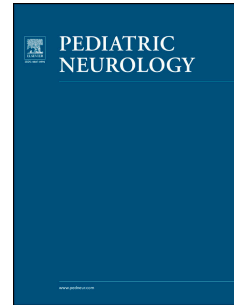


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COL4A1 Mutation in a Neonate with Intrauterine Stroke and Anterior Segment DysgenesisShaheen Durrani-Kolarik^c, Kandamurugu Manickam^{b,c,d}, Bernadette Chen^{a,c,d}^aCenter for Perinatal Research, The Research Institute at Nationwide Children's Hospital,
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43240 USA^cNationwide Children's Hospital, Columbus, OH 43205 USA^dDepartment of Pediatrics, The Ohio State University, Columbus, OH 43210 USARunning head: *COL4A1* mutation in neonate

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Abstract

COL4A1, located on chromosome 13q34, encodes the alpha 1 chain of type IV collagen that is a component of basal membranes. It is expressed mainly in the brain, muscles, kidneys and eyes. *COL4A1* mutations can vary in presentation, from asymptomatic carriers to severe devastating disease secondary to the effects on small vessels, including porencephaly, intracerebral hemorrhage, infantile hemiparesis in neonates and children, intracerebral hemorrhage, intracranial aneurysms and retinal arteriolar tortuosities in adults. Given its limited description in the medical literature, diagnosis of this mutation can be overlooked. This is important, as identification of this mutation in affected individuals has implications for perinatal management and genetic counseling with availability of prenatal testing to determine inheritance in additional family members. In addition, making this diagnosis may help tailor appropriate screening tests for organs typically involved with *COL4A1* mutations. In this case report, we describe a term infant with an extensive intrauterine stroke and anterior segment dysgenesis with a de novo mutation in *COL4A1*.

Keywords: fetal porencephaly; hemorrhagic stroke; cataract; Axenfeld-Rieger

Introduction

COL4A1, located on human chromosome 13q34, is a gene that encodes the alpha 1 chain of collagen type IV.¹ Type IV collagens are basement membrane proteins that are expressed in all tissues, including the vasculature. *COL4A1* plays an important role in the cohesiveness of the basement membrane, maintenance of vascular tone and endothelial cell function.² Vascular defects caused by a semidominant mutation in *COL4A1* that inhibits the secretion of mutant and normal type IV collagen, were first shown in a mutant mouse model that developed severe perinatal cerebral hemorrhage and porencephaly secondary to focal disruptions of vascular basement membranes.³ In addition, the mutant mice were found to be smaller than controls and had multiple pleiotropic phenotypes including ocular and renal abnormalities and reduced fertility.³ Thus, one of the main characteristics of patients with a *COL4A1* mutation is the presence of a multisystemic phenotype, with variable involvement of the brain, eyes, kidneys and muscles.^{1,2} Herein we present a newborn female with fetal ultrasound findings of extensive hemorrhagic stroke with cystic conversion, who was diagnosed postnatally with a *COL4A1* mutation due to her clinical findings, multi-organ involvement and a high index of suspicion.

Patient Description

The patient is a 39 weeks, small for gestational age [birth weight 2325 g (< 3rd %); length 16.5 inches (< 3rd %), head circumference 32 cm (5th %)] Caucasian female infant born to a 24 year-old gravida 1 para 0 now 1 mother. The mother had good prenatal care with negative serologies, with a past medical history remarkable for well-controlled asthma and obesity. There were no known exposures to human teratogenic agents such as tobacco smoke, alcohol, drugs, or toxins. The pregnancy was uneventful with a normal fetal ultrasound at 16

weeks and only complicated by type A1 gestational diabetes mellitus. At 31 weeks of gestation, a fetal ultrasound was performed and showed fetal growth less than expected, biparietal diameter/head circumference and abdominal circumference all < 3rd percentile. In addition, abnormal head ultrasound imaging was seen with a cranial appearance consistent with semilobar holoprosencephaly with fluid filled frontal lobe with absent CSP/falx cerebri, and enlarged posterior horns of the lateral ventricles. The midline hypothalamus and posterior fossa appeared normal, although the cerebellum measured small for gestational age. The fetal MRI (Figure 1) performed at 32 weeks gestation, revealed a very abnormal brain parenchyma with near complete loss of parietal lobes and the majority of frontal and temporal lobes, involving both cortex and white matter. There was a small detectable ribbon of cortex in the occipital lobes bilaterally, with absence of white matter, and no evidence of normal 3rd ventricle. The cerebellum was small with abnormal T2 signal and small cysts were visualized, suggesting hemorrhage or mineralization with abnormal remaining tissue. There was suggestion of residual hemorrhagic products versus mineralization in the calvarium along the ependymal margins of the frontal lobes and in the dependent right cerebrum based on T1 signal. The small amount and abnormality of the remaining cerebral tissue was suggestive of an infarct. The thalamus and brainstem were relatively preserved. The remainder of the fetal MRI was otherwise unremarkable except that the globes of the eyes appeared long. The family declined amniocentesis. Instead, an extensive prenatal work-up was performed including cell free DNA for trisomy 13, 18, 21, monosomy X, 22q11.2 deletion, and platelet reactivity testing which were all negative. The family history did not reveal any members with similar congenital problems; no history of birth defects, eye abnormalities, genetic diseases intellectual disability or learning disorders. There was no history of frequent miscarriages,

bleeding disorders, infertility, infant or childhood deaths or known familial disorders. The father reported a history of migraines and the mother had a history of headaches along with a distant family history of stroke.

The infant was delivered via scheduled primary low-transverse cesarean section for breech presentation and required minimal oxygen support with an intact respiratory drive. Apgar scores were 8 and 8 at 1 and 5 minutes, respectively. Pertinent findings on examination included small for gestational age, eye abnormalities with eyelid edema, proptosis, minimal visibility of the sclera, cloudy cornea, absent red reflexes, and abnormal size and shape of the pupil and iris bilaterally (Figure 2). Otherwise no gross dysmorphism was noted. Ophthalmological exam was consistent with anterior segment dysgenesis bilaterally, with complete malformation and agenesis of the anterior segment with cornea, anterior chamber, trabecular meshwork and iris involvement, elevated intraocular pressure, megalocornea and cataract on the right eye. B-Scan ocular ultrasonography also demonstrated an absence of lens bilaterally. Postnatal MRI of the brain (Figure 3) was performed on her second day of life showing diffuse hemorrhagic brain injury with bilateral hemorrhagic infarction with severe cystic encephalomalacia and no residual brain parenchyma. Mass effect on the brainstem was present with cerebral aqueduct obstruction and cervical medullary kinking, markedly abnormal cerebral parenchyma in the remaining supratentorial anterior cerebral artery and posterior cerebral artery territory with gross hemorrhage, bilateral cerebellar hemispheric infarction with severe cystic encephalomalacia, and bilateral globe abnormalities.

Postnatal work-up included testing of coagulation factors as well as microarray. Additionally, based on the patient's clinical features, sequencing on *COL4A1* was sent. The hospital course was uneventful and the infant was taking full oral feeds and discharged home

with parents. Following discharge, results of the testing demonstrated that a point mutation was found at Gly785Glu on the *COL4A1* gene. The infant had close follow-up with neurology, ophthalmology, genetics, and the Advanced Illness Management (AIM) team. The infant was seen at our Neurology Stroke Clinic at four months of age and physical examination was consistent with severe developmental and visual impairments, as well as an increasing head circumference and irritability with the concern for obstructive hydrocephalus. The patient was subsequently admitted to the hospital for neuroimaging, followed by a ventriculoperitoneal shunt placement. Genetic testing was performed on both the mother and father who were found to be negative for the single site *COL4A1* point mutation. Thus the risk of the parents to have another child in the future with the same mutation is less than 1%.

Discussion

Our patient's presentation consisting of significant intrauterine bilateral hemorrhagic infarctions with resulting cystic encephalomalacia and anterior segment eye anomalies was consistent with a *COL4A1* mutation. *COL4A1* plays a role in the initial formation of basement membranes during embryonic development and forms a sheet-like network beneath the endothelium and surrounding smooth muscle cells of the vascular basement membranes.^{2,4} Glycine residues in collagen proteins like *COL4A1* are essential for the formation of the triple-helical structure of collagen.² Thus the *de novo* Gly785Glu mutation in *COL4A1* found in our patient would explain the destabilization of collagen protein in the vascular basement membranes that predisposed her to the clinical findings of small-vessel disease that she demonstrated.

At present, no diagnostic criteria have been established for *COL4A1*-related disorders. There is a wide spectrum of clinical symptoms that variably include brain, ocular, renal, and

muscle involvement, as well as reports of Raynaud phenomenon and supraventricular arrhythmia.^{1,5} Brain involvement can include infantile hemiplegia, migraines with or without aura, seizures, dementia, intellectual disability, intracerebral hemorrhage presenting at various ages, including antenatal⁶⁻¹⁰ and recurrent episodes, and ischemic stroke.¹¹ Neuroimaging demonstrates the features of brain small-vessel disease such as porencephaly characterized by a fluid-filled cavity in the brain; leukoencephalopathy, usually bilateral and symmetric, located mainly in the supratentorial posterior periventricular areas; cerebral microhemorrhages; lacunar infarcts; deep intracerebral hemorrhages; dilated perivascular spaces; and single or multiple intracranial aneurysms.^{2,3,12} On ophthalmologic examination, a multitude of findings have variably been described including bilateral tortuosity of the second- and third-order arteries, hemorrhagic spots, and Axenfeld-Reiger anomaly with microcornea, congenital or juvenile cataract, increased intraocular pressure, iris hypoplasia, retinal detachment and optic nerve excavation.^{13,14} Renal involvement includes hematuria and renal cysts.¹⁴ Muscle cramps have been reported involving a variety of muscles with associated persistent elevation of serum creatine kinase concentrations.¹⁴

The inheritance of *COL4A1* mutation is autosomal dominant with near 100% penetrance with expression varying in age of onset and severity of clinical symptoms, even within the same family. Therefore, if a parent of the proband is affected, the risk to the siblings is 50%. There may also be *de novo* mutations or low-level parental mosaicism; however, the proportion of these cases in the population remains unknown.

In our patient and as others have shown,⁷⁻¹⁰ the clinical onset of small vessel disease in the brain as a result of *COL4A1* mutations can occur as early as the antenatal period. In neonatology and pediatrics, patients diagnosed with stroke, both in utero and postnatal, often

do not have an etiology identified. Therefore, a detailed family history as well as ophthalmologic exam may be warranted to determine other small-vessel organ involvement, as *COL4A1* mutations may be grossly underestimated in this patient population. Despite not having a family history of small-vessel disease with the associated clinical spectrum of *COL4A1* mutations, this mutation needs to be recognized by practitioners and be considered on the list of differential diagnoses in patients with no known etiology for an in utero or postnatal stroke. In one case series, three of the four neonates with extensive prenatal porencephaly had no known family history and were found to have sporadic *COL4A1* mutations.¹⁰

The implications for making a diagnosis of *COL4A1* mutation in a patient such as the one presented herein are considerable. Prenatal testing can be performed by chorionic villus sampling or by amniocentesis if one of the parents is known to carry the mutation. Preimplantation genetic diagnosis may also be an option for these families. In animal studies of mice with the *COL4A1* mutation, it was demonstrated that none of the surgically delivered mutant pups had severe cerebral hemorrhage as was observed in the heterozygous mutant pups that were born naturally.¹⁵ Therefore, preventive measures could be taken in cases of known familial *COL4A1* mutation, specifically cesarean delivery.

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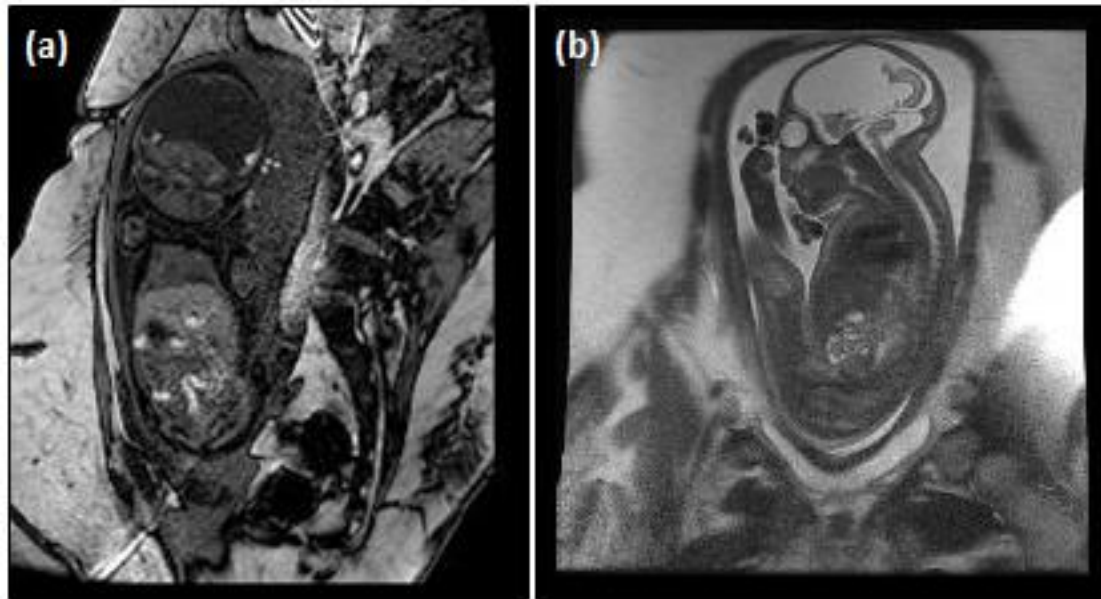
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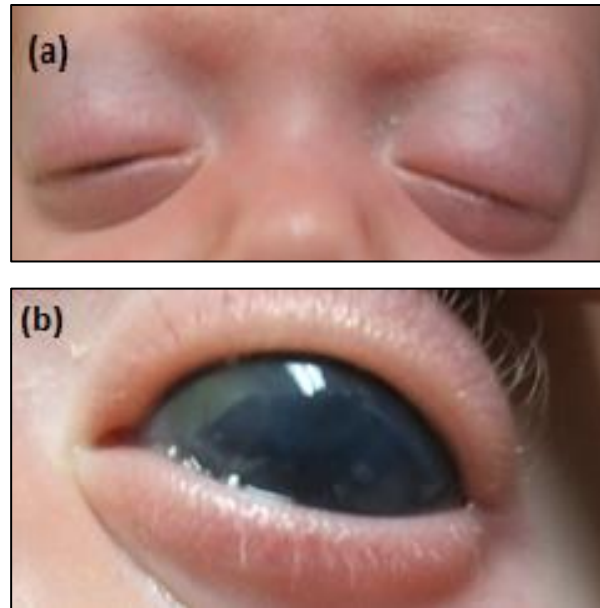
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Figure 1



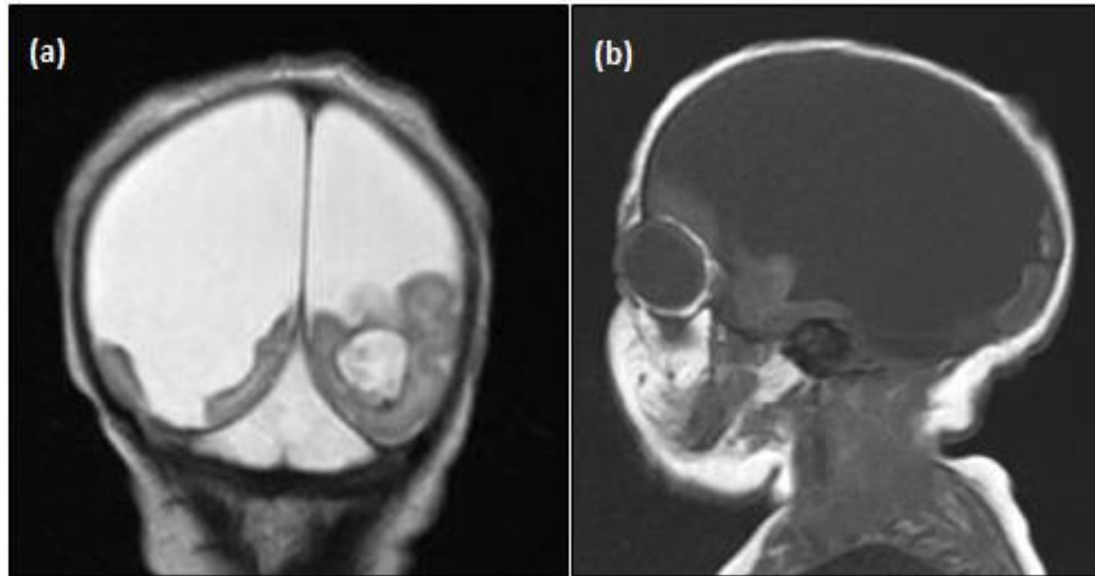
Fetal MRI with coronal T1-weighted (a) and sagittal T2-weighted imaging (b) demonstrates an extensive intrauterine stroke with near complete loss of parietal lobes and majority of frontal and temporal lobes, involving both cortex and white matter.

Figure 2



Eye exam demonstrates eyelid edema and proptosis (a) and minimal visibility of the sclera, cloudy cornea, abnormal size and shape of the pupil and iris (b).

Figure 3



Non-contrast Brain MRI with coronal fast spin echo (FSE) T2-weighted (a) and sagittal T1-weighted (b) imaging demonstrating bilateral hemorrhagic MCA infarction with severe cystic encephalomalacia/absent parenchyma with mass effect on brainstem. Bilateral cerebellar hemisphere infarction with severe cystic encephalomalacia also demonstrated.