Hb Gunma ($\beta^\text{Gunma}$) with Pulmonary Embolism

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A 67-year-old woman with pulmonary embolism was suspected to have $\beta$-thalassemia based on microcytosis, hemolysis and a negative red cell stability test. The DNA sequencing analysis of $\beta$-globin gene, however, revealed the deletion of three nucleotides within codon 127–128, leading to substitution of glutamine and alanine residues at 127 and 128 by proline, namely Hb Gunma. This mutant is characterized by the fact that no abnormal hemoglobin is detected in the circulating blood, and is classified as a thalassemic hemoglobinopathy. The present case showed a relatively hemolytic manifestation.

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Introduction

Hemoglobinopathy is defined as mutations that change the amino acid sequence of one of the globin chains. In contrast to hemoglobinopathy, $\beta$-thalassemia mutations cause the absence or reduction of $\beta$-globin synthesis due to affected DNA processing or non-functioning m-RNA. Clinically, most variants of hemoglobinopathy are asymptomatic. Some variants, however, show clinical symptoms such as anemia and jaundice due to decreased stability of the hemoglobin molecule; these are known as unstable hemoglobinopathy. For the differential diagnosis, it is noted that circulating abnormal hemoglobin is detected in unstable hemoglobinopathies but not in thalassemia (1). However, the highly unstable globin mutants represent $\beta$-thalassemia phenotype, because they are degraded soon after their synthesis and undetectable in the circulating blood. These globin variants are known as thalassemic hemoglobinopathies, which include $\beta^\text{Kowata}$-Yakushiji, $\beta^\text{Yakata}$ and $\beta^\text{Gunma}$ (Hb Gunma) in Japan. We report here a case of $\beta^\text{Gunma}$ with pulmonary embolism, which showed a more severe hemolytic manifestation compared to a previously reported case (2). The cause of embolization is also discussed.

Case Report

The proband was a 67-year-old Japanese woman who was admitted to the hospital because of sudden onset of general malaise and shortness of breath on February 2, 1993. She had undergone splenectomy at the age of 29 years, because of the presence of anemia and marked splenomegaly. Her previous medications included furosemide, denopamine, ferrous sulfate and vitamin B complex under the diagnosis of heart disease and anemia. Prior to admission she had often been sitting to take care of her ill husband. The patient was tentatively diagnosed as pulmonary embolism by a pulmonary perfusion scan, performed after the intravenous injection of $^{99mTc}$ macroaggregated human albumin. Intravenous infusion of urokinase considerably improved her symptoms. Warfarin potassium was then substituted and she was discharged 19 days after the admission.

Laboratory studies at the time of admission were as follows; red blood cell count (RBC) 388x10$^4$/µl, hemoglobin (Hb) 9.7 g/dl, hematocrit (Ht) 28.5%, reticulocytes 7.1%, mean corpuscular volume (MCV) 73.4 fl, mean corpuscular hemoglobin (MCH) 24.9 pg, mean corpuscular hemoglobin concentration (MCHC) 33.8 g/dl, white blood cell count (WBC) 8,500/µl, platelet count 385x10$^3$/µl, total bilirubin 1.9 mg/dl, unconjugated bilirubin 1.5 mg/dl, glutamate oxaloacetate transaminase (GOT) 21 IU/l (reference interval; 7–38), glutamate pyruvate transaminase (GPT) 32 IU/l (10–35), lactic dehydrogenase (LDH) 237 IU/l (150–450), Hepaplastin test 88%, antithrombin III 95%, protein C 71% and protein S 8.7 µg/ml (6.6–11.5).

One month later, the patient was re-admitted for further examination of anemia. She appeared well; her height was 143.1 cm, weight 47.3 kg, body temperature 36.1°C, pulse 54/minute and blood pressure 130/60 mmHg. On physical examination she was slightly anemic and subicteric. The heart was of normal size and no murmur was heard. The lungs were clear. The liver was not palpable. There was slight edema in the lower From the Niigata Prefectural Yoshida Hospital, Yoshida, Niigata, *Niigata Prefectural Kakizaki Hospital, Kakizaki, Niigata and **the Department of Biochemistry, Kawasaki Medical College, Okayama

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The laboratory studies at the time of the second admission were as follows; RBC 390×10⁴/μl, Hb 10.1 g/dl, Ht 29.4%, MCV 75.3 fl, MCH 25.9 pg, MCHC 34.4 g/dl, reticulocytes 7.1%, WBC 7,600/μl and platelet count 531×10³/μl. Blood film examination showed an aniso-poikilocytosis containing Howell-Jolly bodies and erythroblasts (67/100 WBC). No Heinz body was observed in red cells on staining with brilliant cresyl blue (Fig. 1). Serum Fe was 124 μg/dl, total iron binding capacity (TIBC) 240 μg/dl, ferritin 480 ng/ml, haptoglobin 35 mg/dl (reference interval; 45–320), hemopexin 20 mg/dl (63–109), vitamin B₁₂ 834 pg/ml and folic acid 1.6 mg/ml. The GOT was 19 IU/l, GPT 18 IU/l, LDH 335 IU/l, total bilirubin 2.0 mg/dl and unconjugated bilirubin 1.6 mg/dl. The direct and indirect Coombs tests were negative. The bone marrow smear showed erythroid hyperplasia (myeloid erythroid ratio 0.18) with a moderate degree of megaloblastic change. The hemostasis tests were as follows: prothrombin time 14.4 seconds (69.4%),

Fig. 1. Peripheral blood film stained with brilliant cresyl blue (×740). The red cells show aniso-poikilocytosis. Erythroblasts (E), Howell-Jolly bodies (arrows) and reticulocytes (R) are seen. No Heinz body is found.

Fig. 2. Hemoglobin electrophoresis by polyacrylamide gel isoelectric focusing of the hemolysate (pH range 6.7–7.7). It shows that HbF is below 1% and HbA₂, 4.54%. No abnormal hemoglobin is detected.

Fig. 3. DNA sequence of the β-globin gene (clone A) derived from the β¹_gunma and normal alleles (β¹_globein gene) by dideoxy method. The nucleotide sequence of the sense strand, corresponding amino acids and codon numbers are also shown. AGG deletion within codons 127 and 128 results in substitution of glutamine and alanine residues by proline at codon.
activated partial thromboplastin time 34.5 seconds and serum fibrinogen 316 mg/dl. The platelet aggregation tests by adenosoine diphosphate and collagen were normal. The serum platelet factor-4 was 11 ng/ml (reference interval; below 20) and β-thromboglobulin 48 ng/ml (below 60).

Isoelectric focusing of the hemolysate (pH 6.7–7.7) revealed that HbF was below 1% and HbA2 4.54%; no abnormal hemoglobin was detected, however (Fig. 2). The isopropanol test was negative. The globin chain synthesis ratio (β/α) by reticulocyte was 0.49. The DNA was obtained from peripheral blood of the patient. After amplification by PCR-related techniques, the DNA sequence analysis was performed by the direct cloning and dideoxy methods. Two different genes were, then, identified; one was normal and the other was AGG deletion within codons 127 and 128 in exon 3 of the β-globin gene (Fig. 3). The proband has 5 siblings, 3 sons and 2 grandchildren (Fig. 4). Of these family members, 4 were examined hematologically and the data was as follows: elder sister (II-2 Fig. 4); RBC 417×10^4/μl, Hb 12.8 g/dl, Ht 37.8%, MCV 90.7 fl, MCH 30.8 pg, MCHC 33.9 g/dl, reticulocytes 1.4%; youngest son (III-2); RBC 614×10^4/μl, Hb 12.4 g/dl, Ht 39.9%, MCV 65.0 fl, MCH 20.2 pg, MCHC 31.0 g/dl, reticulocytes 1.5%; one of her grandchildren (IV-1); RBC 470×10^4/μl, Hb 13.0 g/dl, Ht 38.9%, MCV 82.7 fl, MCH 27.6 pg, MCHC 33.4 g/dl, reticulocytes 0.9%; another grandchildren (IV-2); RBC 525×10^4/μl, Hb 9.6 g/dl, Ht 30.0%, MCV 57.1 fl, MCH 18.3 pg, MCHC 32.0 g/dl, reticulocytes 2.4%. In Fig. 4, the family members with microcytosis are shown as a closed circle or squares.

**Discussion**

The present patient experienced abrupt onset of shortness of the breath. The radiologic image and other studies suggested pulmonary embolism. At that time however the other labora-

tory data revealed no definitive abnormal findings for hemostasis but showed moderate anemia, hyperbilirubinemia of unconjugated type, low value of haptoglobin, erythroid hyperplasia of the bone marrow and negative Coombs test. Judging from the data, she was tentatively diagnosed as inherited hemolytic anemia. The additional findings of microcytic hypochromic anemia, no Heinz body and negative test for isopropanol stability suggested that she had β-thalassemia. The result of DNA sequencing analysis, however, revealed the mutation of Hb Gunma (β^Gunma) or β127–128Gln→Ala→Pro which expresses a phenotype of β-thalassemia. No abnormal hemoglobin is detected in this case by heat and isopropanol stability tests because, in comparison with other hemoglobinopathies, this structurally changed β-globin molecule is so highly unstable that it is rapidly destroyed after synthesis (2). This globin variant is therefore classified as a thalassemic hemoglobinopathy.

Hattori et al (3) reported the first case of this variant in four unrelated families in 1989. In 1990, Fucharoen et al (2) reported the same variant of a 36-year-old man in Gunma Prefecture in Japan, and named it Hb Gunma after genetic studies. The hematological data of the case reported by Fucharoen et al was as follows; Hb, 11.3 g/dl; Ht, 38.3%; MCV, 64.2 fl, MCH, 19.8 pg; MCHC, 30.8 g/dl; HbF, 3.4%; HbA2, 4.3%; slight anisopoikilocytosis of erythrocyte without inclusion; negative test for heat and isopropanol stability and the globin chain synthesis ratio (β/α), 0.45. Compared to these data, the globin chain synthesis ratio (β/α) of the present case was 0.49, similar to the value of the original case. Otherwise, hemolytic manifestation such as anemia and jaundice was more prominent in the present case. In fact, she had undergone splenectomy because of marked splenomegaly (25 cm spleen size) probably due to severe hemolysis at the age of 29 years. The reason for the differing clinical features between the original and present cases remains unresolved.

The hematological studies of family members revealed microcytosis, aniso-poikilocytosis and a few of nucleated red cells in the peripheral blood smear in her son (III-2, Fig. 4) and one of her grandchildren (IV-1), suggesting that they are heterozygous for the same mutation. The DNA sequence analysis, however, was not undertaken.

In addition to the Hb Gunma, there have been 5 variants ofthalassemic hemoglobinopathy reported, such as β^Terre Haute (codon106 Leu→Arg) (4), β^Houston (codon127 Gln→Pro) (5), β^Geneva (elongated β-chain) (6), β^Showa-Yakushiji (codon110 Leu→Pro) (1) and β^Yaksh (elongated β-chain) (7). The β^Indianapolis (codon112 Cys→Arg) (8) had been also reported as thalassemic hemoglobinopathy. Recent studies, however, revealed that this abnormal hemoglobin was only mildly unstable in contrast to the first case (9, 10). The sequencing of DNA by PCR method was reexamined using the preserved bone marrow sample obtained from the first patient. This study confirmed a substitution of leucine to arginine at 106 residue of β-globin chain. And the mutation is renamed Hb Terre Haute (β^Terre Haute) (4) as previously described. These mutants of thalassemic hemoglobinopathy show clinically moderate to severe anemia and their mutations are linked to a post translational process of β-
globin chain synthesis (2, 7). In these mutants, the inclusion bodies in red blood cells due to the precipitation of free α-chains are confirmed in the β*Geneva* and β*Makabe*, whereas no inclusion body is found in the other thalassemic hemoglobinopathies.

In addition to the hematological features, the occurrence of pulmonary embolism may characterize the present case. The pathophysiology of vasoocclusion has been discussed in HbS (11), and hemoglobinopathies with erythrocytosis are known as a risk factor of embolism. However the pulmonary embolism of the present case did not result from the abnormal hemoglobin, because it did not circulate through blood vessels. The present case showed no definitive data of hypercoagulability, since all hemostatic tests were normal. In the present case, pulmonary embolism may be induced by embolization of venous thrombus in the legs formed by such risk factors as hemoconcentration due to ingestion of diuretics under the diagnosis of heart disease and/or venous congestion caused by a prolonged sitting posture.

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References


