β^+ -Thalassemia with Hemochromatosis

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A 64-year-old man was admitted due to ascites. Laboratory data showed hemoglobin 6.7 g/dl, mean corpuscular volume 82 fl, and ferritin 2,360 ng/ml. Liver biopsy showed hemochromatosis. The diagnosis of β -thalassemia was suggested by a decreased ratio of β/α -globin synthesis *in vitro* (0.26). Cloning of the β -globin gene showed A-to-G mutation in the first base of the ATA box. He was confirmed to be homozygous for this specific allele by β -gene complex analysis and analysis of Southern blot hybridization of the α - and β -globin genes. His two sons were confirmed to be heterozygous for this allele.

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Introduction

β -Thalassemia is a heterogeneous inherited disorder characterized by defective synthesis of β -globin of normal adult hemoglobin A (HbA, subunit structure $\alpha_2\beta_2$) (1). Hemochromatosis is often associated with homozygous β -thalassemia (2). In Japan, however, such concomitance has rarely been reported to date. Recently we experienced a case of β^+ -thalassemia homozygote with hemochromatosis, and clarified the genetic abnormality of the β -globin gene.

Case Report

A 64-year-old Japanese male was admitted to our hospital because of abdominal fullness and pretibial edema. He had no history of blood transfusion or excessive drinking of alcohol. On admission, the conjunctivae were anemic and slightly icteric. The liver was felt 2 finger-breadths below the right costal margin, and the spleen 2 finger-breadths below the left costal margin. The abdomen was distended, and shifting dullness and fluctuation were observed. There was pitting edema of the lower extremities. His skin was not pigmented. Hematologic data on admission revealed hemoglobin 6.7 g/dl, hematocrit 22.2%, RBC $270 \times 10^4/\mu\text{l}$, reticulocytes 5.3%, WBC $6,800/\mu\text{l}$, platelets $22.2 \times 10^4/\mu\text{l}$, mean corpuscular volume (MCV) 82 fl, and

mean corpuscular hemoglobin (MCH) 24.8 pg. Biochemical data revealed total bilirubin 3.1 mg/dl, indirect bilirubin 1.7 mg/dl, albumin 3.4 g/dl, alanine aminotransferase 56 IU/l (normal value: 4–35 IU/l), aspartate aminotransferase 40 IU/l (normal value: 9–35 IU/l), lactic dehydrogenase 471 IU/l (normal value: 180–460 IU/l), serum iron 170 $\mu\text{g/dl}$, total iron binding capacity 194 $\mu\text{g/dl}$, ferritin 2,360 ng/ml, and haptoglobin <6 mg/dl. Hepatitis B surface antigen, antibody to hepatitis B core antigen, and antibody to hepatitis C virus (C100-3) were negative. Urinary sugar was negative and oral glucose tolerance test (75 g) was normal. His ^{51}Cr -labeled erythrocyte life span ($T_{1/2}$) was 14 days (normal value 32–40 days). After i.m. injection of 500 mg desferrioxamine, 24-hour iron excretion was 4.27 mg (normal value: <1.5 mg). Abdominal CT scan showed a marked increase in the density of hepatic parenchyma and splenomegaly (Fig. 1). On laparoscopy, the liver was dark brown with a nodular surface. Histologic examination of a liver specimen revealed remarkable hemosiderin deposition in both hepatocytes and Kupffer cells and pseudolobule formation, revealing the diagnosis of hemochromatosis (Fig. 2). Bone marrow examination was as follows: nuclear cell counts $14 \times 10^4/\mu\text{l}$, erythroblasts 67.5%, and M/E ratio 0.3. Isoelectric focusing of his hemolysate showed no abnormal Hb; Hb F and Hb A₂ comprised 7.45% (normal value: <1.5%) and 7.06% (normal value: 2.2–3.2%),

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Fig. 1. Abdominal CT scan of the patient is shown. Numbers 1 and 2 show the position where the CT numbers of hepatic parenchyma and spleen were measured, respectively. The hepatic parenchymal CT number was markedly elevated (91.1 U).

Fig. 2. Histologic findings of the liver. (A) Pseudolobules were surrounded by fibrous septa (Azan-Mallory stain, $\times 52$). (B) Hemosiderin was deposited in both hepatocytes and Kupffer cells (Perls' stain, $\times 520$).

Fig. 3. Pedigree of the patient's family. The arrow indicates the proband. $\square$: male, $\circ$: female, $\diagup$, $\diagdown$: deceased, $\blacksquare$: male with homozygous state for β -thalassemia, $\blacksquare$: male with heterozygous state for β^+ -thalassemia. The data of the proband and his sons are listed in Table 1.

respectively. The ratio of the β/α -globin chain biosynthesized in reticulocytes of his peripheral blood (3) was determined by urea CM-cellulose column chromatography (4) and liquid scintillation count. The ratio was 0.26 (normal value: 0.9–1.2), demonstrating that the synthesis of the β -globin chain was severely reduced.

Restriction fragment length polymorphisms (RFLP) were determined at the eight sites (Hinc II 5' to the ϵ gene, Xmn I 5' to the $G\gamma$ gene, Hind III within the $G\gamma$ and $A\gamma$ genes, Hinc II within and 3' to the $\Psi\beta$ gene, Ava II within the β gene and Bam HI 3' to the β gene) in the β globin gene cluster (5); a single haplotype (haplotype II) for RFLP at eight polymorphic sites was found (Fig. 4A). An about 7.5-kilobase (kb) fragment obtained from the digestion of the genomic DNA with Hind III was cloned in phage Charon 28 (6). About half a million of the recombinant phages were screened using the ^{32}P -labeled β IVS2 DNA fragment as a probe, and then, four clones containing the β -globin genes were obtained. The 2.8 kb Sph I-Pst I fragment containing the β -globin gene was subsequently subcloned in M13mp19 phage vector (6) to determine the nucleotide sequence of the whole β globin gene by the dideoxy method (7). The result revealed one base substitution of A-to-G at position -31 5' to the Cap site (the first base of the ATA box) (Fig. 4B, Fig. 5). The framework of the abnormal β -globin gene was +CTCT- or type 2 (8) (Fig. 4B). The same results were obtained from 4 clones. The direct DNA sequencing of polymerase chain reaction (PCR) products (9) demonstrated the single mutant in the ATA box and the same framework to the abnormal β globin gene described above. These results strongly suggested that the patient was homozygous for β thalassemia globin gene.

Furthermore, to rule out deletion of one of the β globin genes, genomic DNA obtained from the proband was digested with Bgl II, and hybridized with ^{32}P -labeled β IVS

(A) RFLP:

(B) Framework:

Fig. 4. (A) RFLP of the β -globin gene clusters at eight restriction sites. The markers (+) or (-) indicate the presence or the absence of the restriction site. (B) The framework of β -globin genes in the propositus and the sons.

Fig. 5. Autoradiogram of the sequencing gel in the probandus and the 1st son. The 2nd son showed the same sequence as the 1st son. Only the ATA box and adjacent regions are shown.

Fig. 6. Radiogram obtained by use of β IVS2 fragment and α -globin gene DNA fragment as probes after digesting DNA obtained by Bgl II. 1: normal control, 2: propositus, 3: 1st son, 4: 2nd son.

Table 1. Relative Intensity of α_1 -, α_2 -, and β -Globin Gene Band on Radiogram

	α_2 -gene	α_1 -gene	β -gene
Propositus	1.00	1.14	1.10
1st son	1.00	0.89	0.97
2nd son	1.00	0.94	1.03
Control	1.00	1.22	1.00

The intensity of the α_2 -gene is expressed as 1.00.

2 DNA fragment and α -globin gene DNA. The radioactive intensities of the gene bands (α_2 , α_1 , and β) on the radiogram of Southern blot hybridization were determined using a Fujix Bio-image analyzer (Model BAS 2000, Fuji Film Co. Ltd.). As shown in Fig. 6 and Table 1, the relative intensities of α_1 -gene and β -gene to α_2 -gene were equivalent between the propositus and the normal control. This result finally confirmed that this patient was homozygous for β -thalassemia globin gene.

Family Study

Our patient's parents had died of diseases except hematological or liver-related; their marriage was not consanguineous. Our patient had three healthy children (two sons and one daughter) (Fig. 3). We also studied blood of his two sons (Case A and Case B in Fig. 3). The results of the propositus and the sons are summarized in Table 2. Neither son had anemia; their levels of HbF were normal. However, they both had decreased levels of MCV and increased levels of HbA₂. The serum iron and ferritin concentrations were normal in both of them. The ratios of β/α -globin chain biosynthesized in reticulocytes of their peripheral blood were moderately reduced. Both normal (ATAAA) and mutant (GTAAA) sequences in the ATA box were shown by direct sequencing of the PCR products (Fig. 5). The frameworks of the mutant β -globin genes obtained from the sons were the same as the framework of the propositus. On the other hand, the framework of the normal β -globin genes obtained from the sons was T(Hgi AI:-)-G(Ava II:-)-T-C, which was different from that of the mutant gene (Fig. 4B). From these results, their genotypes were confirmed to be heterozygous for this allele.

Discussion

This case was initially admitted to our hospital due to decompensated liver cirrhosis. The increased CT number of hepatic parenchyma and the remarkably high level of serum ferritin suggested the diagnosis of hemochromatosis (10), which was confirmed by liver biopsy. Despite the existence of iron overload, he had hypochromic microcytic anemia with reticulocytosis and high levels of Hb F and Hb A₂. The suppressed ratio of β/α -globin chain biosynthesis confirmed the diagnosis of β -thalassemia. Furthermore, cloning of β -globin gene clarified A-to-G mutation in the first base of the ATA box, and the presented data clearly demonstrated that the propositus was homozygous for this specific allele. In addition, his two sons were asymptomatic and heterozygous for this allele.

In patients with β -thalassemia, deficient synthesis of β -globin leads to small red blood cells, which contain less hemoglobin than normal. The two phenotypes, β^0 - and β^+ -thalassemia, denote the absent and reduced β -globin production, in cis to the abnormal chromosome, respectively. In β^+ -thalassemia homozygotes, β -globin synthesis is decreased to 5–30% of that of normal individuals (1).

Recently, various molecular mechanisms of this decreased β -globin synthesis, including abnormalities of β -globin gene transcription and of post-transcriptional processes, have been identified (11–14). In this patient, a single base substitution within the ATA box was identified by the gene cloning, which could result in decreased transcription of the β -globin gene and a consequent reduction of β -globin synthesis. Other types of mutations in the ATA box have been reported (14–18), but the type of mutation identified in this case has not been reported in any other ethnic group, and it has been considered to be unique to Japanese (18). This ATA box mutation was first described in a Japanese patient in 1986 (19). Since then, three additional families have been reported (20), and only three cases, including the present case, were homozygous (19, 20).

The association of a particular haplotype of β -globin gene cluster with a specific β -thalassemia mutation and β -gene polymorphisms has been reported (8). In the present patient, the haplotype of β -thalassemia gene cluster was haplotype II and the β -globin gene framework was type 2. This association is inconsistent with the finding that

Table 2. Family Data

	Age (years)	RBC ($\times 10^4/\mu\text{l}$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	Hb F (%)	Hb A ₂ (%)	Fe ($\mu\text{g/dl}$)	TIBC ($\mu\text{g/dl}$)	ferritin (ng/ml)	β/α ratio
Proband	64	270	6.7	22.2	82	24.8	7.45	7.06	170	194	2,360	0.26
1st son (Case A)	39	516	13.7	36.6	79	25.4	0.36	5.22	70	278	52	0.69
2nd son (Case B)	37	540	14.7	33.6	80	25.6	0.91	5.06	131	319	54	0.74

β/α ratio: ratio of β -chain to α -chain biosynthesized in reticulocytes.

subjects with haplotype II usually have framework 1 (21). This unusual association has already been reported in patients with the same type of β -thalassemia (20).

Until recently, thalassemia has been considered to be rare in Japan, but recent study showed that the prevalence of β -thalassemia in the Japanese population is approximately 1:1000 (22). Since patients with thalassemia in Japan are usually heterozygous, their symptoms are mild or absent, and thalassemics with the complication of hemochromatosis are extremely rare in Japan (22). Previously, the causes of hemochromatosis in thalassemics had been considered to be due to excessive iron administration and frequent blood transfusion (2, 23, 24). Recent study showed that the increased iron absorption secondary to ineffective erythropoiesis could be the major cause of iron overload in thalassemics (2, 24, 25). Our patient had no history of blood transfusion or iron administration, and the cause of hemochromatosis was likely attributable to increased iron absorption. Other studies utilizing HLA typing have suggested that in patients with thalassemia minor, iron overload may be partially attributed to the concomitant inheritance of an idiopathic hemochromatosis gene (26, 27). In the present patient, the iron overload status was not revealed in his children by biochemical findings, and such concomitance is considered unlikely.

Further clinical and molecular biological studies in additional cases with thalassemia are necessary to clarify the relation between clinical phenotypes and genetic abnormalities.

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