Brief Report

Novel *COL4A1* Mutation in an Infant with Severe Dysmorphic Syndrome with Schizencephaly, Periventricular Calcifications, and Cataract Resembling Congenital Infection

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Background: A clinical case is described of growth retardation, severe developmental delay, facial dysmorphic features with microcephaly, as well as congenital cataract, schizencephaly, periventricular calcifications, and epilepsy. **Methods:** TORCH infection was suspected, but all tests for toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus were negative for the child and her mother; however, an increased level of antibodies against parvovirus B19 was detected in the proband. **Results:** Chromosomal analysis and array-CGH showed no aberration. Target capture sequencing for *COL4A1* and *COL4A2* revealed a de novo *COL4A1* mutation (c.2123G>T [p.Gly708Val]). The mutation occurred at a highly conserved Gly residue in the Gly-X-Y repeat of the collagen triple helical domain, suggesting that these mutations may alter the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers. The mutation was predicted to be damaging. **Conclusion:** We suggest that *COL4A1* testing should be considered in patients with schizencephaly as well as with phenotype suggesting TORCH infection without any proven etiological factors.

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Key words: *COL4A1*; Schizencephaly; periventricular calcifications; cataract; developmental delay; congenital infection

Case Study

The female patient is the only child of healthy nonconsanguineous parents. She was born at 37 weeks of gestation by Caesarean section due to suspicion of asphyxia. There was no history of prenatal exposure to any recognizable teratogens or any mother's infection. Ultrasound examination at 12 weeks of gestation was normal, including nuchal translucency result. At 32 weeks of gestation, intrauterine growth restriction (IUGR) was pointed out. No fetal edema symptom was observed.

Her birth weight was low (2060 g, -2,1 SD), length was 49 cm, and occipitofrontal circumference was 20 cm. Apgar score was 8 at 1 min. She demonstrated microcephaly. An audiology exam was normal. Bilateral cataracts were noted. Neurological examination revealed abnormal muscle tone, exaggerated deep tendon reflexes, and poor movement. The placenta examination did not reveal any visible and histopathological abnormalities. Additionally, she was diagnosed with mild umbilical hernia.

TORCH testing was performed (toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, parvovirus B19), and a markedly elevated number of DNA copies for parvovirus B19 (122,450 copies/ml) was found. Complete blood cell counts performed several times were within the normal range. Moreover, tests for metabolic diseases (gas chromatography-mass spectrometry and tandem mass spectrometry) were negative. Abdomen ultrasonography examination detected no internal organ defects. Kidney function was within the normal range. Brain imaging studies (cranial ultrasound, computed tomography, magnetic resonance imaging) revealed many abnormalities: schizencephaly with two clefts (open-lip cleft between the right Sylvian fissure and the anterior horn of the right ventricle, and closed-lip cleft in the left frontal lobe) was a prominent feature (Fig. 1A–C); other abnormalities included hypoplasia of the corpus callosum, agenesis of septum pellucidum, ventriculomegaly, and multiple periventricular calcifications.

At the age of 4 months, she developed epilepsy. Her electroencephalography showed extensive foci of abnormal brain activity.

A clinical evaluation at the age of 4, 7, and 12 months revealed microcephaly with occipitofrontal circumference 5 cm below the 3rd centile, growth retardation, and severe developmental delay. Furthermore, the patient demonstrated many dysmorphic features, such as microcephaly, dysplastic right auricle, thick lips, broad and depressed

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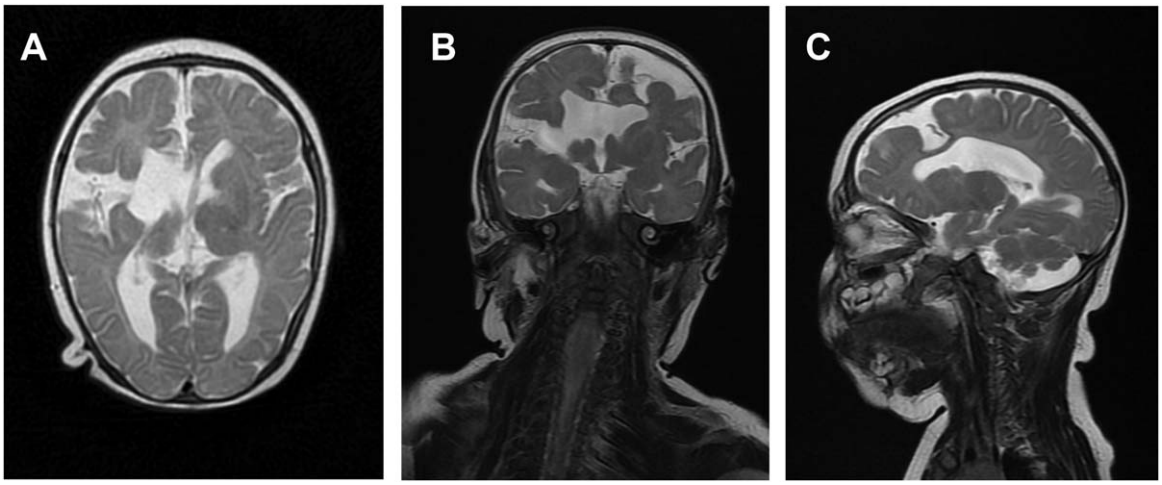

FIGURE 1. *MR study of the proband brain, T2-weighted axial (**A**), coronal (**B**), and sagittal (**C**) images. Schizencephaly with open-lip cleft between the Sylvian fissure and lateral ventricle is seen on the right (A,B), while on the left, there is schizencephaly with closed-lip cleft in the frontal lobe (B,C). There is also a visible absence of septum pellucidum (A) as well as enlargement of the ventricles (especially right lateral ventricle) and subarachnoid spaces.*

nasal root, and puffy feet as well as capillary malformation on the skin of occiput, anterior fontanel, and both eyelids (Fig. 2).

Genetic Investigation

The chromosomal analysis revealed a normal female karyotype (46,XX). No subtelomeric aberrations or microdeletion syndromes were found on multiplex ligation-dependent probe amplification testing (P070-A2 human telomere-5, P245-A2 microdeletion syndromes-1). Moreover, array-comparative genomic hybridization (array-CGH) was ordered and did not disclose any alterations. To screen for *COL4A1* and *COL4A2* mutations, we performed target capture sequencing for 50 epileptic genes including *COL4A1* and *COL4A2* as previously described (Kodera et al., 2013). *EMX2*, *NBN*, *SHH*, *TUBB2B*, which have been associated with schizencephaly, were not included in the target capture. In this analysis, 100% of coding sequences of *COL4A1* and *COL4A2* were covered by 20 reads or more in the patient, and a *COL4A1* mutation [c.2123G>T (p.Gly708Val]) was identified. The mutation occurred at a highly conserved Gly residue in the Gly-X-Y repeat of the collagen triple helical domain, suggesting that these mutations may alter the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers (Engel and Prockop, 1991; Khoshnoodi et al., 2008). The mutation was novel and was absent in Exome Variant Sever (http://evs.gs.washington.edu/EVS/) or ExAC database (http://exac.broadinstitute.org/). Web tools predicted the mutation to be damaging: SIFT (score 0.00; http://sift.jcvi.org/), PolyPhen2 (score 0.997; http://genetics.bwh.harvard.edu/pph2/), and MutationTaster (score 0.999; http://www.mutationtaster.org/). The mutation was absent in her parents, indicating that the mutation occurred de novo.

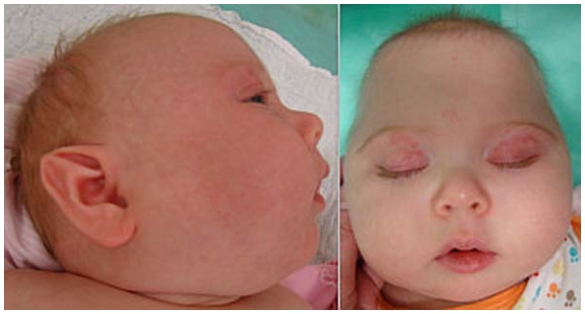

FIGURE 2. *The proband at the age of 4 months. Note microcephaly, dysplastic right auricle, thick lips, broad and depressed nasal root, and capillary malformation on the eyelids.*

Discussion

COL4A1 (OMIM*120130) encodes type IV collagen α-1 chain protein, which is expressed in all human tissues, especially in the vasculature. To date, many pathogenic *COL4A1* mutations have been identified in different disorders (Yoneda et al., 2013). A wide spectrum of clinical manifestations associated with *COL4A1* mutations may be caused by aberration of basement membranes of blood vessels. Blood vessels of the brain, eye, and kidney seem to be the mostly affected. Brain changes caused by both prenatal and postnatal vascular events are porencephaly, small vessels disease of a brain resulting in ischemic strokes or more often intracerebral hemorrhages, and susceptibility to intracranial hemorrhages (Livingston et al., 2011; Rødahl et al., 2013; Yoneda et al., 2013).

Leukoencephalopathy and calcification in a brain also have been described (Livingston et al., 2011; Rødahl et al., 2013).

Eye involvement consists of many developmental disorders targeting structures of the anterior segment of an eye, known as anterior segment dysgenesis including Axenfeld-Rieger anomaly (Livingston et al., 2011). Kidney disorders present as hematuria and bilateral cysts (Plaisier et al., 2007; Yoneda et al., 2013). In addition, multisystem phenotypes of *COL4A1* mutation are HANAC syndrome (hereditary angiopathy with nephropathy, aneurysm, and muscle cramps) and Walker-Warburg syndrome (Plaisier et al., 2007; Labelle-Dumais et al., 2011; Takenouchi et al., 2015). The latest investigation by Yoneda et al. expanded the clinical phenotypes of *COL4A1* mutations to include schizencephaly with hemolytic anemia (Yoneda et al., 2013).

Prenatal and postnatal phenotype with mild IUGR, microcephaly, congenital cataract, calcification in the brain, and developmental delay presented by our female patient was highly suggestive for TORCH infection. Negative results of array-CGH and other genetic tests seemed to confirm the diagnosis. Even though the TORCH testing disclosed elevated parvovirus B19 antibodies, the proband's clinical status was inconsistent with a typical phenotype of parvovirus B19 infection, especially with ophthalmological symptoms and neuroimaging signs. Parvovirus infection can cause fetal anemia, fetal hydrops, and fetal death as well as neonatal hepatitis, viral myocarditis, and cardiomegaly, which were not observed in our case (Lamont et al., 2011; De Jong et al., 2012). However, some IUGR cases caused by parvovirus B19 have been described (Brandenburg et al., 1996; Weiner and Naides, 1992).

What is more, there were noted neurologic findings in fetuses with parvovirus B19 infection, such as parenchymal calcifications, arterial and venous infarction, cerebellar hemorrhage, and cortical malformations (Courtier et al., 2012). Vertical transmission of parvovirus to the fetus occurs in approximately 30% of infected pregnant women, although most neonates are born healthy. Children having successfully survived intrauterine transfusion for parvovirus B19-induced fetal anemia and hydrops fetalis usually have a good neurodevelopmental prognosis. Nevertheless, neurodevelopmental delay in hydropic fetuses that required intrauterine red blood cell transfusion is observed in 30% of affected children (Lamont et al., 2011; De Jong et al., 2012). Moreover, the rate of congenital anomalies associated with parvovirus B19 infection is not higher than in the normal population (Lamont et al., 2011). Taking together the above information and the clinical history of our patient, it could not be excluded that our proband presented overlapping symptoms of COL4A1-related disease and parvovirus infection.

In further diagnostic analyses, the schizencephaly detected in the magnetic resonance examination was established as a primary feature. Target capture sequencing for *COL4A1* and *COL4A2* revealed a novel, pathogenic de novo c.2123G>T (p.Gly708Val) mutation in *COL4A1*. Many features presented by our patient, including brain anomalies with neurological symptoms, eye disorders, and intellectual disability, were separately described by other authors (Livingston et al., 2011; Rødahl et al., 2013; Yoneda et al., 2013). Of interest, Deml et al. described a patient with ophthalmological defects (congenital cataract, bilateral microcornea, Peters anomaly, unilateral microphthalmia, and unilateral retinal detachment) in which a different mutation (c.2122G>A) in the *COL4A1* gene was diagnosed but resulted in the same amino acid change (Gly708Arg) (Deml et al., 2014).

To date, there were no reports of prenatal diagnosis of IUGR and postnatal growth retardation with severe developmental delay associated with a *COL4A1* mutation. Additionally, we predict the study of future cases of schizencephaly related to a *COL4A1* mutation may help us to better understand neuronal migration disorder (Yoneda et al., 2013).

In conclusion, we report a phenotype resembling TORCH infection caused by a novel mutation in *COL4A1*. *COL4A1* testing is warranted in patients with schizencephaly as well as suspected TORCH infection without any proven etiological factors.

Acknowledgments

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