



Original article

Expanding the phenotype of *COL4A1*-related disorders—Four novel variants

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Received 1 April 2020; received in revised form 27 May 2020; accepted 28 May 2020

Abstract

Objective: *COL4A1* variant causes severe central nervous system (CNS) anomalies, including hydranencephaly. However, the pathogenic mechanism underlying the *COL4A1* phenotype remains unclear. Here, we report *de novo* *COL4A1* variants in four Japanese patients with typical or rare CNS involvement and exhibiting diverse phenotypes.

Methods: We identified and enrolled four patients with white matter abnormalities and cerebral structural defects suggestive of cerebrovascular disease. Genetic analysis was performed using panel sequencing.

Results: All the patients were perinatally asymptomatic during the infantile period but exhibited developmental delay and growth retardation later. All the patients exhibited CNS symptoms, including psychomotor disability, spastic paralysis, and epilepsy. Brain magnetic resonance imaging revealed hydranencephaly (n = 1), ventriculomegaly (n = 4) associated with cerebral hemorrhage, and atretic encephalocele (n = 1). Three patients had developed congenital cataract, while two had hematuria. We identified two *COL4A1* missense variants [exon32:c.2555G > A p.(Gly852Asp), exon40:c.3407G > A p.(Gly1136Asp)] and two in frame variants [exon32:c.2603_2609delinsATCCTGA p.(Ala868_Gly870delinsAspProGlu), exon36:c.3054delinsTG TAGAT p.(Leu1018delinsPheValAsp)]. The in frame variants were associated with severe CNS anomalies, hydranencephaly, and severe ventriculomegaly. Atretic encephalocele has never been reported in individuals with *COL4A1* variants.

Conclusions: Our findings suggest that *COL4A1* variants cause variable CNS symptoms. Association between clinical phenotypes and each *COL4A1* variant would clarify their underlying etiologies.

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Keywords: *COL4A1*; Hydranencephaly; Atretic encephalocele

1. Introduction

COL4A1-related disorders, including cerebrovascular disease, ophthalmic abnormalities, renal impairment, and muscle abnormalities, are complicated multisystem

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<https://doi.org/10.1016/j.braindev.2020.05.009>

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disorders caused by *COL4A1* variants [1]. The *COL4A1* polypeptide chain comprises an N-terminal 7S domain, a triple-helical domain containing the conserved repeat Gly–Xaa–Yaa, and a C-terminal noncollagenous (NC1) domain. *COL4A1* variants, mainly those of Gly in the Gly–Xaa–Yaa repeat, cause hydranencephaly and severe ventriculomegaly [2]. Hydranencephaly, which is a rarely diagnosed collagenopathy of the central nervous system (CNS), is characterized by absence of the cerebral hemispheres and their replacement with fluid-filled cysts in the brain [3]. Although several studies have reported *COL4A1* variants and their phenotypic expressions, the correlation between the genotypic abnormalities and their corresponding phenotypic expression in case of *COL4A1*-related disorders remains unclear [2,4]. Hence, to elucidate the underlying pathogenic mechanism and clinical prognosis of *COL4A1*-related disorders, further assessment of their clinical characteristics and genotypes is required by including patients with variable neurological features. Here, we report *de novo COL4A1* variants in four Japanese patients with typical or rare CNS involvements, representing the phenotypic diversity of *COL4A1*-related disorders.

2. Materials and methods

We analyzed four patients with white matter abnormalities and cerebral structural defects including hydranencephaly and ventriculomegaly suggestive of cerebrovascular disease. Clinical and neurological assessment was performed by child neurologists. After genetic counseling, genomic DNA was extracted from peripheral blood samples of the patients and family members. The study design was approved by the Kanagawa Children's Medical Center Review Board and Ethics Committee. Informed consent was obtained from the parents of each patient. DNA libraries were enriched for sequences using the TruSight One sequencing panel kit (Illumina, Inc., San Diego, CA, USA) and sequenced using the MiSeq platform (Illumina, Inc.) with 150-bp pair-end reads. Exome data alignment, variant calling, and variant annotation were performed as described previously [5]. Calling copy number variation was determined according to the log-ratio analysis and read depth (*z*-score) of each exon. Variants identified by targeted sequencing were confirmed by Sanger sequencing.

3. Results

We diagnosed four patients with novel variants of *COL4A1* that corresponded with their cerebral structural defects (Table 1).

Patient 1 who had no detectable perinatal abnormality was born at 39 weeks of gestation uneventfully with a birth weight of 2524 g [−1.3 standard deviation (SD)],

length of 48.0 cm (−0.4 SD), and occipitofrontal circumference (OFC) of 31.0 cm (−1.6 SD). He had experienced spastic paralysis and tonic seizure at 6 months of age. At 8 months of age, he visited our hospital for the treatment of developmental delay due to disability of rolling over and sitting unaided. Proportional growth failure was noted with a weight of −2.3 SD, length of −3.0 SD, and OFC of −2.8 SD. Brain magnetic resonance imaging (MRI) showed asymmetrical ventriculomegaly with multiple periventricular parenchymal destruction suggestive of previous hemorrhage or infarctions (Fig. 1-A, B). At 18 months of age, he could not walk unaided and speak meaningful words. Genetic analysis revealed a *de novo COL4A1* variant, c.3407G > A p.(Gly1136Asp) in the Gly–Xaa–Yaa repeat of exon 40 (Fig. 2-A).

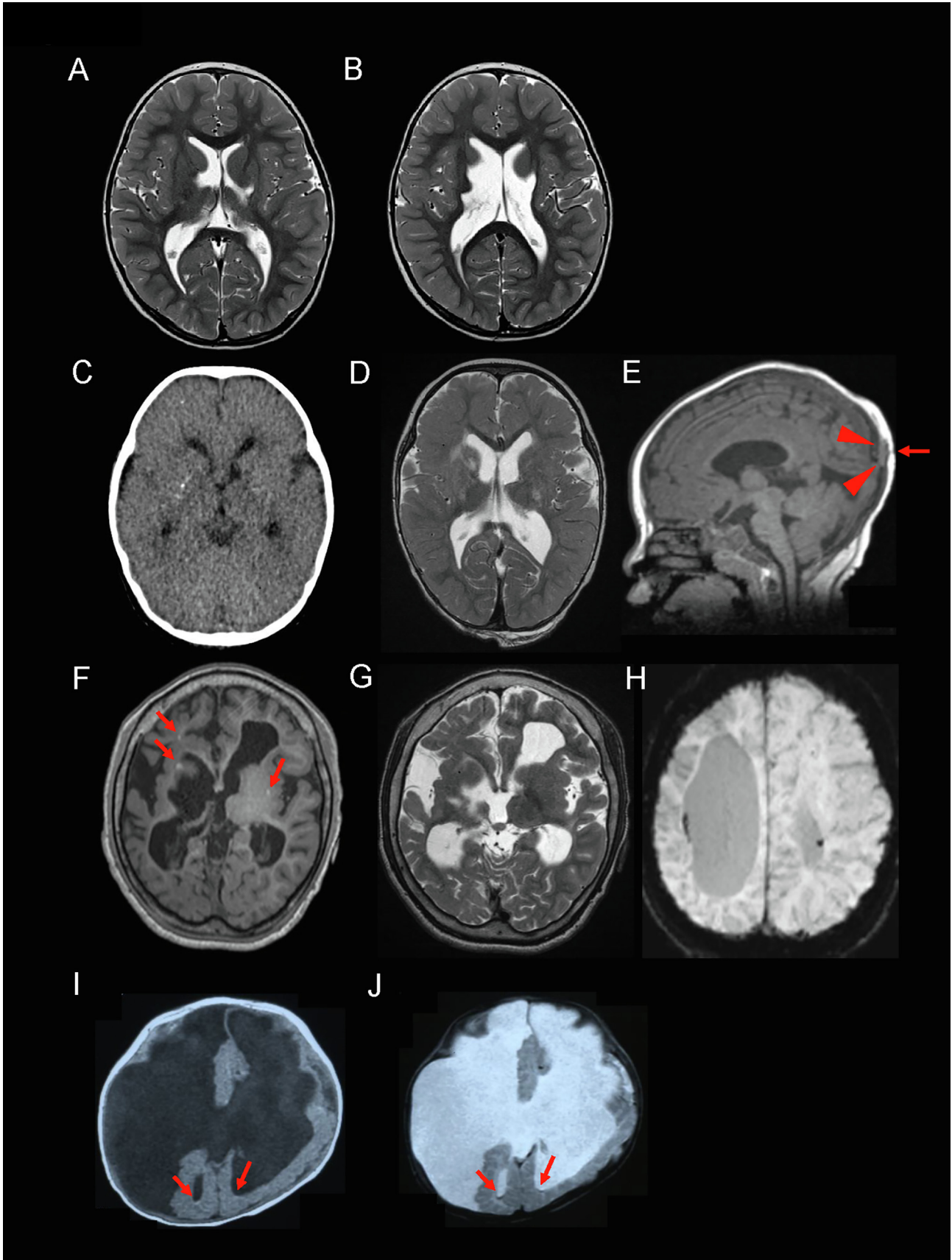
Patient 2 had perinatal intrauterine growth restriction (IUGR). He was born at 37 weeks of gestation with a birth weight of 2132 g (−1.8 SD), length of 45.0 cm (−1.0 SD), and OFC of 30.4 cm (−1.5 SD). After birth, he was transferred to our hospital for a subcutaneous mass. Computed tomography (CT) showed punctate calcifications of the bilateral pallidum and right frontal lobe (Fig. 1-C). Brain MRI showed mild ventriculomegaly with mild white matter volume loss, T2-prolonged area in the deep gray matter (Fig. 1-D), and an atretic encephalocele for a subcutaneous mass (Fig. 1-E). He attained head control and rolling over at 3 and 7 months of age, respectively. He experienced spastic paralysis and growth retardation, and microcephaly (weight −2.1 SD, length −1.6 SD, and OFC −2.3 SD at 12 months of age). At 18 months of age, he could not sit unaided and speak meaningful words. Further examination showed congenital cataract and hematuria. Genetic analysis revealed a *de novo COL4A1* variant, c.2555G > A p.(Gly852Asp) in the Gly–Xaa–Yaa repeat of exon 32 (Fig. 2-B).

Patient 3, who had no detectable perinatal abnormality, was born at 37 weeks of gestation uneventfully with a birth weight of 2588 g (−0.5 SD), length of 47.5 cm (+0.1 SD), and OFC of 32.0 cm (−0.4 SD). He was admitted at 8 months of age because of developmental delay due to disability of rolling over and sitting unaided. Brain MRI showed severe asymmetrical ventriculomegaly with multiple periventricular parenchymal destruction, some high signal spots in the periventricular region and left basal ganglia consistent with calcifications, and white matter volume loss (Fig. 1-F, G). Susceptibility-weighted imaging (SWI) showed signal loss along with periventricular white matter (Fig. 1-H). He developed epilepsy when he was 1 year old. At 17 years of age, he could not walk unaided and speak meaningful words. Further examination showed congenital cataract. Genetic analysis revealed a *de novo COL4A1* variant, c.3054delinsTG TAGAT p.(Leu1018delinsPheValAsp) in exon 36 (Fig. 2-C).

Table 1
Synopsis of clinical features, *COL4A1* variants, and radiological investigation.

	Patient 1	Patient 2	Patient 3	Patient 4
Age at exome (years)/ Sex	1y/M	1y/M	17y/M	16y/M
exon	exon40	exon32	exon36	exon32
c-Notation	c.3407G > A	c.2555G > A	c.3054delinsTGTAGAT	c.2603_2609delinsATCCTGA
p-Notation	p.(Gly1136Asp)	p.(Gly852Asp)	p.(Leu1018delinsPheValAsp)	p.(Ala868_Gly870delinsAspProGlu)
<i>De novo</i>	+	+	+	+
IUGR	-	+	-	-
Psychomotor disability	+: moderate acquired rolling over and sitting unaided at 10 and 15 months, but showed absence of unaided walk at 18 months	+: moderate acquired head control and rolling over at 3 and 7 months, but showed absence of unaided walk at 18 months	+: severe never acquired the ability to walk	+: severe never acquired the ability to walk
Growth retardation	+	+	+	+
Microcephaly	+	+	+	+
Neurological symptom	Spastic paralysis Tonic seizure	Spastic paralysis	Spastic paralysis Myoclonic seizure	Spastic paralysis Myoclonic seizure
Cataract	-	+	+	+
Laboratory findings				
Creatine kinase elevation	-: 133 U/L (mean 59–248)	-: 94 U/L (mean 49–240)	-: 215 U/L (mean 49–240)	-: 147 U/L (mean 49–240)
Hematuria	-	+	-	+
Radiological investigations				
Ventriculomegaly	Asymmetrical ventriculomegaly with multiple periventricular parenchymal destruction	Mild ventriculomegaly	Severe asymmetrical ventriculomegaly with multiple periventricular parenchymal destruction	Hydranencephaly
White matter volume loss	+	+	+	+
Signal loss in SWI	NA	NA	+	NA
Other findings	-	Calcifications of the bilateral pallidum and right frontal lobe Atretic encephalocele	Calcifications of the left basal ganglia	-

NA: not available. SWI: Susceptibility-weighted imaging.



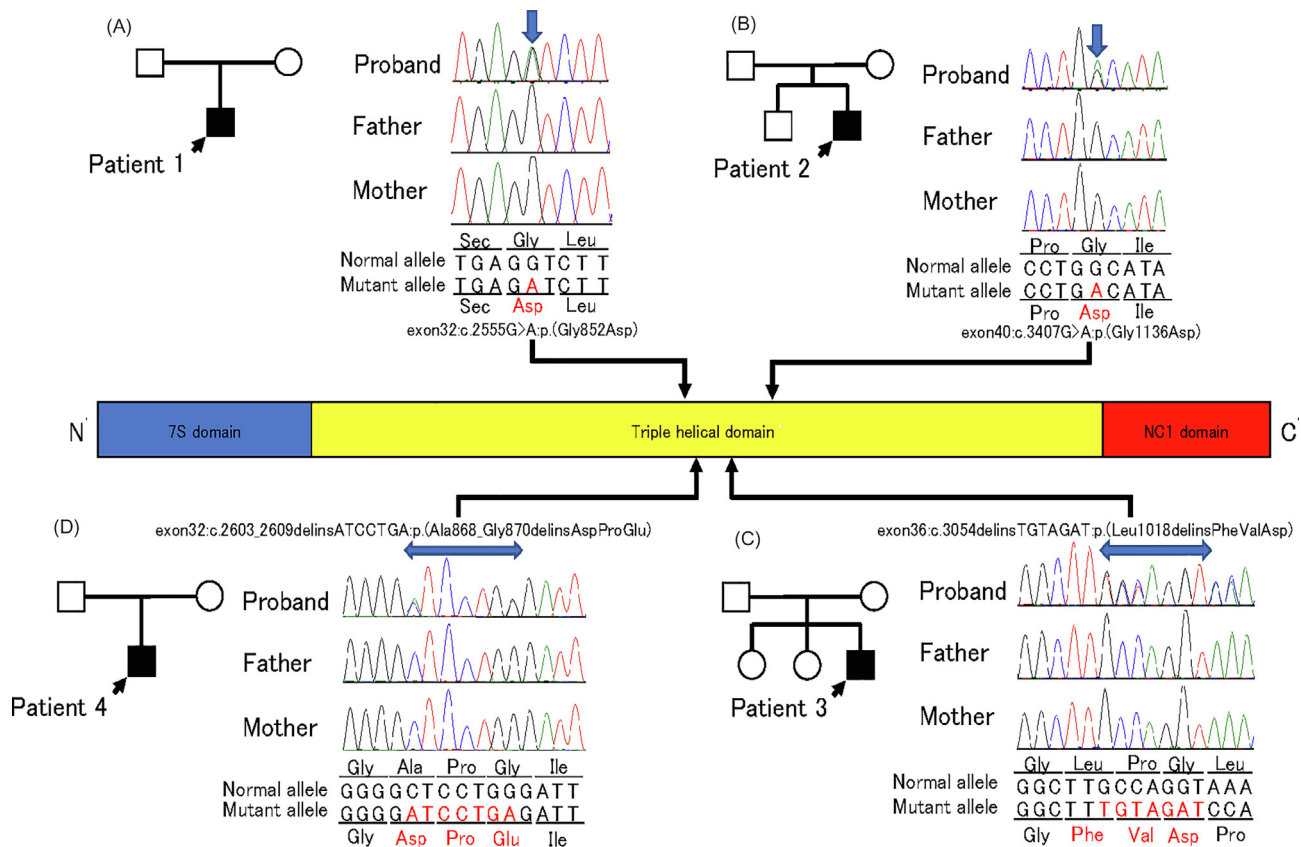


Fig. 2. Nucleotide sequence analysis of patients, their family pedigree, and the *COL4A1* domain. Black boxes indicate probands.

Patient 4 who had no detectable perinatal abnormality was born at 40 weeks of gestation uneventfully with a birth weight of 2,585 g (−1.5 SD), length of 47.0 cm (−1.3 SD), and OFC of 34.3 cm (+0.8 SD). He was admitted at 2 months of age because of microcephaly (<−2.0 SD). Brain MRI showed hydranencephaly with signals suggestive of previous intraventricular hemorrhage (Fig. 1-I, J) and a ventriculoperitoneal shunt was placed because of papilledema suggestive of increased intracranial pressure. He developed myoclonic seizures at 10 months of age. At 17 years of age, he could not walk and speak meaningful words. Further examination showed congenital cataract and hematuria.

Genetic analysis revealed a *de novo COL4A1* variant, c.2603_2609delinsATCCTGA p.(Ala868_Gly870delinsAspProGlu) in exon 32 (Fig. 2-D).

All the variants reported in our study were not found in the large population databases [The Genome Aggregation Database (<https://gnomad.broadinstitute.org/>)] and are novel variants. In silico assessment by Provean [Protein Variation Effect Analyzer (provean.jcvi.org)] prediction suggests that all these variants would be deleterious and SIFT [Sorting Tolerant From Intolerant (<http://sift.jcvi.org/>)] prediction suggests that exon32:c.2555G > A p.(Gly852Asp) (patient 1) and exon40:c.3407G > A p.(Gly1136Asp) (patient 2) would be

Fig. 1. Brain images of Patients 1 to 4. A, B: Patient 1; Axial T2-weighted images showing asymmetrical ventriculomegaly with multiple periventricular parenchymal destruction. C–E: Patient 2; C: CT image showing punctate calcifications of the bilateral pallidum and right frontal lobe. D: Axial T2-weighted image reveals irregular high signal areas in the bilateral deep gray matter, mild ventriculomegaly, and mild white matter volume loss. E: Sagittal T1-weighted image showing atretic encephalocele (arrowheads). A small bone defect is present beneath the subcutaneous mass (arrow). F–H: Patient 3; F, G: Axial T1 and T2-weighted images showing severe asymmetrical ventriculomegaly with some periventricular parenchymal destruction. Some high signal spots in the periventricular area and left lenticular nucleus compatible with calcifications (arrows) are seen on T1-weighted image. H: Susceptibility-weighted imaging showing loss signals along the dilated lateral ventricles. I, J: Patient 4; Axial T1 and T2-weighted images showing widespread hemispheric parenchymal destruction and its nearly complete replacement by cerebrospinal fluid. Small high signals on T1-weighted image and low signals on T2-weighted image along the lateral ventricle (arrows) suggestive of old intraventricular hemorrhage are seen.

damaging. According to the American College of Medical Genetics and Genomics guidelines, all the variants reported in our study are likely to be pathogenic [6].

4. Discussion

We identified two *COL4A1* missense [Patient 1: exon32:c.2555G > A p.(Gly852Asp), Patient 2: exon40:c.3407G > A p.(Gly1136Asp)] and two in frame [Patient 3: exon36:c.3054delinsTGTAGAT p.(Leu1018delinsPheValAsp), Patient 4: exon32:c.2603_2609delinsATCCTGA p.(Ala868_Gly870delinsAspProGlu)] variants in our study. All the patients were admitted to the hospital because of developmental delay and growth retardation. The brain MRI findings of hydranencephaly and severe ventriculomegaly were suggestive of *COL4A1* variants in two patients (Patients 3 and 4). SWI is much more sensitive than T2*-weighted MRI for detecting cerebral microbleeds [7], and it showed signal loss along with periventricular white matter in Patient 3 in our study, further proving that SWI is an effective method for investigating a previous episode of intracranial hemorrhage caused by a *COL4A1* variant. Hence, we emphasize that SWI combined with MRI is useful for diagnosing cerebral microbleeds caused by *COL4A1* variants.

In this study, our patients showed rare clinical phenotypes, including an asymmetrical and mild ventriculomegaly, calcifications of basal ganglia, and an atretic encephalocele, for patient 1 and patient 2, respectively. *COL4A1* variants identified by performing a large cohort study have been associated with a variety of phenotypes including microcephaly, intellectual impairment, motor disability, epilepsy, porencephaly, ocular defects, increased serum creatine kinase level or muscle cramps, and kidney abnormalities in 45%, 81%, 55%, 86%, 49%, 38%, 14%, and 6% of the study populations, respectively [4]. Ventriculomegaly and calcifications of basal ganglia are observed in 2% and 11% of the patients, respectively [8]. Given the presence of eye features or a family history, a broad range of clinical features such as white matter change may require variant analysis of *COL4A1* [9]. However, an isolated occurrence of these complications could not warrant the clinical diagnosis of *COL4A1* variant.

The ventriculomegaly of Patient 1 and 2 was suggestive of previous hemorrhage or infarctions leading to cerebral palsy that occurred during the fetal stage. These results indicated that the *COL4A1* variants identified in our study may contribute to a differential diagnosis of cerebral palsy of unknown origin. To find patients with *COL4A1*-related disorders among those with cerebral palsy of unknown origin, a detailed investigation of extracranial complications is important.

The truncating variants in *COL4A1*-related disorders are associated with more severe CNS anomalies, porencephaly, severe hydrocephaly, severe developmental delay, and epilepsy [2,8]. Our rare phenotype *COL4A1*-related patients exhibited relatively milder CNS symptoms than typical patients. A *COL4A1* mutant mice study suggested that the neuromuscular phenotype was linked to *COL4A1* variant but the underlying molecular mechanisms differed between tissue types [10]. If we clarify the major question whether pathogenesis is caused by *COL4A1* deficiency or by the presence of mutant proteins in the basement membrane, such a finding can explain the association between CNS symptoms and each *COL4A1* variant.

We found an atretic encephalocele and *COL4A1* variant (Patient 2). Atretic encephalocele has never been described in patients with *COL4A1* variant. Encephalocele is characterized by congenital herniations of intracranial structures. Atretic encephalocele is a nonpedunculated nodular lesion involving the intracranial tissue of parietal or occipital areas. Migration and regression faults of the neural crest have been proposed to be the origin of atretic encephalocele [11]. *COL4A1* is associated with neurulation in which different cell adhesion molecules are involved [12]. Encountering a rare complication in a rare disease may not be a coincidence. It will be interesting to pursue this finding through retrospective analyses of *COL4A1* variants by using tissues of patients with this condition. These analyses would contribute to explain the complex neurodevelopmental mechanism in *COL4A1*-related disorders.

In conclusion, the discovery of these novel variants may help in better understanding the biochemical basis of the pathogenesis of *COL4A1* variants and their specific associations with distinct CNS symptoms. Molecular analysis using exome sequencing could expand the related phenotype of *COL4A1* variants. An important novelty of our study is represented by identification of both typical and rare phenotypes of *COL4A1*-related disorders that contribute to finding a pathogenic variant in molecular analysis. Further analysis is required to clarify the underlying mechanisms of the clinical spectrum of *COL4A1*-related disorders.

Acknowledgements

We appreciate the patients' family members for granting permission to publish this study. This research was supported by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan, JSPS KAKENHI 17K10069 (KK); Project support of Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences; The Initiative on Rare and Undiagnosed Diseases (IRUD) 18ek0109301 from Japan Agency for Medical Research and Development (AMED).

Conflict of interest disclosures

None.

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