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BRIEF REPORT
Hemoglobin Hammersmith [β 42(CD1) Phe → Ser] Causing Severe Hemolytic Anemia in a Japanese Girl

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Hemoglobin Hammersmith, a rare, unstable hemoglobin variant, was diagnosed in a 9-year-old Japanese girl. She presented with the typical manifestations of this disorder, including neonatal hyperbilirubinemia, followed by progressive hepatosplenomegaly, jaundice, and bilirubinuria. Because of severe hemolytic anemia, she received transfusions of red blood cells every 3 to 4 weeks. However, she underwent splenectomy at the age of 4 years and has continued to be in partial remission without requiring further transfusions. DNA sequence analysis of the polymerase chain reaction-amplified β-globin gene revealed a point mutation (T → C) in the second nucleotide of the 42nd codon of the β-globin chain (β 42(CD1) Phe → Ser). Pediatr Blood Cancer 2006;47:839–841.
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CASE REPORT

Unstable hemoglobin usually presents as autosomal-dominant hereditary hemolytic anemia with inclusion bodies in the red blood cells. Patients are usually asymptomatic and do not require treatment, although severe hemolytic crisis can occur during infections and under condition of oxidative stress. Hemoglobin Hammersmith, an unstable β-chain variant, was first described in two unrelated children with severe hemolytic anemia [1]. Subsequently, this unstable hemoglobin has been described in 11 other children of different ethnic backgrounds who presented with severe hemolytic anemia (Table I) [2–10]. It has been reported that Hemoglobin Hammersmith was associated with congenital anomalies [10] and Turner syndrome [6]. We describe a 9-year-old Japanese girl in whom severe hemolytic anemia developed at the age of 3 months and hemoglobin Hammersmith was diagnosed through sequence analysis of polymerase chain reaction (PCR)-amplification of parts of the β-globin gene at the age of 9 years.

The patient was born by normal vaginal delivery at 38 weeks' gestation as the second child of non-consanguineous parents, with Apgar scores of 9 and 10 at 1 and 5 min, respectively. Birth weight was 2,974 g (−0.31 SD), length was 49.0 cm (+0.05 SD), and occipitofrontal circumference was 34.6 cm (+1.15 SD). On the fourth day after birth, she presented with jaundice and poor milk intake. Total bilirubin concentrations were 17.1 and 16.4 mg/dl on the seventh and ninth days after birth, respectively. However, phototherapy was not performed for neonatal jaundice. At the age of 3 months, she was found to have anemia and was admitted to our hospital for further evaluation. Results of laboratory studies on admission were as follows: hemoglobin, 6.8 g/dl; hematocrit, 23.0%; red blood cell count, $216 \times 10^4/\mu l$; reticulocyte count, 23.1%; platelet count, $45.2 \times 10^4/\mu l$; white blood cell count, 9,700/μl; aspartate aminotransferase, 41 IU/dl; alanine aminotransferase, 17 IU/dl; lactate dehydrogenase, 862 IU/L; total bilirubin, 1.3 mg/dl; direct bilirubin, 0.2 mg/dl; indirect bilirubin, 1.1 mg/dl; and haptoglobin, <10 mg/dl. The karyotype was 46,XX. Moreover, Coombs' test was negative.

Physical examination on admission showed no anomalies, such as short stature, nail dystrophy, and bone and urogenital tract malformations. Bone marrow examination revealed erythroid hyperplasia without abnormal cells. The family history was unremarkable. These findings led to a diagnosis of hemolytic anemia, for which the patient was routinely treated by us. She received transfusions of red blood cells every 3 to 4 weeks because of severe anemia (hemoglobin concentration, <6.0 g/dl) from the age of 9 months.

At the age of 9 months, hemoglobin electrophoresis, an isopropanol test (instability test), isoelectric focusing (IEF), and automated high-performance liquid chromatography (HPLC) analysis for hemoglobin A_{1c} (TSK gel G7HSi, HLC-723G7, Tosoh, Tokyo, Japan) were performed to study the abnormal hemoglobin. Hemoglobin electrophoresis revealed normal hemoglobin A_2 (2.2%) and increased hemoglobin F (25.2%). The isopropanol test showed significant flocculent precipitation in the proposita's hemolysate after 5 min of contact with 17% isopropanol solution at 37°C. The IEF showed an indistinct band with a smear, and the profile of HPLC was nondiagnostic. Results of hemoglobin analysis were normal in both parents. These results did not lead

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TABLE I. Summary of Reported Cases of Hemoglobin Hammersmith

Age at diagnosis/sex	Ethnic origin of patient	References
10 year/female	English	[1]
6 year/female	English	[2]
3 year/female	Tunisian	[3]
1 year/female	Japanese	[4]
5 year/female	Caucasian	[5]
5 month/female	North American Indian and Anglo-Saxon	[6]
4 year/female	Chinese	[7]
25 year/female	Not specified	[8]
15 year/female	Chipewayan Indian	[9]
5 month/female	African American twins	[10]

to a definitive diagnosis of an underlying hematologic disorder.

Results of laboratory studies at the age of 3 years 4 months were as follows: hemoglobin, 6.1 g/dl; hematocrit, 19.1%; red blood cell count, $175 \times 10^4/\mu l$; reticulocyte count, 33.4%; platelet count, $25.8 \times 10^4/\mu l$; white blood cell count, 9,800/μl; aspartate aminotransferase, 77 IU/dl; alanine aminotransferase, 20 IU/dl; lactate dehydrogenase, 1,189 IU/L; total bilirubin, 2.1 mg/dl, being direct form, 0.1 mg/dl; hemoglobin F, 17.9%; and haptoglobin, <10 mg/dl. The red cell examination showed target cells. Physical examination showed anemia and icterus. A systolic ejection murmur was audible at the apex. The liver was palpable 1 cm below the right costal margin, and the spleen was palpable 7 cm below the left costal margin. The patient underwent laparoscopic splenectomy at the age of 4 years 6 months because of severe hemolytic anemia exacerbated by acute infections. A peripheral blood smear stained with Brilliant Green showed many cells containing single or multiple inclusion bodies consistent with Heinz bodies, which are considered to be precipitated hemoglobin. She has continued to be in partial remission without requiring blood transfusions (hemoglobin concentration, 6.0–12.0 g/dl).

At the age of 9 years, Hemoglobin Hammersmith was diagnosed with the following procedure. DNA was extracted from the patient's white blood cells, and the β-globin gene was amplified with PCR. The DNA abnormality was surveyed by single-strand conformation polymorphism (SSCP) analysis, and an abnormal SSCP band was detected. Sequencing of DNA extracted from this abnormal band revealed a TTT (Phe) → TCT (Ser) mutation at codon 42 of the β globin gene (Fig. 1). This heterozygous mutation gives rise to a phenylalanine-to-serine substitution near the heme pocket and weakens heme-globin bonding, which increases heme loss from the affected subunits. The heme loss induces denaturation and subsequent precipitation of the hemoglobin molecule, which results in membrane damage and finally premature red blood cell destruction.

Hemoglobin Hammersmith is an electrically silent variant, i.e., the substitution of an amino acid is associated with no net change in the electrical charge of the hemoglobin molecule [7]. In addition, the presence of hemoglobin F in hemolysates may produce a false-positive isopropanol test. Thus, routine clinical laboratory method cannot detect such an abnormal hemoglobin, and the correct diagnosis may not be made. The diagnosis in our patient was obtained by analysis at the DNA level. Sequence analysis of β-globin, even if electrophoretically silent, was useful in diagnosing such a hemoglobinopathy. Because familial transmission has not been reported, Hemoglobin Hammersmith appears to be a de novo mutation. Interestingly, all 11 reported patients with Hemoglobin Hammersmith have been female, as was our patient (Table I). It may occur a negative, fatal intrauterine selection against male [10]. The significance of this observation remains unclear.

Sequence analysis of globins is a powerful tool for the direct identification of hemoglobin variants and is suitable for

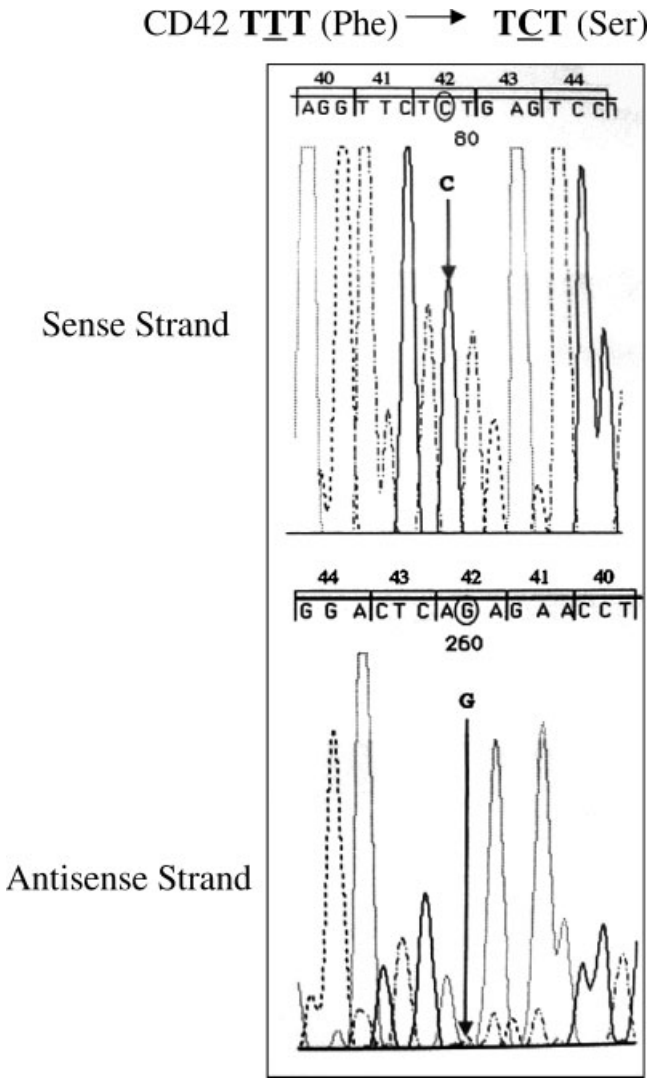

Fig. 1. DNA sequence analysis showing the TTT → TCT mutation at codon 42 of β-globin gene resulting in the amino acid substitution Phe → Ser.

routine use. Identification of a heritable defect may be useful for both patient management and future genetic counseling.

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