

A 6-year-old Girl with Hemoglobin H Disease

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Abstract

Hemoglobin H (HbH) disease is the severe nonfatal form of α -thalassemia syndrome. It is usually caused by molecular defects of 3 of 4 α -globin genes ($-/\alpha$) which cause α -globin expression to be decreased. HbH disease is rare in Japan. Here, we report on a 6-year-old girl with HbH disease who had profound hypochromatic and microcytic anemia. Analysis of the α -globin genes of the patient's family showed that the father, who was Japanese, had an abnormal gene with a 3.7-kb deletion ($-\alpha^{3.7}/\alpha\alpha$), and the mother, who was Filipino, had a deletion removing both α -globin genes of the Filipino type ($-\text{FIL}/\alpha\alpha$). Neither parent had anemia. The patient was found to have HbH disease with a heterozygous genetic abnormality ($-\text{FIL}/-\alpha^{3.7}$). Recently, the number of marriages of Japanese to natives of areas where thalassemia is epidemic has increased. Therefore, the incidence of HbH disease can be expected to increase in Japan. Long-term follow-up will be needed to evaluate the long-term complications and to improve the quality of life of patients with HbH disease.

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Key words: α -thalassemia, hemoglobin H disease, microcytic anemia

Introduction

Hemoglobin is composed of 4 globin chains (2 α and 2 β globin chains) bound to the heme molecule. α -Thalassemia is the most common inherited disorder of hemoglobin synthesis in south Asia and southern China. Healthy individuals have 2 α -globin genes linked on each chromosome 16 ($\alpha\alpha/\alpha\alpha$). α -Thalassemia is associated with a variable degree of α -globin chain deficit that reflects the number of affected α -globin genes¹. The frequency of α -thalassemia alleles is 5% to 10% in the Mediterranean basin and is as high as 60% to 80% in

parts of Saudi Arabia, India, and Thailand. The frequency of carriers among the Chinese population has been reported to be 5% to 15%. On the other hand, the frequency of α -thalassemia is less than 0.01% in Japan^{2,3}. In hemoglobin H (HbH) disease, 3 of the 4 α -globin genes are affected. Here, we report on a 6-year-old girl with HbH disease.

Case Report

A 6-year-old girl with a fever of 37.6°C and cough for 1 week came to our hospital with her paternal grandmother. Acute bronchitis and anemia were diagnosed. The patient's father was Japanese, and

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Table 1 Laboratory data of the patient and her family

		Patient	Mother	Father
RBC	$\times 10^{12}/L$	4.70	5.34	5.29
Ht	%	26.8	34.9	44.7
Hb	g/dL	7.9	11.0	14.9
MCV	fL	57.0	65.4	84.5
MCH	Pg	16.8	20.6	28.2
MCHC	%	29.5	31.5	33.3
Ret	‰	22	8.7	8.0
Fe	$\mu\text{g}/\text{dL}$	73	93	ND
TIBC	$\mu\text{g}/\text{dL}$	268	319	ND
Ferritin	ng/mL	89	39.8	ND
HbA2 (2.2–3.2)*	%	0.57	1.80	2.43
HbF (0.1–1.2)*	%	<1	<1	<1

ND: not done, *: normal range

her mother was Filipino. Her growth and development had been normal. A physical examination revealed mild pallor and mild jaundice but no hepatosplenomegaly or other abnormalities.

Laboratory findings were as follows: red blood cell (RBC) count $470 \times 10^4/\mu\text{L}$; hemoglobin (Hb) level 79 g/L; mean corpuscular volume (MCV) 57.0 fL (normal, 80–94 fL); mean corpuscular hemoglobin (MCH) 16.8 pg (normal, 26.0–31.0 pg); serum iron level 73 $\mu\text{g}/\text{dL}$ (normal, 70.0–180 $\mu\text{g}/\text{dL}$); total iron binding capacity 268 $\mu\text{g}/\text{dL}$ (normal, 251–398 $\mu\text{g}/\text{dL}$); serum ferritin 89 ng/mL (normal, 12–301 ng/mL); and total bilirubin 1.8 mg/dL (normal, 0.2–1.2 mg/dL). These results indicated marked hypochromatic and microcytic anemia. The morphological examination of RBCs showed anisopoikilocytosis, target cells, and hypochromasia (**Fig. 1**). Because of the normal transferrin saturation rate ($\text{Fe}/\text{total iron-binding capacity}$: $73/268 = 0.272$) and the normal serum ferritin level, we did not diagnose iron-deficiency anemia (**Table 1**). Because the MCV/RBC ratio was low (12.1) and the patient's mother was Filipino, a thalassemia syndrome was suspected. Red cell inclusion bodies were observed after Brilliant cresyl blue staining (**Fig. 2**). Electrophoresis of Hb on cellulose acetate membranes revealed HbH (**Fig. 3**).

We then performed genotypic analysis after we had obtained informed consent for DNA analysis from the family. After the extraction of DNA from the peripheral blood of both the parents and the patient, gap-polymerase chain reaction (PCR) assay

revealed the amplification of only $-\alpha^{3.7}$ (single gene deletion) in the father and only amplification of $- \text{-FIL}$ (double gene deletions) in the mother. The patient was confirmed to be a compound heterozygote for $-\alpha^{3.7}$ and $- \text{-FIL}$ ($-\text{-FIL}/-\alpha^{3.7}$)⁴, and HbH disease was diagnosed (**Fig. 4**).

The patient was followed up for 2 years after diagnosis. She had compensated for hemolytic anemia with average Hb levels of more than 8 g/dL and did not require blood transfusions or even standard iron chelation therapy, except during episodes of mild hemolysis activated by high fever or acute infections.

Discussion

α -Thalassemias are divided into 4 clinical subsets that reflect the extent of impairment of α -globin chain production. Thus, inactivation of a single α -globin gene on a chromosome has traditionally been designated as α -thalassemia-2 ($-\alpha/\alpha\alpha$). Homozygosity for α -thalassemia-2 or heterozygosity for an allele causing more severe disease in which both α -globin genes are inactivated leads to α -thalassemia-1 ($-\alpha/-\alpha$), ($-\alpha/\alpha\alpha$). HbH disease reflects heterozygosity for α -thalassemia-1 and α -thalassemia-2 ($-\alpha/-\alpha$), whereas Hb Bart syndrome, which is usually not compatible with postnatal life, is caused by a complete absence of α -globin genes ($-/-$)⁵. In Japan, the apparent frequency of α -thalassemia-2 is 1 in 400. Thus, α -thalassemia-1 ($-\alpha/-\alpha$) is rare. The frequency of another α -

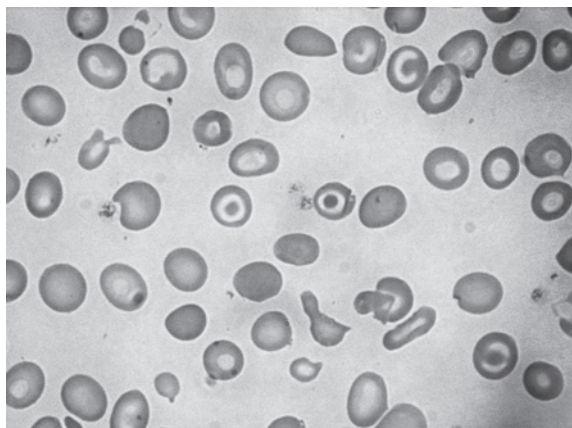


Fig. 1 Peripheral blood smear from a patient who showed hypochromatic red cells, target cells, and anisopoikilocytosis.

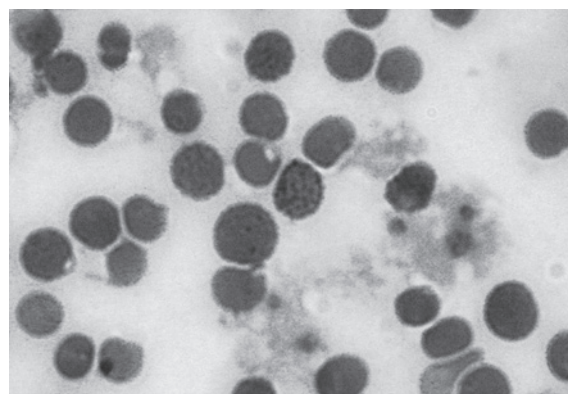


Fig. 2 Red cell inclusion body in the peripheral blood (Brilliant cresyl blue stain)

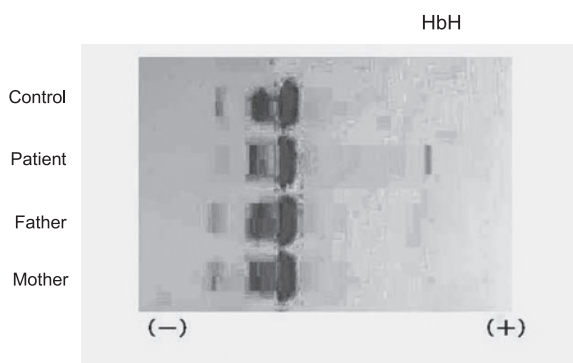


Fig. 3 Isoelectric focusing of hemolysates from a family member of a proband with HbH disease was performed on a polyacrylamide gel plate containing 3 kinds of carrier ampholytes (pH 3.5–10, pH 7–9 and pH 6.7–7.7) to make the pH range 6 to 9 at 10°C, 500 V, for 16 hours.

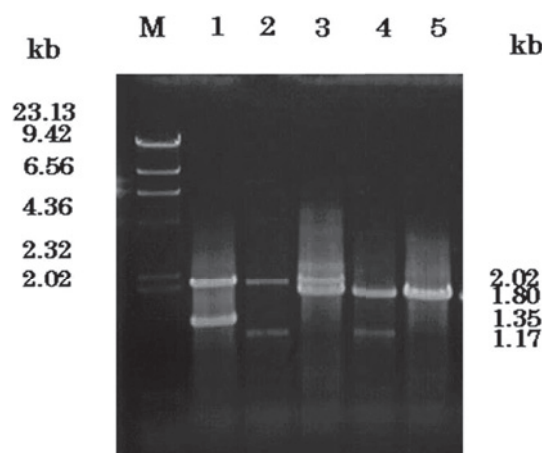


Fig. 4 Analysis of α -globin gene deletions with multiplex gap-PCR in the patient and her family

M: molecular marker; lane 1: positive control ($-^{SEA}/-\alpha^{3.7}$), HbH; lane 2: patient ($-^{FIL}/-\alpha^{3.7}$), HbH; lane 3: patient's father ($\alpha\alpha/\alpha^{3.7}$), α -thalassemia-2; lane 4: patient's mother ($-^{FIL}/\alpha\alpha$), α -thalassemia-1; and lane 5: control (healthy donor). Product size: $-\alpha^{3.7}$: 2.02 kb; $\alpha 2$: 1.8 kb; $-SEA$: 1.35 kb; $-FIL$: 1.17 kb. Information of primer sequences and PCR conditions has been published previously⁴

thalassemia-1 ($-/\alpha\alpha$) would be 1 in 3,000 to 5,000. Just 17 families with HbH disease have been identified³.

The clinical variability of HbH disease depends on a considerable genetic heterogeneity. Two main types of HbH disease—deletional HbH disease and nondeletional HbH disease—have been described¹⁵. Deletional HbH disease is commonly caused by a deletion removing both α -globin genes on a single chromosome 16 such, as the Southeast Asian (SEA) type ($-SEA$), the Thai type ($-THAI$), and the Filipino type ($-FIL$)⁶, plus a deletion removing only a single α -globin gene on the other chromosome 16, such as the ($-\alpha^{3.7}$) or ($-\alpha^{4.2}$) deletions⁷.

In the present case, we analyzed α -globin genes isolated from members of the patient's family. The gap-PCR method⁴ showed that the patient was heterozygous for α -thalassemia-1 of Filipino type ($-^{FIL}/\alpha\alpha$) and α -thalassemia-2 with a ($-\alpha^{3.7}/\alpha\alpha$) genotype, and HbH disease was diagnosed.

In general, most patients with HbH disease, particularly deletional HbH disease, show normal growth and development. This condition has long been thought to be a mild clinical condition because

most patients with HbH rarely require blood transfusions, splenectomy, or even standard iron chelation therapy and rarely become transfusion-dependent¹⁵. However, the major complications result from iron overload. The increase in serum ferritin levels is correlated with age but is unrelated to the genotype⁸. Furthermore, the severity of anemia usually increases during pregnancy⁹.

Thalassemia syndromes in children are frequently misdiagnosed as iron deficiency anemia because the microcytic anemia is similar, and iron deficiency anemia is much more prevalent in Japan. Many discrimination indices have been reported for distinguishing thalassemia from iron deficiency anemia¹⁰. The Mentzer index is the simplest¹¹. If the MCV/RBC ratio is 13 or less, thalassemia should be considered. On the other hand, iron-deficiency anemia should be suspected if the MCV/RBC ratio is greater than 13. Furthermore, family history is important in the diagnosis of patients. The present patient's mother was Filipino, and her father was Japanese. The patient came to our hospital with her maternal grandmother, and her name was Japanese. At first we did not realize that she was the child of an international marriage.

According to the vital statistics of Ministry of Health, Labour and Welfare of Japan, international marriages numbered 12,529 in 1986 and accounted for 1.8% of marriages, but they increased to 44,701 in 2006 and accounted for 6.1% of marriages¹². In addition, in nearly 80% of international the wives are non-Japanese, 70% of whom are from China or the Philippines, where thalassemia is prevalent. Therefore, the incidence of HbH disease can be expected to increase in Japan. When we encounter patients with microcytic anemia, we should determine whether the anemia is due to iron deficiency or to thalassemia. Long-term follow-up

will be needed to evaluate long-term complications and to improve quality of life.

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