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SHORT RESEARCH ARTICLE

COL4A1 mutation-related disorder presenting as fetal intracranial bleeding, hydrocephalus, and polymicrogyria

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Abstract

Fetal intracranial hemorrhage represents a rare event with an estimated prevalence of 1:10 000 pregnancies. We report a patient diagnosed prenatally with intracranial hemorrhage and ventriculomegaly carrying a novel, previously unreported, likely pathogenic variant in *COL4A1*. At the gestational age of 27 weeks, dilation of lateral ventricles was detected during a routine prenatal ultrasound scan, confirmed by prenatal MRI at 30 + 3 weeks of gestation. Prenatal examinations included amniocentesis with conventional G-band karyotyping and arrayCGH, and maternal testing for TORCH and parvovirus B19 infections. Virtual gene panel based on whole-exome sequencing data was performed postnatally. At the age of 2.5 months, the patient manifested epileptic seizures that remain difficult to control. Postnatal MRI showed partial thalamic fusion and polymicrogyria, in addition to severe enlargement of lateral ventricles, multiple deposits of hemosiderin in cerebral and cerebellar hemispheres, and thin optic nerve and chiasma. Virtual gene panel based on whole-exome sequencing data led to a detection of a de novo previously unreported *in-frame* deletion

Jan Janota and Miroslava Balaščaková contributed equally to this study.

NM_001845.5:c.4688_4711del in *COL4A1* located in the highly conserved NC1 domain initiating collagen helix assembly. The presented case lies one a more severe end of the COL4A1 mutation-related disease spectrum, manifesting as fetal intracranial bleeding, malformation of cortical development, drug-resistant epilepsy, and developmental delay.

KEYWORDS

COL4A1, *COL4A1* mutation-related disorders, intracranial hemorrhage, malformation of cortical development, polymicrogyria

1 | INTRODUCTION

Fetal intracranial hemorrhage represents a rare event with an estimated prevalence of 1:10 000 pregnancies, associated with a significant risk for long-term neurological sequelae and fetal demise in the most severe cases.[1,2] Apart from various maternal and fetal factors, the majority of cases remain unexplained.[2] Currently known genetic factors predisposing to fetal intracranial hemorrhage involve mostly pathogenic variants in *COL4A1* and *COL4A2* genes.[2] The patients present clinically with epileptic seizures, motor deficits, and developmental delay, in addition to widespread organ involvement: renal, muscular, cardiac, and ophthalmological symptoms that altogether constitute the "*COL4A1* mutation-related disorders".[3] The most common MRI findings included periventricular leukoencephalopathy, porencephaly, cerebral calcifications, microbleeds and intracranial bleeding; rarer findings comprised focal cortical dysplasia (FCD), lissencephaly, and gyral abnormalities.[3]

Although genetic causes of the majority of malformations of cortical development (MCD) have been identified,[4,5] precise genotype–phenotype correlation and interpretation of novel genetic variants remains challenging. In addition to genetic causes, one needs to consider other possible etiologies in the diagnostic process,[4] such as maternal infection or ischemic episode. Polymicrogyria (PMG) is caused by various genetic and non-genetic factors that include ischemia, CMV infection, and pathogenic variants in *PIK3R2*, *CCND2*, *DYNC1H1*, and *WDR62*.[6] Polymicrogyria occurs frequently on the borders of porencephaly or hydranencephaly[7] or in association with hereditary hemorrhagic telangiectasia, probably as a result of localized intrauterine cerebral hypoxia.[8] However, to our knowledge, PMG represents a rather less common presentation of *COL4A1* mutation-related disease, often lining schizencephalic clefts.[9–11] It is of interest, however, that a pathogenic in-frame deletion in *COL4A2* was observed in a three-generational family with some members affected with PMG, intraventricular hemorrhage and white matter changes.[12]

We report a patient diagnosed prenatally with intracranial hemorrhage and dilation of lateral ventricles; postnatal MRI showed PMG, severe enlargement of lateral ventricles and multiple deposits of hemosiderin in cerebral and cerebellar hemispheres and partial thalamic fusion. At the age of 2.5 months, the patient manifested epileptic seizures that remain difficult to control. Virtual gene panel based on whole-exome sequencing data led to a detection of novel, previously unreported de novo *in-frame* deletion NM_001845.5:c.4688_4711del located in the highly conserved NC1 domain of COL4A1 protein that initiates collagen helix assembly.

2 | CASE PRESENTATION

The patient came to medical attention at the gestational age of 27 weeks when dilation of lateral ventricles was detected during a routine prenatal ultrasound scan. The finding was confirmed by prenatal MRI at 30 + 3 weeks of gestation (shown in Figure 1A–C) that showed grade III intraventricular hemorrhage (enlargement of lateral ventricles, resulting from subependymal hemorrhage into germinal matrix and choroid plexus bleeding); no other major anomalies were detected. Prenatal examinations included amniocentesis with conventional G-band karyotyping and arrayCGH that showed 46, XX and normal female profile, respectively. The mother was negative for active TORCH and parvo B19 virus infections. Family history was negative for neurological conditions, except for intracerebral hemorrhage in paternal grandmother (no additional information was available).

The patient was born via an elective Caesarian section at 38 + 0 weeks of gestation with a good postnatal adaptation—Apgar score in 1, 5 and 10 minutes was 9-9-10. Postnatal brain MRI confirmed the prenatal diagnosis of ventriculomegaly, and detected multiple hemosiderin depots, corpus callosum atrophy and partial thalamic fusion raising the suspicion of lobar holoprosencephaly. Visual evoked potentials failed to elicit response to flash

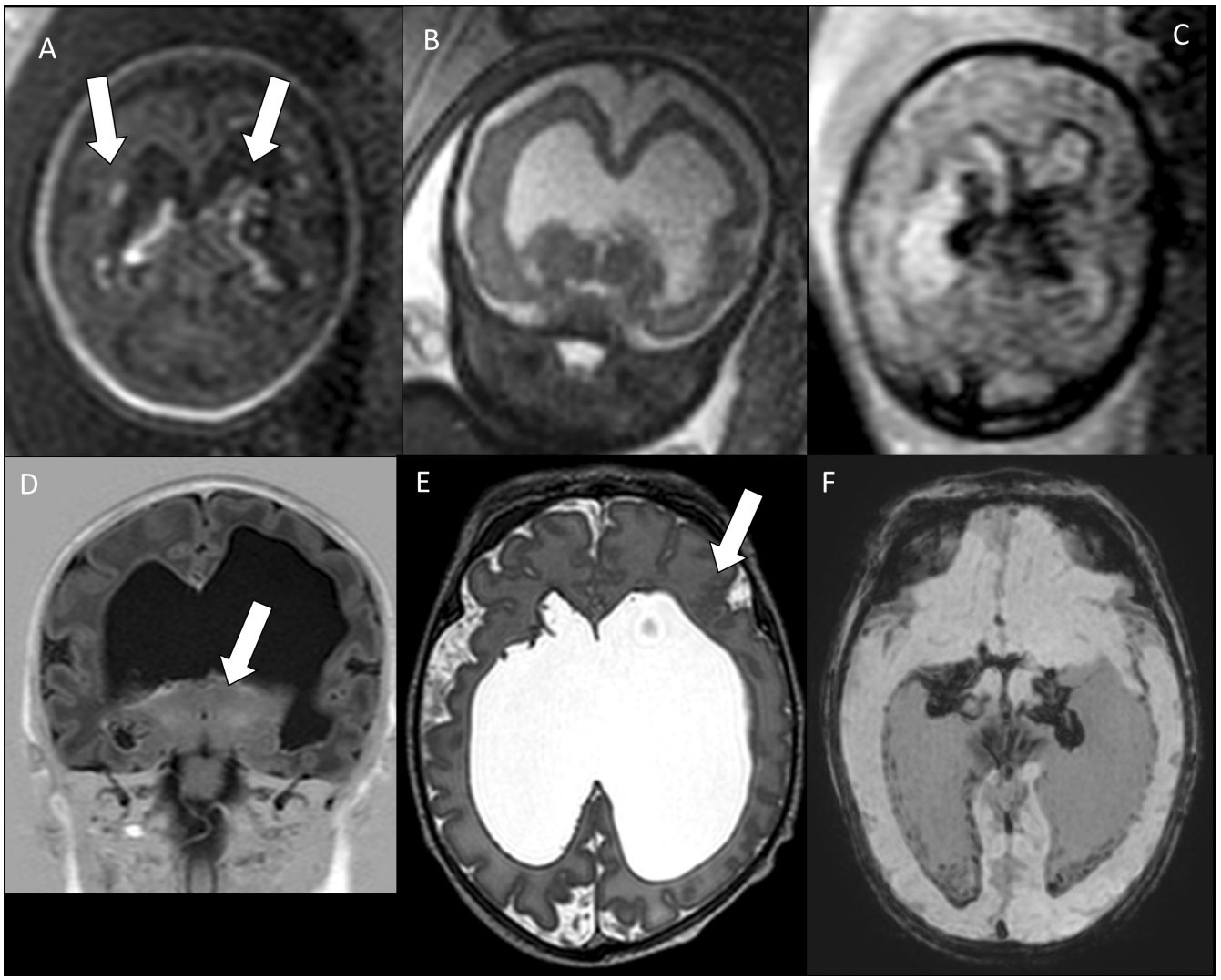

FIGURE 1 30 + 3-wk-old female fetus with intracranial in utero hemorrhage, fetal MRI (A-C). Preterm infant girl aged 3 d (same child as in fetal imaging), postnatal MRI (D-F). Axial T1 sequence on the brain shows hyperintense periventricular and choroidal plexus hemorrhage (A, arrows). Coronal T2-weighted sequence demonstrate lateral ventricles dilatation and septum pellucidum fenestration (B). Axial echoplanar sequence demonstrates hemorrhage with increased susceptibility artifacts (C). IR T1-weighted coronal scan shows progressive hydrocephalus, partial thalamic fusion (arrow) and septum pellucidum fenestration (D). Axial T2-weighted sequence with focal polymicrogyria (arrow) and septum pellucidum fenestration (E). Axial susceptibility weighted sequence demonstrates hypointense ventricular and choroidal plexus hemorrhage (F).

visual stimuli; otoacoustic emissions were elicited bilaterally. Ophthalmological examination showed bilateral optic nerve hypoplasia that prompted endocrinological examination to exclude septo-optic dysplasia; hormonal production was normal. She had no facial dysmorphism and other congenital anomalies.

At the age of 2.5 months, the patient started experiencing episodes of rapid eye blinking and vertical nystagmus of epileptic origin. Electroencephalography showed abnormal activity of unusually high amplitude with slow background activity, along with high-voltage epileptiform discharges; the patient was started on phenobarbital treatment. VideoEEG monitoring confirmed previous findings, in addition to epileptiform discharges and spike–wave complexes over both fronto-centro-temporal regions and left fronto-temporal area; seizures were classified as focal motor with undetermined awareness. The epileptiform activity was more prominent over the left hemisphere, broadly corresponding to the area with maximum structural abnormalities, including PMG. Repeated brain MRI detected progression of hydrocephalus, and consequently, the patient received a ventriculo-peritoneal shunt at the age of 4 months. Her seizures persisted and increased in frequency; therefore, the patient was transferred to the department of pediatric neurology for intravenous treatment with phenobarbital and midazolam. At the same time, her brain MRI underwent an expert re-evaluation by a pediatric neuroradiologist who described, in addition to previous

findings, PMG, most prominent in the left ventral sylvian area and frontal–parietal regions, and thinning of the optic nerve and optic chiasma (shown in Figure 1D–F). The initial suspicion of holoprosencephaly and septo-optic dysplasia was not confirmed. The patient was discharged on the combination of three antiseizure medications (ASM) (phenobarbital, vigabatrin, and topiramate), experiencing brief focal motor seizures and delayed developmental milestones. At the age of one year, the patient's mother did not report any seizures; however, her EEG remained severely abnormal with epileptiform activity and diminished background activity; the patient is severely developmentally delayed with a visual impairment (does not follow objects, little spontaneous activity, lying on her back, does not turn to belly, muscle hypotonia).

Based on the reviewed MRI diagnosis, we proceeded to perform a whole-exome sequencing-based virtual panel examination of 366 genes associated with MCD. Gene variants were filtered based on their frequency in population databases (below 1% in gnomAD) and compared with available databases (ClinVar, human gene mutation databse—HGMD). We detected a novel, previously unreported heterozygous de novo variant NM_001845.5:c.4688_4711del NP_001836.2:p.(Gln1563_Cys1570del); its presence was confirmed by Sanger sequencing, and segregation analysis (also by Sanger sequencing) showed the variant was absent in either parent. We classify it as likely pathogenic, based on the guidelines of American College of Medical Genetics (ACMG): the variant is located in a highly conserved NC1 domain (shown in Figure 2) of the *COL4A1* gene (PM1 criterion), it is absent from controls in gnomAD (PM2 criterion) and results in protein length change (PM4 criterion).[13]

COL4A1 mutation-related disorder is associated with multiple systemic features[3]; in our patient, we observed no typical ophthalmological vascular changes, for example, retinal arterial tortuosity; however, the patient had optic nerve atrophy. Abdominal ultrasound was not performed; however, prenatal MRI showed no gross structural anomalies of intra-abdominal and/or intra-thoracic organs. No skin lesions were observed and echocardiography performed in the first days of life was normal with a small foramen ovale. No gross hematuria was detected; microscopic hematuria was not tested. Hemolytic anemia was not diagnosed either. In the process of future follow-up, the patient warrants regular MRI investigations, full blood count and biochemical urine testing for microscopic hematuria, abdominal ultrasound, echocardiography and ophthalmological examination, in addition to developmental assessment.

3 | DISCUSSION AND CONCLUSION

We report a case of a patient who showed an early and severe presentation of *COL4A1* mutation-related

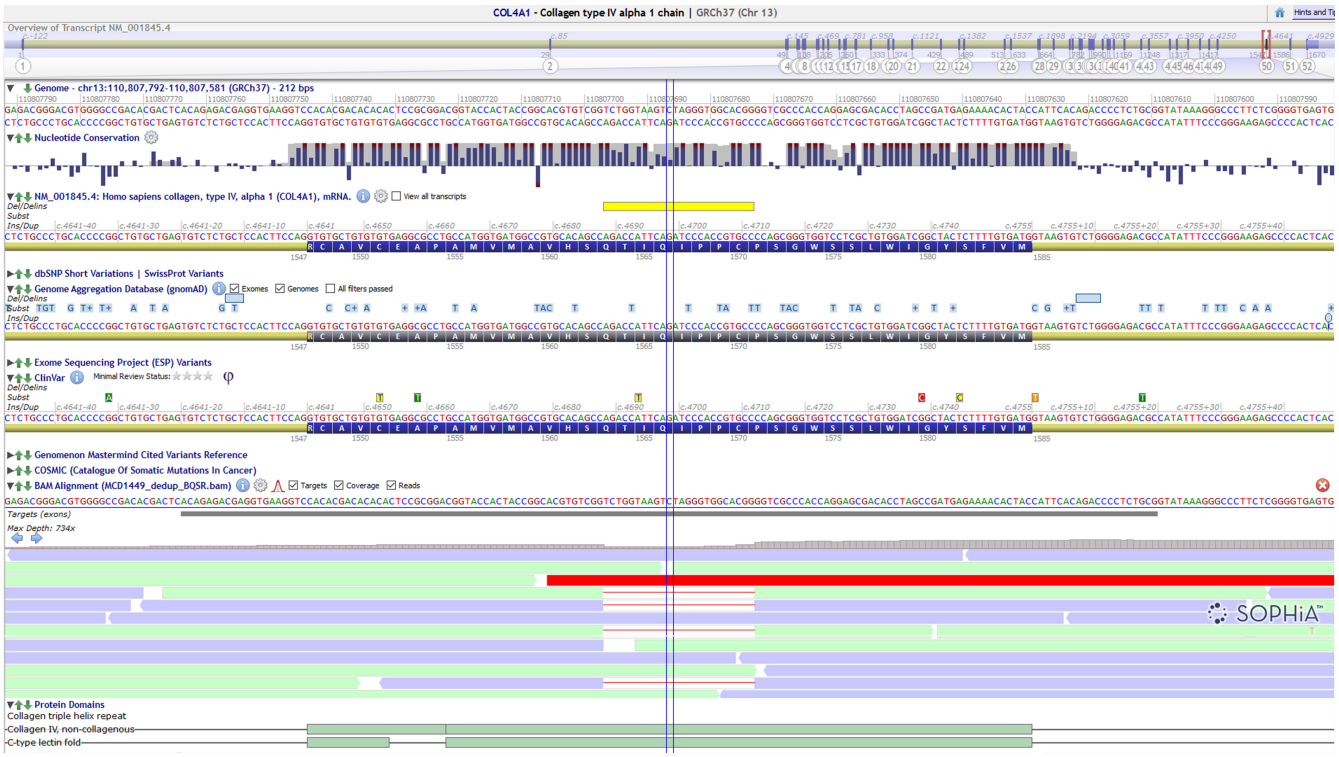

FIGURE 2 Visualization of NM_001845.5:c.4688_4711del variant in the Alamut visual software (SophiaGenetics, version 2.15)

disorder; she presented at the 27 weeks of gestation with ventriculomegaly, and later with in utero intracranial hemorrhage, hydrocephalus, MCD, drug-resistant epilepsy, developmental delay, and optic atrophy. By means of whole-exome sequencing-based virtual gene panel analysis, we discovered a probably pathogenic variant in *COL4A1*; no other putative causal variants were detected.

In our center, we routinely apply virtual gene panel (VP) testing based on exome sequencing (ES) data, and we achieved similar diagnostic yield in patients tested with VP and ES[14]; previous multi- and single-center studies performed genetic testing by targeted gene panel sequencing or ES.[3,10,12]

COL4A1 and *COL4A2* encode for type IV collagens that are important basement membrane proteins, and they form heterotrimers whose assembly is initiated at the C-terminal noncollagenous domains.[15] Three potential pathogenic mechanisms have been hypothesized; (a) cytotoxic effect of mutant heterotrimers, (b) extracellular deficiency of normal heterotrimers and (c) dominant-negative effect of extracellular mutant proteins.[16] Experiments on mice models support the dominant-negative effect of mutant heterotrimers.[15] Further in silico and functional studies could prove whether the *in-frame* deletion located in the NC1 domain, reported here, might lead to the formation of aberrant protein possibly disrupting heterotrimer assembly and also ascertain the relationship between mutation type and phenotypic severity as we still lack clear genotype–phenotype correlations in *COL4A1* mutation-related disease.[11]

Although initially certain MRI features prompted diagnostic suspicion for holoprosencephaly spectrum, later review of brain MRI led us to initiate genetic testing of genes associated with MCD. In some patients with complicated pre- and perinatal history, their MCD may be incorrectly diagnosed as a direct consequence of pre/perinatal insult, and genetic testing may not even be recommended. This case report highlights the importance of early diagnostic genetic evaluation even in patients with seemingly "non-genetic" cause of the MCD and especially in cases of otherwise unexplained fetal intracerebral bleeding.

This case represents one of the most severe prenatal and neonatal manifestations of *COL4A1* mutation-related disease, and describes a novel, previously unreported, likely pathogenic *COL4A1* variant.

AUTHOR CONTRIBUTIONS

Barbora Straka, Pavel Kršek, and Miroslava Balaščaková were involved in conceptualization, investigation, data curation, and writing. Markéta Vlčková, Zuzana Libá, Barbora Heřmanovská, Martin Kynčl, Jan Janota, and Jana Dorňáková were involved in investigation, data curation, and writing. Alena Musilová was involved in genomic data analysis and curation.

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CONFLICT OF INTEREST

None declared. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this study is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The research data are not publicly available on legal and ethical grounds (patient-identifying data); specific data are available on reasonable request from the investigators.

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