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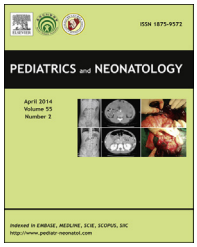

CASE REPORT

Severe Hemolytic Jaundice in a Neonate with a Novel *COL4A1* Mutation

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We report our experience with a preterm infant with severe hemolytic jaundice who required exchange transfusion just after birth. The patient was negative for alloimmune hemolysis as a result of maternal–fetal blood type incompatibility, and tests for inherited defects in erythrocyte metabolism, membrane function, and hemoglobin synthesis were normal. We also performed a bone marrow examination, but could not identify the cause of hemolysis. The patient had several other complications, including porencephaly, epilepsy, elevated serum levels of creatine kinase, and persistent microscopic hematuria. Later, we detected a genetic mutation in *COL4A1*, which was recently found to be associated with hemolytic anemia. We therefore believe that all of the patient's clinical features, including hemolytic anemia, were due to the mutation in *COL4A1*. Genetic testing for *COL4A1* mutations is recommended in neonates who exhibit hemolytic disease of unknown etiology, especially when other complications compatible with *COL4A1*-related disorders are present.

1. Introduction

Hemolysis is the most important cause of severe hyperbilirubinemia during the early neonatal period. Without appropriate treatment, hyperbilirubinemia can develop into kernicterus, particularly in preterm infants. The major causes of neonatal hemolytic jaundice are alloimmunization (maternal–fetal blood type incompatibility) and congenital

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disorders of red blood cells, such as hereditary spherocytosis and glucose-6-phosphate dehydrogenase deficiency.

Here, we present a preterm small-for-date infant with severe hyperbilirubinemia and anemia who required exchange transfusion just after birth. The patient had several other complications, including imaging abnormalities in the central nervous system, epilepsy, elevated serum levels of creatine kinase, and persistent microscopic hematuria. All of these clinical features were attributed to a novel mutation in *COL4A1*, which was detected when the patient reached 2 years of age.

2. Case Report

A male infant was delivered at 34 weeks of gestation by elective cesarean section in response to fetal growth arrest, and had Apgar scores of 8 and 9 at 1 minute and 5 minutes, respectively. The patient was the first son of unrelated healthy parents. His family members had no medical history of hemolytic disease. At birth, he was symmetrically growth retarded with a weight of 1422 g [−2.4 standard deviation (SD)], length of 40.0 cm (−1.7 SD), and head circumference of 27.6 cm (−1.9 SD). On admission, the neonate's total bilirubin level was high (4.7 mg/dL), and he was started on intensive phototherapy immediately after birth. However, 12 hours later, his total bilirubin level rose further to 10.5 mg/dL and the hemoglobin level decreased from 11.8 g/dL to 9.6 g/dL. Therefore, we performed exchange transfusion on Day 0 and Day 1. Both the patient's and mother's blood were type B (RhD+), and yielded negative results with the direct Coombs test. Reticulocyte levels were elevated by 89‰, carbon monoxide—hemoglobin concentration was elevated by 3.8%, and a peripheral blood smear showed moderate anisocytosis and polychromasia. There was no massive hemorrhage in the cerebral ventricles, liver, adrenal glands, or other intraperitoneal organs. After exchange transfusion, hyperbilirubinemia and hemolytic anemia improved. Although recombinant erythropoietin was administered from Day 2, anemia progressively worsened (hemoglobin level decreased from 13.2 g/dL to 5.1 g/dL in 20 days), and the patient required a red blood cell transfusion on Day 29 (Figure 1). Inherited defects of erythrocyte metabolism (glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, and other erythrocyte enzyme defects) were not detected by genetic analysis. Defects in membrane function (hereditary spherocytosis and hereditary elliptocytosis) and hemoglobin synthesis (sickle cell disease, thalassemia, and other hemoglobinopathies) were also not detected. A bone marrow specimen on Day 52 showed erythroid hyperplasia, but no signs of malignant disease. Tests for specific antibodies against toxoplasmosis, rubella, cytomegalovirus, and herpes simplex were negative, and no cytomegalovirus DNA was found in the patient's urine. Blood and urine cultures were negative for viral pathogens. Chromosomal analysis revealed a normal male karyotype. No abnormalities were found in diagnostic tests for common inborn errors of metabolism. We were unable to identify the cause of hemolysis in the neonatal period. His anemia improved after red cell transfusion, and there was no recurrence of anemia thereafter. The patient was discharged at age 2 months.

Cranial magnetic resonance imaging on Day 39 as a matter of routine for very low birth weight infants revealed dilated lateral ventricles with irregular walls (porencephaly; Figure 2). During follow-up, hemiplegia with mild motor and mental retardation, strabismus, and epilepsy were observed. Occasionally, high serum creatine kinase levels (500—2,000 IU/L) and persistent microscopic hematuria were noticed. Later, genetic analysis of *COL4A1* revealed a heterozygous mutation in the *COL4A1* gene: c.3245G>A (p.Gly1082Glu).

Informed consent to report this case was obtained orally from the neonate's parents.

3. Discussion

Here, we present the case of a preterm small-for-date infant who exhibited severe hyperbilirubinemia and prolonged anemia. The clinical course and laboratory data strongly suggested hemolytic disease. Hemolytic disease in newborns can have multiple causes, such as alloimmune hemolytic disease and inherited defects in erythrocyte metabolism, membrane function, or hemoglobin synthesis (Table 1).[1,2] Despite our systematic approach, we could not identify the cause of the patient's hemolysis during the

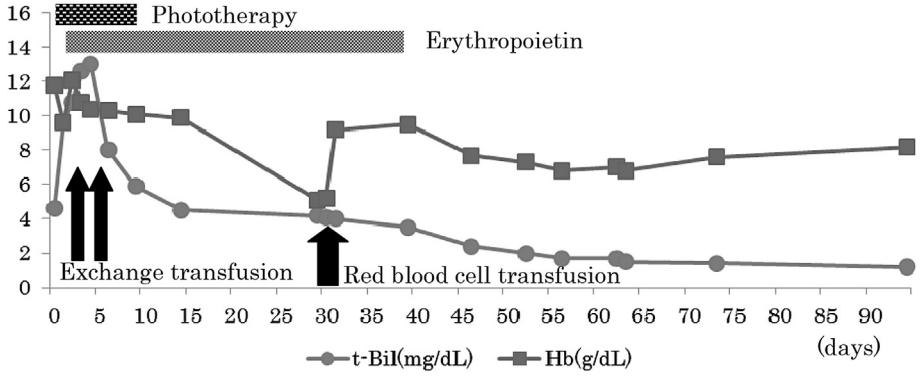

Figure 1 Clinical course. Exchange transfusion was performed on Day 0 and Day 1 and red blood cell transfusion was performed on Day 29. Hyperbilirubinemia improved after exchange transfusion. Anemia improved after red cell transfusion and did not recur. Hb = hemoglobin; t-Bil = total bilirubin.

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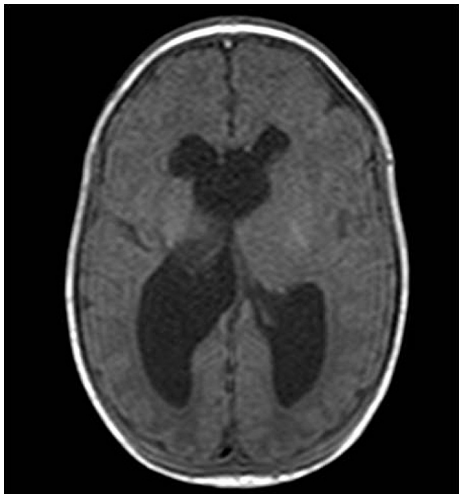

Figure 2 Cranial magnetic resonance imaging on Day 39. T1-weighted imaging shows enlarged lateral ventricles with irregular walls.

neonatal period. There was concern that the patient's anemia would worsen with viral infections, especially human parvovirus B19 infection; however, despite several infections, anemia did not recur until later. The transient nature of his anemia could not be explained.

Recently, we reported the clinical features of 15 Japanese patients with *COL4A1* mutations. One-third of them suffered from hemolytic anemia that spontaneously resolved within several months.[3] Other clinical features of *COL4A1*-related disorders include intracerebral hemorrhage, ischemic stroke, porencephaly detected by neuroimaging, infantile hemiplegia, seizures, intellectual disability, hematuria, muscle cramps with elevated creatine kinase levels, and ophthalmologic disorders.[3–6] Therefore, the clinical findings of this case, such as persistent microhematuria, elevated serum levels of creatine kinase, and porencephaly with central nervous system-related symptoms, in addition to hemolytic anemia in the neonatal period, were consistent with the presence of a *COL4A1* mutation.

Type IV collagens are basement membrane proteins that are expressed in all tissues, including the vasculature, and can dynamically influence a broad range of biological processes.[7] COL4A1 (α1 chain) and COL4A2 (α2 chain) form a triple-helical structure by combining as heterotrimers with a 2:1 stoichiometry ($\alpha1\alpha1\alpha2$).[1] COL4A1 forms a sheet-like network found beneath both the endothelium and the surrounding smooth muscle cells, and has important roles in maintaining the cohesiveness of basement membranes and in endothelial cell function.[8] COL4A1 contains domains consisting of glycine-X-Y amino acid repeats, which are essential for the formation of the triple-helical structure. In the present case, a mutation in the *COL4A1* gene (c.3245G>A) resulted in the substitution of a

Table 1 Causes of hyperbilirubinemia in neonates.[1,2]

Hemolysis	Nonhemolysis
Alloimmune hemolytic disease (maternal–fetal blood type incompatibility)	Extravascular blood
ABO incompatibility	Cephalhematoma
Rh isoimmunization	Pulmonary hemorrhage
Minor blood group incompatibility	Cerebral hemorrhage
Inherited defects of erythrocyte metabolism	Intra-abdominal hemorrhage
Glucose-6-phosphate dehydrogenase deficiency	Prematurity including late-preterm gestation
Pyruvate kinase deficiency	Infections
Pyrimidine 5'-nucleotidase deficiency	Sepsis
Glucose phosphate isomerase deficiency	Enhanced enterohepatic bilirubin circulation
Phosphofructokinase deficiency	Intestinal obstruction
Phosphoglycerate kinase deficiency	Ileus, meconium plugging, cystic fibrosis
Aldolase deficiency	Swallowed blood
Hexokinase deficiency	Breast milk feeding
Enolase deficiency	Hormonal deficiency
Adenylate kinase deficiency	Hypothyroidism
Adenosine deaminase overproductive disease	Hypopituitarism
Inherited defects of erythrocyte membrane function	Polycythemia
Hereditary spherocytosis	Twin–twin transfusion
Hereditary elliptocytosis	Delayed cord clamping
Stomatocytosis	Maternofetal transfusion
Pyknocytosis	Infant of diabetic mother
Inherited defects of hemoglobin synthesis	Impaired hepatic bilirubin uptake
Sickle cell disease	Patent ductus venosus
Thalassemia	SLCO1B1 gene polymorphisms
Hemoglobinopathy	Disorders of bilirubin conjugation – UGT1A1 gene variants
	Crigler–Najjar syndrome type I
	Crigler–Najjar syndrome type II
	Gilbert disease

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glycine for a glutamic acid residue in one of the glycine-X-Y repeats. Therefore, this mutation is likely to result in disruption in the triple-helical structure of the protein and cause several symptoms.

Here, we reported that a *COL4A1* mutation can cause hemolytic anemia. Van Agtmael et al[9] revealed that red blood cell number and hematocrit of *COL4A1* mutant mice were significantly lower than those of wild type mice (8.55×10^{12} RBC/L and 38% hematocrit vs. 9.50×10^{12} RBC/L and 45% hematocrit). However, the mechanisms via which *COL4A1* mutations lead to hemolysis are not yet known. One possibility is that *COL4A1* mutations result in structural or functional changes to basement membranes, which can lead to defective transmigration of red blood cells or their progenitors, and the subsequent destruction of these cells. Prior to being released into the peripheral blood, blood progenitor cells, including CD34+ cells, transmigrate to the basement membranes of the bone marrow vasculatures. In this process, blood progenitor cells have to breach the basement membranes by matrix-degrading enzymes capable of degrading type IV collagen.[10] Janowska-Wieczorek et al[10] reported that peripheral blood CD34+ cells, but not steady-state bone marrow CD34+ cells, strongly express collagen type IV degrading gelatinases, matrix metalloproteinases-2 (MMP-2), and MMP-9. This suggests that type IV collagens can dynamically influence the transmigration of blood progenitor cells. We also speculate that fetal hemoglobin may be more vulnerable to this damage than adult hemoglobin, which may explain why hemolysis is transient in patients with the *COL4A1* mutation. Further studies are required to clarify the role of *COL4A1* mutations in hemolytic anemia.

In conclusion, we report a preterm small-for-date infant who experienced severe hemolytic jaundice soon after birth. Genetic testing for *COL4A1* mutations is recommended in neonates with hemolytic disease of unknown cause, especially when other complications compatible with *COL4A1*-related disorders are present.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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