—**Report on Experiments and Clinical Cases**—

Two Children with Thalassemia Identified During Screening for Anemia in Junior High School

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Abstract

We present two Japanese students with thalassemia identified during screening for anemia in their junior high school. Blood test results revealed marked hypochromic and microcytic erythrocytosis in one patient and microcytic anemia in the other. Both cases showed a mean corpuscular volume/red blood cell (MCV/RBC) ratio less than 13. Their β/α synthesis ratio was elevated. Deletion of ψα2, ψα1, α2, α1 and θ1 genes in the α-globin gene clusters were noted in the first case. This pattern of gene deletion was consistent with heterozygous α-thalassemia 1 of the Southeast Asian type. On the other hand, an increased hemoglobin A_2 level and reduced β/α synthesis ratio were found in the second case. Direct cloning and DNA sequencing identified a point mutation (guanine to adenine) at position 1 of intervening sequence II in the β-globin gene (IVS II-1 G→A). These results suggest that this patient had heterozygous β^0-thalassemia.

Diagnosis of thalassemia should be confirmed by molecular analysis in cases with microcytic anemia or hypochromic microcytosis with a MCV/RBC ratio of 13 or less.
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Key words: thalassemia, α-thalassemia, β-thalassemia, screening, anemia

Introduction

The thalassemias are characterized by abnormalities in the production of α or β-globin chains. α-thalassemia is caused by reduced or absent production of α-globin chains, and β-thalassemia is caused by reduced or absent production of β-globin chains.

Thalassemia primarily affects people of Mediterranean, Southeast Asian, and African ancestry in malaria-endemic regions. The prevalence of thalassemia is not high in northeast Asia, including Japan, where malaria is not common. Recently, the prevalence of β-thalassemia minor in Japan has been reported to be approximately 1 per 1,000[1]. This rate is much higher than earlier estimates. Because most thalassemia minor patients are relatively asymptomatic, the recognized disease rate among these patients is less than the true incidence. Some cases have reportedly been found serendipitously. We describe two cases with hypochromic microcytosis, one with anemia and one without, who were diagnosed with thalassemia through anemia screening in junior high school.

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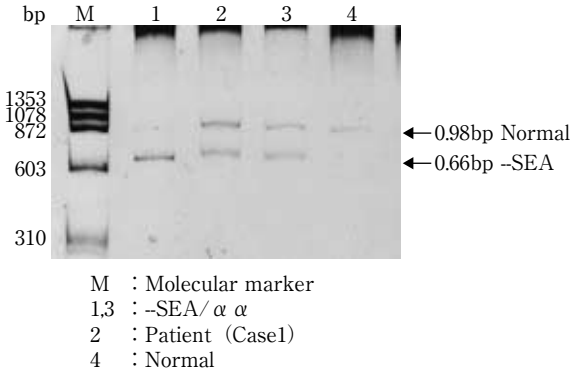
Table 1 Laboratory data of the patient and his family in case 1

		Patient	Mother
RBC	$\times 10^{12}$/L	6.67	5.65
Ht	%	44.1	37.7
Hb	g/dL	13.2	11.3
MCV	fL	66.1	66.7
MCH	pg	19.8	20.0
MCHC	%	29.9	30.0
Ret	%	1.1	1.6
Fe	μg/dL	94	
TIBC	μg/dL	342	
Ferritin	ng/mL	42	
Hb A_2	%	2.6	2.4
Hb F	%	< 1	< 1
β/α globin synthesis ratio		1.65	1.8



M : Molecular marker
1,3 : --SEA/$\alpha\alpha$
2 : Patient (Case1)
4 : Normal

Fig. 1 Electrophoresis on a 5% polyacrylamide gel of PCR products obtained by use of primer set to detect the α-thalassemia-1 gene (--SEA), which was visualized by silver-staining.

Case Reports

Case 1

A 12-year-old Japanese boy was found to have marked hypochromic and microcytic erythrocytosis when his blood was examined during routine screening for anemia in junior high school. The patient had no complaints. A physical examination revealed no hepatosplenomegaly or other abnormal findings.

His red blood cell (RBC) count was 6.67×10^{12}/L, hemoglobin (Hb) level was 132 g/L, and mean corpuscular volume (MCV) was 66.1 fL. RBC morphology showed a slight aniso-poikilocytosis, target shapes, and hypochromasia. His serum iron level was 94 μg/dL, total iron binding capacity 342 μg/dL, and serum ferritin 42 ng/mL. Because of his low MCV/RBC ratio (9.91), normal transferrin saturation rate (94/342 = 0.275), and normal serum ferritin level, we did not diagnose him as having iron-deficiency anemia. Hb electrophoresis on cellulose acetate membranes revealed normal Hb A_2 and Hb F. Analysis of Hb synthesis showed suppressed synthesis of α-globin compared with β-globin (β/α ratio = 1.65) (**Table 1**). The deletion of about 18 kb, including ψα2, ψα1, α2, α1 and θ1 from α-globin gene clusters were recognized on DNA analysis by polymerase chain reaction (PCR) using special primer sets. The amplified DNA products were electrophoresed in 5% polyacrylamide gel and then photographed (**Fig. 1**)[2,3]. The pattern of gene deletion was consistent with heterozygous α-thalassemia 1 of the Southeast-Asian type. His mother showed an increased β/α synthesis ratio and the same gene deletion pattern.

Case 2

A 14-year-old girl was found to have microcytic anemia during routine screening for anemia in her junior high school. The patient reported no complaints. Physical examination revealed no hepatosplenomegaly or other abnormal findings. Her RBC count was 5.32×10^{12}/L, hemoglobin level was 106 g/L, and MCV 64.2 fL. Her MCV/RBC ratio was 12.27. RBC morphology showed a slight aniso-poikilocytosis and hypochromasia. Serum iron level was 114 μg/dL, total iron binding capacity 267 μg/dL, and serum ferritin 63 ng/mL. Hb electrophoresis on cellulose acetate membranes revealed elevated Hb A_2 and Hb F to 5.87% and 2.11%, respectively. Her β/α globin synthesis was reduced to 0.75 (**Table 2**).

The β-globin gene of the patient was amplified by the PCR. The DNA fragments were cloned directly using phage vector M 13 mp 19[4], and subjected to sequence analysis by the dideoxy chain termination method[5] from the 5' promoter region to the 3' end. A guanine to adenine transition at position 1 of the intervening sequence II in the β-globin chain (IVS II-1 G→A) was observed (**Fig. 2**). This substitution

Table 2 Laboratory data of the patient and her family in case 2

		Patient	Mother	Brother
RBC	$\times 10^{12}$/L	5.32	5.49	6.6
Ht	%	34.2	35.7	39.7
Hb	g/dL	10.6	11.2	12.3
MCV	fL	64.2	65.0	60.2
MCH	pg	19.9	20.4	18.6
MCHC	%	31.0	31.4	31.0
Ret	%	1.0	0.9	1.0
Fe	μg/dL	114	73	38
TIBC	μg/dL	267	234	338
Ferritin	ng/mL	63	130	17
Hb A_2	%	5.87	4.92	5.33
Hb F	%	2.11	3.59	< 1
β/α globin synthesis ratio		0.75	0.64	0.8

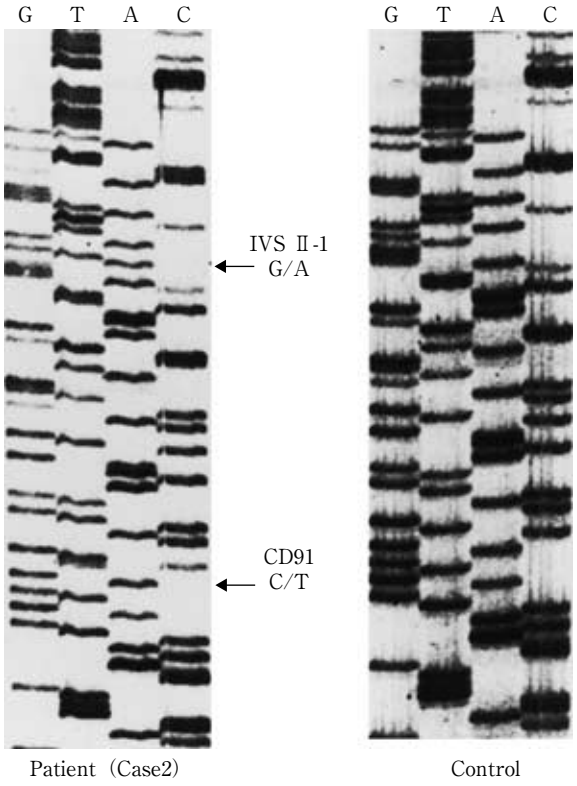


Fig. 2 Sequence of the normal control (right) and case 2 patient (left). The G→A mutation at position 1 of IVS II and the C→T mutation in codon 91 of the β-thalassemia genes were observed.

would cause the splicing region to disappear, resulting in β^0-thalassemia. Also, a silent mutation of cytosine to thymine was observed at codon 91 (**Fig. 2**).

The patient's mother and a brother had similar microcytic anemia. Her mother's Hb level was 115 g/L and her brother's was 123 g/L; respective MCV values were 65.0 fL and 60.2 fL. Both had high Hb A_2 and Hb F levels. The ratio of β-globin to α-globin of her mother and brother were 0.64 and 0.8, respectively. The same mutations were found on the βIVS II-1 region in both.

Discussion

Thalassemia is characterized by abnormalities in the production of the α or β-globin chain. Thalassemia minor usually is not associated with any severe clinical disabilities. Patients with some types of α-thalassemia do not show any clinical signs or hematologic abnormalities. Although high levels of Hb A_2 and F are seen with β-thalassemia, these findings have not been shown in heterozygous α-thalassemia.

Case 1, with α-thalassemia, showed normal levels of Hb A_2 and Hb F, but case 2, with β-thalassemia, showed high levels of Hb A_2 and Hb F.

The incidence of thalassemia is higher than previous estimates for Japan. According to the reports, the frequency of β-thalassemia varies between 0.1%[1] and 0.03%[6] in Japan. The exact frequency of α-thalassemia is not known. Thus the -α/-α thalassemia is extremely low incidence. However, the –/αα type seems to be one fifth that of β-thalassemia[7]. One half of patients in Japan with α-thalassemia had the South East Asian type, the same as case 1. Up to now, 262 families with 35 different mutations (excluding Hb E) have been discovered in Japan[6]. IVS II 1 and codon 91 mutations also have been found in Japan[8]. IVS II-1 mutations, the fourth most frequent mutation in Japan, represent about 10% of all β-thalassemia mutations in Japan[6].

Anemia is detected is about 3% of male and 5% to 6% of female students during routine blood screening in junior high school[9]. Most patients experience anemia from iron deficiency. Thalassemia may be distinguishable from other microcytic anemias, such as iron-deficiency anemia, by blood test results, which show normal or slightly increased serum iron concentrations and normal total iron

binding capacity in thalassemia. Normal or slightly increased serum ferritin levels also seem to be indicative of thalassemia. Iron deficiency anemia is one of the most common diseases in female junior and senior high school students; so we should always keep the possibility in mind that some students may suffer from both iron deficiency anemia and thalassemia at the same time when we find them with severe microcytic anemia.

Many discrimination indices have been reported to distinguish between thalassemia and iron-deficiency anemia[10]. The Merzter index is the simplest index[11]. If the MCV/RBC ratio is 13 or less, thalassemia should be considered. On the other hand, iron-deficiency anemia should be suspected whenever the MCV/RBC ratio is greater than 13.

Diagnosis of thalassemia, therefore, should be confirmed by molecular analysis when one encounters a case of microcytic anemia or hypochromic microcytosis with a MCV/RBC ratio of 13 or less.

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