** Dysplastic double cortex, left parietal lobe (asterisks, H&E) (**H**) White matter heterotopias, left temporal lobe (arrows, H&E) (**I**) polymicrogyria-like cortex, left Sylvian fissure (arrows, H&E) (**J**) Transverse reduction defect of fingers 1,3, 5, left hand.

Other family members

The parents of the 2 fetuses (II-2 and II-5, Fig. 1) are non-consanguineous Caucasians in their 30s. They have a 4-year-old healthy son. The mother (II-5) had an uneventful medical history apart from medically treated and well-controlled hypothyroidism. She had two prior spontaneous miscarriages at 6- and 8-weeks' gestation and underwent an extensive workup for repeated miscarriages, including a thrombophilia workup, TORCH serology, and karyotyping, all of which were normal. The father (II-2), who had the same *COL4A2* deletion, was born at term after an uneventful pregnancy as the second son to parents of Jewish-Moroccan ancestry. At 7 months of age, he was noted to have left-sided hemiparesis and later developed high myopia. Computed tomography revealed an enlarged right lateral ventricle (images not available). He was diagnosed with hemiplegic CP due to presumed perinatal injury, and no further workup was carried out. He had febrile seizures in early childhood, with no subsequent clinical seizures. He had learning difficulties and attended a special education school in early childhood with normal intellect in adult life. Following the diagnosis of the affected fetuses, he underwent brain magnetic resonance imaging (MRI) at 35 years of age, which revealed right-sided porencephaly, reduced volume of the right frontal WM, and an irregular, thickened cortex suggestive of focal opercular PMG (Fig. 4). An ophthalmological examination showed high myopia and tilted discs. His renal US, urinalysis, and creatine phosphokinase levels were normal.

A clinical review of the extended paternal family revealed at least four other affected individuals, including his 65-year-old father (individual I-1), who has a history of diabetes mellitus, hypertension, and aortic stenosis but is neurologically intact. His brain MRI shows a remote hemorrhagic infarct in the left lentiform, with adjacent porencephalic dilatation of the lateral ventricle (Fig. 4). Remote lacunar infarcts in the left frontal area, and the deep WM as well as mild PVWM T2-hyperintesities are also evident.

The other affected family member is a 9-year old female (III-6), the niece of individual II-2 (Fig. 1) who was born at $37 + 6$ gestational age following a normal pregnancy, with normal Apgar scores, birth weight, and head circumference. At the age of 6 months, she was diagnosed with right-sided mild hemiparetic CP. An MRI of the brain at age 10 months revealed left-sided porencephaly with bilateral signal abnormalities in the deep PVWM in both frontal and parietal regions, with loss of PVWM volume sparing of subcortical WM. SWI showed low-signal intensity on the ventricular margins suggestive of presumed perinatal GMH (Fig. 4). A thrombophilia workup revealed a maternally inherited heterozygous prothrombin variant (G21210A). She attended regular schools, had normal verbal and cognitive

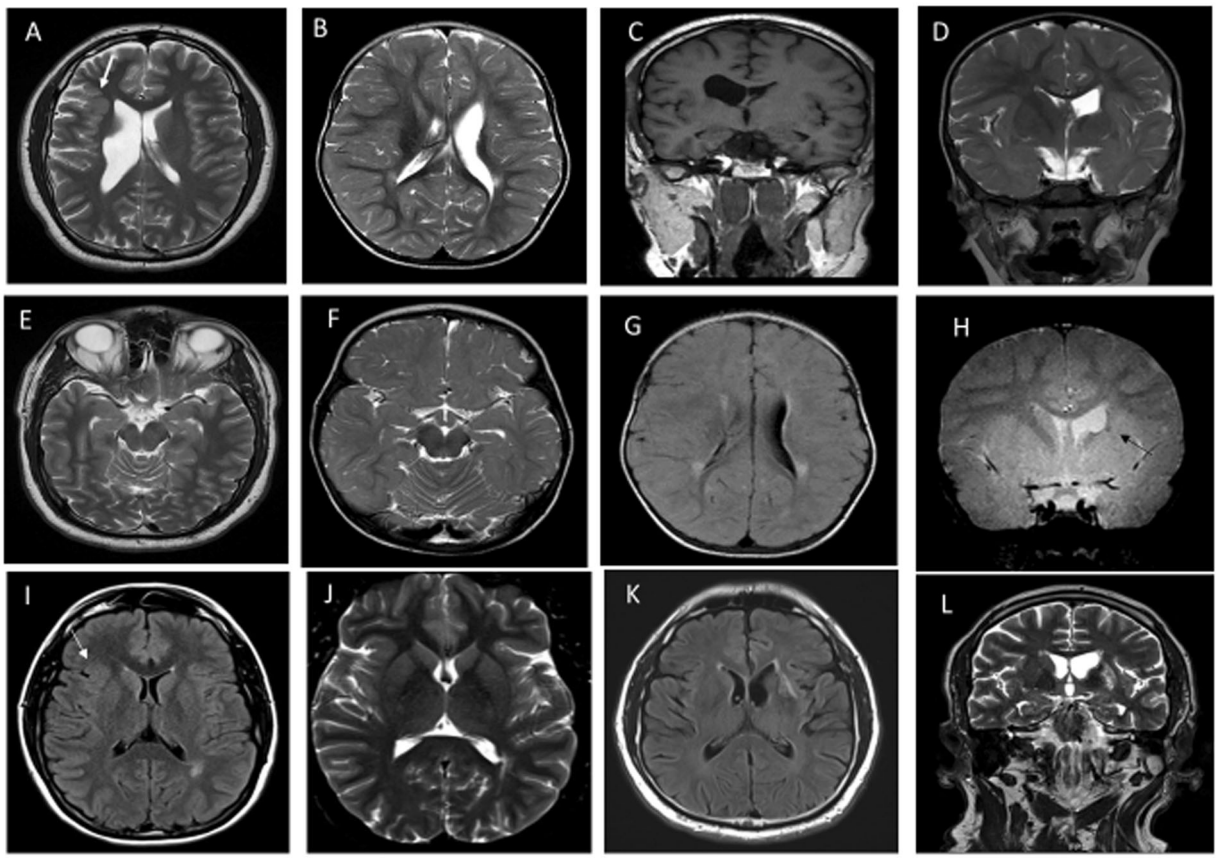

Fig. 4 Brain MRI of patients with *COL4A2* intragenic deletion. Brain MRI of individuals II-2 at 35 years of age (**A**, **C**, **E**), III-6 at 10 months of age (**B**, **D**, **F**, **G**, **H**), II-3 at 32 years of age (**I**, **J**) and I-1 at age 65 years (**K**, **L**). Axial T2, coronal T1, and coronal T2 of individual II-2 (**A**, **C**) and III-6 (**B, D**) demonstrating frontal porencephalic dilation of the right lateral ventricle associated with loss of PVWM volume. Note the perisylvian cortical thickening-polymicrogyria (solid arrow). Wallerian degeneration is apparent through the corticospinal tracts in individuals (**E**, **F**). Axial T2-Flair of individual III-6 (**G**) illustrating a multifocal, predominantly posterior, PVWM hyperintensity, expanding into the centrum semiovale and associated with PVWM loss, sparing the cortex and basal ganglia, and ventricular dilatation suggesting periventricular venous infarction (PVI). Coronal susceptibility weighted image showing subtle linear hyperintensity lining the dilated frontal horn suggesting hemosiderin rim (**H**, black arrow). Axial T2-FLAIR MRI of individual II-3 (**I**, **J**) showing a peri-atrial left T2-weighted hyperintense lesion and a right remote frontal peri-insular focal cortical thickening suggestive of PMG (arrow). Axial T2-FLAIR and coronal T2 of individual I-1 (**K**, **L**) shows a remote hemorrhagic infarct in the left lentiform.

development, and had no clinical seizures. Her neurological examination showed right-sided spastic hemiparesis (GMFCS-2). She had no other systemic involvement.

The mother of individual III-6 was 32-year-old healthy female (individual II-3) with normal perinatal history, development, and normal intellect. Her brain MRI showed a peri-atrial left T2-weighted hyperintense lesion as well as a right frontal peri-insular focal cortical thickening suggestive of PMG. A few punctuate hyperintense T2-lesions were also noted in the left frontal lobe, centrum semiovale, and the cerebellum. She had no other systemic involvement.

Discussion

Although reported for *COL4A1* [11, 19], almost all evidence for an antenatal origin of ICH associated with *COL4A2* has been demonstrated through postnatal imaging. In this report, we highlight the variable phenotypic spectrum of *COL4A2*-related disease throughout the life span in a three-generation pedigree. By providing antenatal human radiological and pathological evidence, we show, for what we believe is the first time, fetal origin of ICH, as well as isolated PMG and heterotopia due to a *COL4A2* disease-causing variant. The detection of this *COL4A2* variant in the two reported fetuses led to a diagnosis and an explanation for clinical features in other family members, highlighting a valuable role of genetic testing in fetal ICH.

In opposition to the reported understanding that *COL4A2* variants are associated with milder phenotypes and later age at onset [30], we show that prenatal severe manifestations may occur as well. The wide phenotypic inter-familial variance, previously documented for both *COL4A1 and COL4A2* [13, 34], suggests incomplete penetrance of the

disease and the potential role of other modifying factors. The highly variable phenotypic spectrum of *COL4A*-related disease is a challenge in both diagnostic and genetic counseling perspectives. It emphasizes that the assessment of fetal ICH should include a multidisciplinary team approach involving gynecologists, pediatric neurologists, and geneticists. Importantly, asymptomatic carriers should also undergo MRI since they may harbor SVD or aneurysms even in early adulthood [13].

A review of the literature and the listed genetic variants to date do not report any other pathogenic in-frame deletions in the *COL4A2* gene [30, 34]. Although the deletion reported here is an in-frame deletion, we show segregation of the variant throughout three generations and partial penetrance in extended family members. The possibility that another disease-causing variants arbitrarily segregating with affected family members without being detected in two WES analyses is ~3%. The deletion is at the distal end of the collagenous domain of the protein (position 1384-1389/1713 amino acids) in the triple helix domain. Numerous pathogenic missense variants located near the end of the triple helical part of *COL4A2* have been reported, suggesting that this area is a "hot spot". Although the deletion may cause an abnormal protein function by different pathogenic mechanism compared to the well-described G-Xaa-Yaa missense variants, a deletion may cause loss of *COL4A2* chain synthesis from one allele, predicted to cause a loss of function of the heterotrimer. This, along with the segregation of the deletion with affected members, is highly supportive of its pathogenicity. Importantly, our results show that the location of the variant cannot be the sole factor for determining genotype–phenotype correlation in regard to the Cerebrovascular disease (CVD). Plausible explanations include genetic modifiers, mosaicism, or environmental influences. Notably, and contrary to what had previously been hypothesized about non-glycine variants being likely low-penetrance risk alleles for late-onset intracranial hemorrhage [33], we now show that *COL4A2* deletions can cause a severe early-onset phenotype, similar to *COL4A1*. Of note, the prothrombin variant (G21210A) that had been identified in proband III-6 and individual II-3 was not found in any other family members. Given that thrombophilia is very uncommon in presumed perinatal stroke [38], and the phenotype was observed in family members that do not carry this variant, it is highly unlikely that this variant can serve to explain the neuroimaging findings identified in these two patients.

The modification of the BM structural properties due to variants in *COL4A1/2* increases the fragility of the vessel wall when exposed to environmental factors. Although pathological changes in BM also occur in other tissues, the major site of vessel damage is the brain [39]. The hemorrhage observed in the fetal brain is thought to be caused by rupture of the weakened small vessels in the GM due to the altered BM. Brain MRIs for individuals II-2, II-3, and III-6 were performed later in life, challenging the exact timing of the insult. However, the PMG demonstrated in individuals II-2 and II-3, taken together with the lack of gliosis, clearly points to an early insult, probably before 24 weeks of gestation [1, 40]. Although malformations of cortical development were associated with *COL4A1* and *COL4A2* in both animal models and human [18, 20, 28, 41–43], isolated PMG not associated with schizencephaly, and heterotopias have never been associated with *COL4A2*. Although vascular injury may induce PMG [44, 45], the pathophysiology of migration anomalies in *COL4A* genes may not only be related to concrete vascular insult but can be explained by dysfunction of the glial-limiting membrane, disturbing the proper migration of neurons [31]. Of note, no other pathogenic variant associated with brain malformations was identified during the WES data annotation. Indeed, it is currently considered that hydranencephaly, schizencephaly, porencephaly, and PMG represent a continuum of brain injury, depending upon the timing and the severity of the insult [1, 40]. Thus, the family describe herein represents similar pathology occurring at different timelines of brain development.

The pathogenicity of *COL4A1* and *COL4A2* is not completely understood and can be related to both the reduction in extracellular-to-intracellular ratio and accumulation of mutant proteins, depending upon the specific variant. These may induce an intracellular cytotoxic effect and induce apoptosis [33, 34]. Currently, there is no targeted treatment for *COL4A1/2*-vasculopathy. Better understanding of the natural history as well as genotype-phenotype correlations and genetic-environmental interactions may eventually emerge, thus facilitating appropriate surveillance protocols and the development of targeted novel therapies. Performing an elective cesarean section is not sufficient to prevent brain trauma as antenatal hemorrhage may occur, even weeks before the anticipated delivery [25, 28]. Although optimal antenatal and perinatal management of *COL4A*-related disease has not been delineated and requires further investigation in humans, preventive measures might prevent additional injury and may also impact disease expression for late-onset disease. For adult-onset CVD, careful surveillance and management of modifiable environmental risk factors, including hypertension, diabetes, avoidance of risky physical activities, constipation and prolonged labor and even nutritional supplements (e.g., vitamin E, fish oil) which may increase the risk of bleeding, and the use of anticoagulants, should be discussed in counseling [26].

We suggest considering genetic testing for *COL4A1* and *COL4A2* in cases for which there is radiologic evidence of fetal/perinatal venous hemorrhagic infarctions with

porencephaly, as well as when there is evidence of accompanying leukoencephalopathy, microhemorrhages or malformations of cortical development, particularly when associated with typical ocular or renal involvement. Since stroke is the most common cause of hemiparetic CP, patients with hemiparetic CP should undergo MRI with SWI sequence to detect remote bleeding, and genetic investigation should be considered in cases with the above-mentioned radiographic imaging patterns. In addition, testing for *COL4A1/COL4A2* should be considered for patients with multiple lacunar infarcts or intracranial aneurysms.

Conclusions

Our study demonstrates, for what we believe to be the first time, a documentation of the antenatal occurrence of fetal autosomal dominant *COL4A2*-related ICH and provides histopathological human evidence of isolated PMG and heterotopia in association with *COL4A2*. This report extends and complements earlier studies on the *COL4A2*-associated phenotype. Our findings highlight the diverse clinical presentation of *COL4A2* vasculopathy and emphasize the important role of genetic diagnosis in fetal ICH as well as in patients with hemiplegic CP, particularly those with a non-supporting perinatal history and characteristic MRI findings.

Acknowledgements We are grateful to the patients for consenting to participate in this study. Variantyx Genomic Intelligence® is thanked for their assistance in the interpretation of the genetic analysis. Esther Eshkol is thanked for editorial assistance.

Funding This study was funded by the Brain Center at Tel Aviv Sourasky Medical Center.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval All experiments were performed in accordance with relevant guidelines and regulations. The study protocols were reviewed and approved by the Institutional Review Board. Informed consent was obtained from all participants prior to genetic testing.

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