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Novel *COL4A1* mutation in a fetus with early prenatal onset of schizencephaly

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Abstract

Porencephaly and schizencephaly are congenital brain disorders that can be caused by *COL4A1* mutations, though the underlying mechanism and developmental processes are poorly understood. Here, we report a patient with schizencephaly, detected by fetal ultrasonography and fetal magnetic resonance imaging, with a de novo novel mutation in *COL4A1* (c.2645_2646delinsAA, p.Gly882Glu). Our results suggest that the onset of damage that potentially results in schizencephaly occurs mid-pregnancy.

Schizencephaly is a congenital anomaly characterized by an abnormal gray matter-lined defect extending from the pial surface to the lateral ventricles. Porencephaly is a congenital brain disorder characterized by cavitation or a cerebrospinal fluid-filled cyst in the brain without gray matter lining. Both conditions are caused by an encephaloclastic process[1]. *COL4A1* mutations have been suggested to cause both schizencephaly and porencephaly, indicating a similar pathological mechanism for both disorders[2–4].

COL4A1 encodes the alpha 1 chain of type IV collagen, which is a basement membrane component. The basic unit of type 4 collagen is a heterotrimer formed by the α1-4 isoform. A α1α1α2 heterotrimer is universally expressed in basement membranes of all tissues. *COL4A1* is mainly expressed in the brain, muscles, kidney, and eyes. COL4A1 protein plays a key role in the inhibition of endothelial cell proliferation, migration, tube formation, and Matrigel neovascularization[5].

In addition to schizencephaly and porencephaly, *COL4A1* mutations also cause vascular membrane vulnerability, resulting in varied phenotypes associated with small vessel disease including retinal arterial tortuosity, Axenfeld-Rieger anomaly, cataracts, Raynaud phenomenon, diffuse periventricular leukoencephalopathy, and hereditary angiopathy with nephropathy, aneurysms, hemolytic anemia, and muscle cramps[3]. Furthermore, *Col4a1* homozygous mutant mice are lethal[2, 6], while heterozygote mutant mice show brain malformations, myopathy, ocular dysgenesis, and glaucoma[7, 8]. However, the mechanisms and developmental processes underlying the phenotypic variability of these disorders are poorly understood[3, 9].

Here, we report a Japanese patient with schizencephaly, determined by serial fetal ultrasonography and fetal magnetic resonance imaging (MRI),with a de novo novel mutation in *COL4A1*.

The patient is a male infant born at 39 weeks of gestation via vaginal delivery to nonconsanguineous healthy parents. His mother was 36 years old (gravida 1, para 1) and his father was 37 years old. The mother was hospitalized for 7 days due to hyperemesis during pregnancy at 9–10 weeks of gestation. There was no known exposure to any teratogens. Maternal infectious screenings were all negative. Ultrasonography at 26 weeks of gestation showed a biparietal diameter (BPD) of 61 mm

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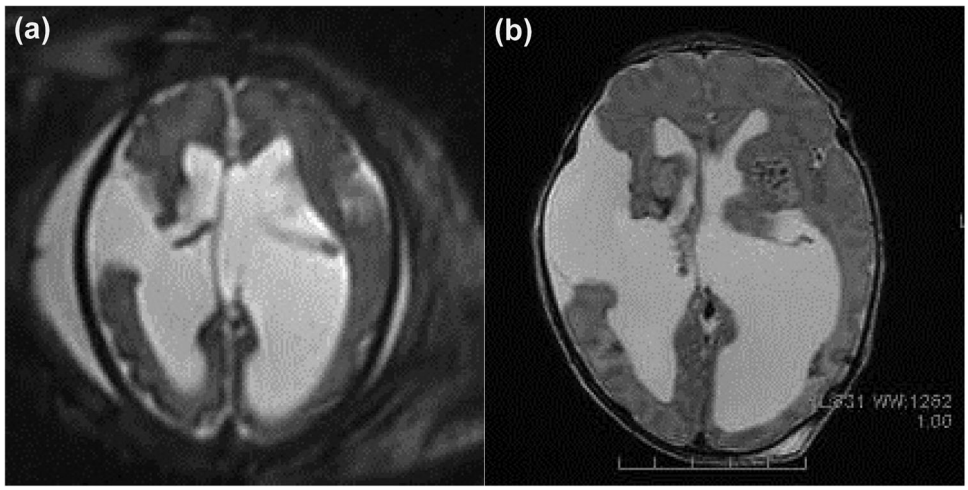

Fig. 1 a Fetal brain magnetic resonance imaging (MRI) at 32 weeks of gestation. An axial half-Fourier-acquired single-shot turbo spin echo (HASTE) image shows ventriculomegaly and a large cleft in the right cerebral hemisphere. **b** Brain MRI on day 1. An axial T2-weighted image shows a large right hemispheric cleft lined by abnormal gray matter with ventriculomegaly. This is characteristic of schizencephaly

(−1.2 SD) and a lateral ventricle width of 10 mm (normal < 10 mm, mild 10–11.9 mm, moderate 12–14.9 mm, and severe ≧ 15 mm). Both measurements were within the normal range at 18–26 weeks of gestation.

At 28 weeks of gestation, follow-up ultrasonography showed bilateral ventriculomegaly, with a BPD of 68 mm and a lateral ventricle width of 25 mm. A fetal brain MRI at 32 weeks of gestation showed bilateral ventriculomegaly and an enlarged right cerebral hemisphere cleft (Fig. 1a). The mother underwent an uneventful vaginal delivery. The patient had a birth weight of 2879 g (−0.85 SD), length of 51 cm (+1.0 SD), and head circumference of 33.4 cm (+0.10 SD). Ophthalmological examination at day 4 revealed bilateral microphthalmia and cataracts. A brain MRI on day 1 confirmed right open-lip schizencephaly with multiple low-intensity lesions and defects in the T2-weighted images, which suggested multiple recurrent bleeds and damage in the bilateral basal ganglia and ventricle wall (Fig. 1b). There were some signal loss lesions in the susceptibility-weighted imaging, which suggest hemosiderin deposition. The patient developed epilepsy on day 10 and does not have head control at 7 months of age. At the age of 6 months, an electroencephalogram showed high voltage slow with multiple spikes. He had treatment with levodopa for rigospasticity.

Genomic DNA from the patient was captured using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA). Captured DNA was sequenced on a MiSeq platform (Illumina) with 151-bp paired-end reads. The paired reads were then aligned to the human reference genome (UCSC hg19, NCBI build 37.1) using the Burrows–Wheeler Aligner (BWA; version 0.7.10) (http://bio-bwa.sourceforge.net/). PCR duplications were removed using Picard (version 1.118) (https://broadinstitute.github.io/picard/). The Genome Analysis Toolkit (GATK; version 3.2-2) (https://software.broadinstitute.org/gatk/) was used to call variants (single-nucleotide variants and small insertions and deletions). Finally, the called variants were annotated using ANNOVAR (22 March 2015) (http://annovar.openbioinformatics.org/en/latest/).

The average coverage depth of the entire panel was 58.51 reads, with 97.7% of the targeted bases covered at 10 × sequence reads. Targeted resequencing identified a novel heterozygous mutation in *COL4A1*, which is a known schizencephaly causing gene (NM_001845.5; c.2645_2646delinsAA, p.Gly882Glu). Sanger sequencing confirmed that this variant occurred as a de novo event (Fig. 2a).

To determine whether there is base substitution in the same allele, polymerase chain reaction (PCR) was performed for these two base substitutions (c.2645_2646delinsAA) using genomic DNA as the template and following primers: (1) forward, 5′-CTGTCAGCATTCAGAGCAAGA-3′ and (2) reverse, 5′-GCACCAGGAATCCTACTGGA-3′. The amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and cloned into the pCR4-TOPO vector (Thermo Fisher Scientific, Inc., Carlsbad, CA, USA). The cloned DNA sequence was confirmed by Sanger sequencing, with two base substitutions identified on the same allele (Fig. 2b).

In this study, we have identified a pathogenic *COL4A1* missense mutation in exon 33 in an infant with schizencephaly. Meuwissen et al. reported cases that had

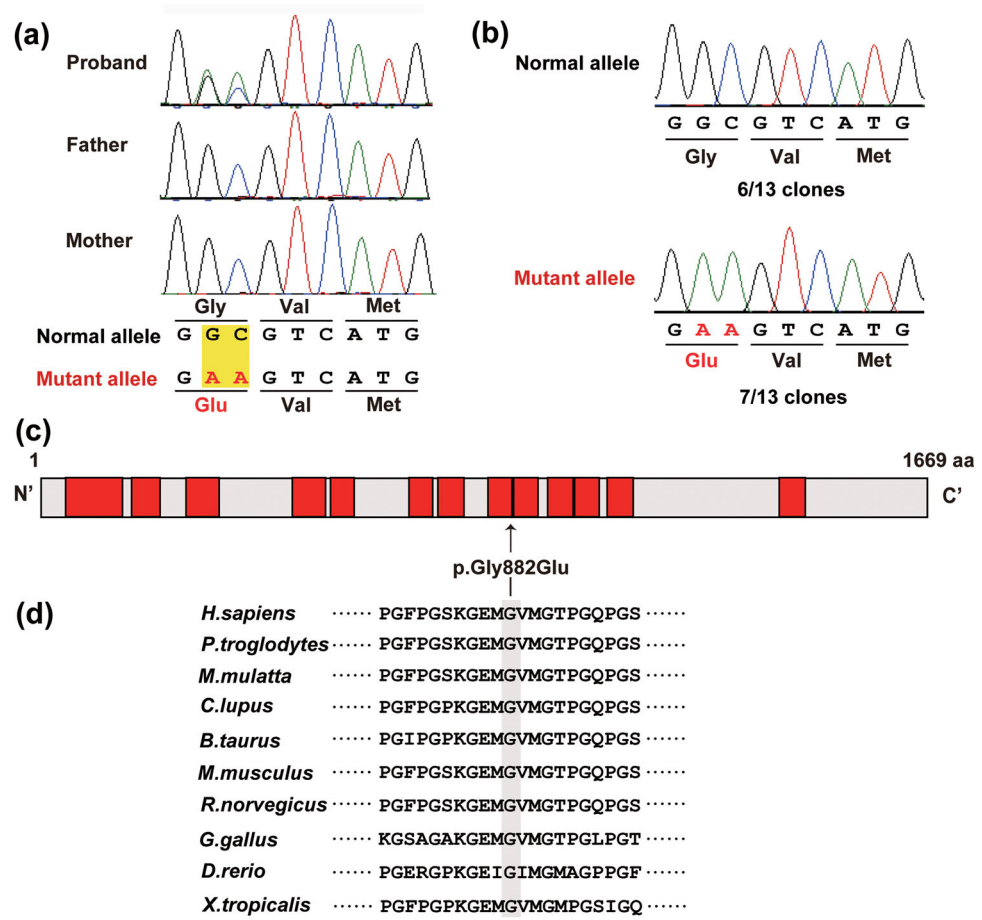

Fig. 2 a Sequence electropherogram for *COL4A1* in the patient and parents. A de novo event was identified in this study. **b** Sequence electropherogram. The amplified PCR product was cloned into the pCR4-TOPO vector and each clone was subjected to sequencing. The two base substitutions are indicated on the same allele. **c** A novel heterozygous mutation in *COL4A1* (NM_001845.5; c.2645_2646delinsAA, p.Gly882Glu) was identified in the patient. The COL4A1 protein contains 13 repeating (Gly-X-Y)*n* amino acid sequences, as registered in InterPro (https://www.ebi.ac.uk/interpro/). The amino acid sequences are highlighted with red boxes (amino acid positions 46–160, 172–221, 275–331, 474–532, 541–590, 690–735, 743–796, 839–895, 885–937, 948–1005, 999–1056, 1058–1116, and 1384–1440). **d** A mutation at an evolutionarily conserved amino acid in nine species. Amino acid position 882 in the COL4A1 protein is highlighted by a gray box

mutations in exons 31–37 in *COL4A1* and had extensive brain lesions resembling hydrocephaly[10]. They considered the pathogenesis to be extensive venous infarction and subsequent intracranial hemorrhage, as well as brain tissue destruction and thinning. The amino acid substitution (p.Gly882Glu) was localized in the collagen triple-helix domain, which consists of a repeating (Gly-X-Y)*n* amino acid sequence and is evolutionarily conserved among different species (Fig. 2c, d). Glycine residues within the repeating amino acid sequence are highly likely to be pathogenic, as has been reported previously[3, 11, 12]. Another mutation at the same amino acid position (p.Gly882Asp) was reported previously in two families (Case 1 and Case 2) with congenital cataracts and variable neurological findings[11]. Case 1 showed epilepsy, motor disorder, microcephaly, and developmental delay, while her mother only exhibited congenital cataracts. The same heterozygous variant was also observed in her mother. Case 2 showed white matter changes and spastic quadriplegia. His sibling with porencephaly and infantile hemiplegia had the same variant. Case 2 was from a family including siblings with porencephaly and migraine. Together with our patient, these results suggest that highly variable expressivity is observed at this recurrent position, causing a *COL4A1*-related syndrome phenotype that is expressed in patients as a partial or milder phenotype to a more severe porencephaly or schizencephaly phenotype. The genetic diagnosis of congenital malformations, including porencephaly, is crucial in genetic counseling for these disorders[13].

In a *col4a1* mutation mouse model, surgical delivery of pups significantly reduced risk of intracranial

hemorrhage[2]. The risk of intracranial hemorrhage during delivery needs to be considered for the fetus carrying a *COL4A1* pathogenic variant. In our patient, a brain MRI at day 1 showed multiple recurrent bleeds. These results indicated that a prenatal molecular diagnosis would have beneficial effects on patients with schizencephaly.

We detected enlargement of the lateral ventricles at 28 weeks of gestation, which was normal at 26 weeks of gestation. This gestational age of onset for lateral ventricle enlargement is consistent with a previously reported case[14]. The *COL4A1* mutation causes repetitive fetal cerebral hemorrhage, congenital schizencephaly and porencephaly. Destruction of the brain tissue by bleeding is likely to occur before 32–33 weeks[15]. However, the specific timing and duration of cerebral hemorrhage and ventricular enlargement have not been previously reported. The presentation of our current patient suggests that cerebral hemorrhage and ventricular enlargement in *COL4A1*-related schizencephaly occurs before the 28th week of gestation.

In conclusion, we have identified a case of *COL4A1*-related schizencephaly with bilateral ventriculomegaly by follow-up ultrasonography at 28 weeks of gestation. Our results suggest that the onset or timing of damage in schizencephaly or porencephaly occurs mid-pregnancy. Our report may be helpful for determining the mechanism and developmental processes associated with *COL4A1*-related diseases.

HGV Database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at https://doi.org/10.6084/m9.figshare.hgv.1939.

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Conflict of interest

The authors declare that they have no conflict of interest.

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