

Case report

Compound heterozygote for hemoglobin C and β -zero thalassemia in Thailand

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Abstract:

Hemoglobin (Hb) C is a hemoglobinopathy caused by the amino acid substitution of glutamic acid (Glu) to lysine (Lys), (GAG>AAG), at codon 6 of the beta-globin gene. Subjects with Hb C trait have no clinical symptoms, while patients with Hb C disease (homozygous Hb C), or compound heterozygote of Hb C and Hb S, Hb E or other beta-globin mutations may have mild to moderate chronic hemolytic anemia. We investigated cause of anemia in a 30-year-old Thai female. The result from CBC showed Hb of 10.2 g/dL and mean corpuscular volume of 64 fL. Therefore, Hb typing using high performance liquid chromatography (HPLC) was performed. The result from HPLC showed Hb F of 17.6%, Hb A₂ of 3.8%, and Hb C of 74.4%. The β -globin gene sequencing was investigated and showed HBB: c.19G>A (Hb C) and HBB: c.-74_31del (105 bp deletion). As a result, this patient received a diagnosis of compound heterozygosity for Hb C/ β^0 -thalassemia.

Keywords : ● Hemoglobin C ● $\beta(0)$ -thalassemia

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รายงานผู้ป่วย

Hemoglobin C ที่มี β -zero thalassemia ร่วมด้วยในประเทศไทย

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บทคัดย่อ

Hemoglobin (Hb) C เป็นฮีโมโกลบินผิดปกติที่เกิดจากการเปลี่ยนกรดอะมิโนจาก glutamic acid (Glu) เป็น lysine (Lys) หรือมีการเปลี่ยนแปลงของกรดนิวคลีอิก $GAG \rightarrow AAG$ ตรงตำแหน่ง codon ที่ 6 บนสาย β -globin รายงานที่เป็นพาหะของ Hb C จะไม่มีอาการทางคลินิก แต่ในรายที่เป็น Hb C disease (homozygous Hb C) หรือ heterozygous Hb C ร่วมกับ Hb S, Hb E และ β -globin mutation อื่น ๆ จะพบภาวะโลหิตจางจากเม็ดเลือดแดงแตกเรื้อรังระดับความรุนแรงตั้งแต่เล็กน้อยจนถึงปานกลางได้ รายงานฉบับนี้เป็นรายงานของผู้ป่วยหญิงไทยอายุ 30 ปี มีค่าฮีโมโกลบิน 10.2 g/dL และปริมาตรของเม็ดเลือดแดง (Mean Corpuscular Volume หรือ MCV) 64 fL ได้รับการตรวจวิเคราะห์หาชนิดและปริมาณฮีโมโกลบินในเลือด (hemoglobin typing) โดยหลักการ high performance liquid chromatography (HPLC) ผล คือ Hb F = 17.6%, Hb A₂ = 3.8% และ Hb C = 74.4% เมื่อตรวจยืนยันด้วยวิธี β -globin gene sequencing ได้ผลเป็น HBB: c.19G>A (Hb C) ร่วมกับ HBB: c.-74_31del (105 bp deletion) สรุปได้ว่า ผู้ป่วยรายนี้เป็น compound heterozygote ของ Hb C/ β^0 -thalassemia

คำสำคัญ : ● ฮีโมโกลบินซี ● บีตา(0)-ธาลัสซีเมีย

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2568;35:151-7.

Introduction

Hemoglobin (Hb) C is one of the three most common hemoglobinopathy worldwide. It is caused by an amino acid substitution of glutamic acid (Glu) with lysine (Lys) (GAG>AAG) at codon 6 of the β -globin gene.¹⁻¹³ Hb C is found commonly in West Africa, North Africa, the United States and Southern Europe.^{1-3,7,10,12} Individuals with Hb C trait exhibit no clinical symptoms, and their mean corpuscular volume (MCV) ranges from low to normal (74.0-97.9 fL).^{11-13,16} However, patients with Hb C disease (homozygous Hb C) present chronic hemolytic anemia. Hb C can be co-inherited with other hemoglobinopathy such as Hb S, Hb E, Hb D-Punjab or β -thalassemia heterozygotes. Patients with Hb C ,co-inherited with Hb S exhibit symptoms similar to those of sickle cell disease, though typically less severe. Patients with compound heterozygosity for Hb C and other β -globin mutations often manifest moderate anemia and splenomegaly, which is generally categorized as β -thalassemia intermedia, in most reported cases.^{1-3,7-10,16} In this report we discuss a rare case of compound heterozygosity for Hb C with β^0 -thalassemia in a Thai patient.

Case report

A 30-year-old Thai female presented with mild anemia. She had undergone a cholecystectomy at the age of 15, had never received a blood transfusion and reported no

family history of thalassemia. On physical examination, she was not pale but exhibited mild jaundice and increased splenic dullness without hepatomegaly. Blood tests revealed the following: CBC--Hb 10.2 g/dL, Hct 29.7%, RBC $4.65 \times 10^{12}/L$, MCV 64 fL, MCH 22 pg, MCHC 34 g/dL, RDW 21.1%, WBC $8.8 \times 10^9/L$, PLT $328 \times 10^9/L$, reticulocyte count 3.0%; biochemical parameters--total bilirubin 1.74 mg/dL, direct bilirubin 0.61 mg/dL, AST (SGOT) 15 U/L, ALT (SGPT) 13 U/L, alkaline phosphatase (ALP) 43 U/L, albumin 4.8 g/dL, globulin 2.5 g/dL, total protein 7.3 g/dL and ferritin 269.0 ng/mL. A peripheral blood smear (PBS) showed hypochromic microcytic red blood cells, anisocytosis 2+, poikilocytosis 2+, target cells 3+ and microspherocytes 2+ (Figure 1). Clinical findings and laboratory results indicated microcytic anemia with indirect hyperbilirubinemia (65.9% of total bilirubin), splenomegaly and a history of gallstones, prompting further investigation for thalassemia. Hb typing using high performance liquid chromatography (HPLC) showed the following: Hb F 17.6%, Hb A₂ 3.8% and Hb C 74.4% (Table 1, Figure 2). The absence of Hb A suggested the patient was either homozygous for Hb C or a compound heterozygote for Hb C and β -thalassemia. To confirm the genotype, DNA analysis for common β -thalassemia mutations was performed using multiplex ARMS-PCR. Results were negative for 17 common β -thalassemia mutations including: -28 (A>G), -29 (A>G), -30 (T>C),

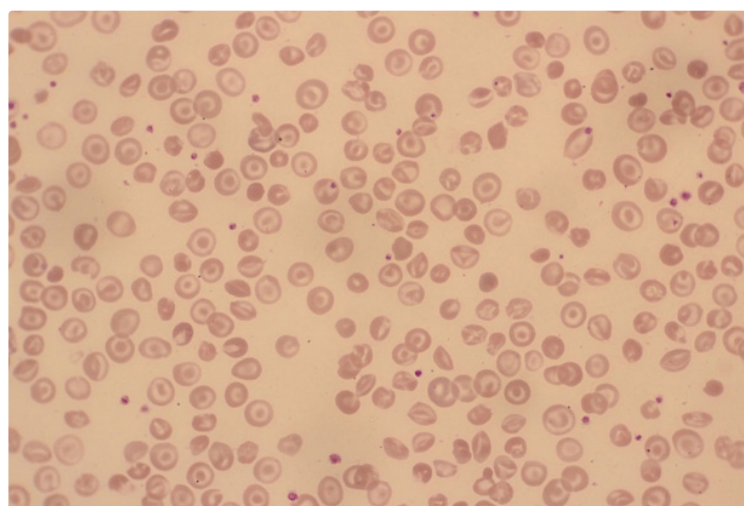


Figure 1 Peripheral blood smear showed hypochromic microcytic red blood cells, anisocytosis 2+, poikilocytosis 2+, target cells 3+ and microspherocytes 2+

Table 1 Results of hemoglobin typing using high performance liquid chromatography of the patient and her family

Parameter	Patient	Mother	Sister
Hb A (%)	-	81.6	80.3
Hb A ₂ (%)	3.8	4.7	4.7
Hb F (%)	17.6	2.8	4.7
Hb C (%)	74.4	-	-

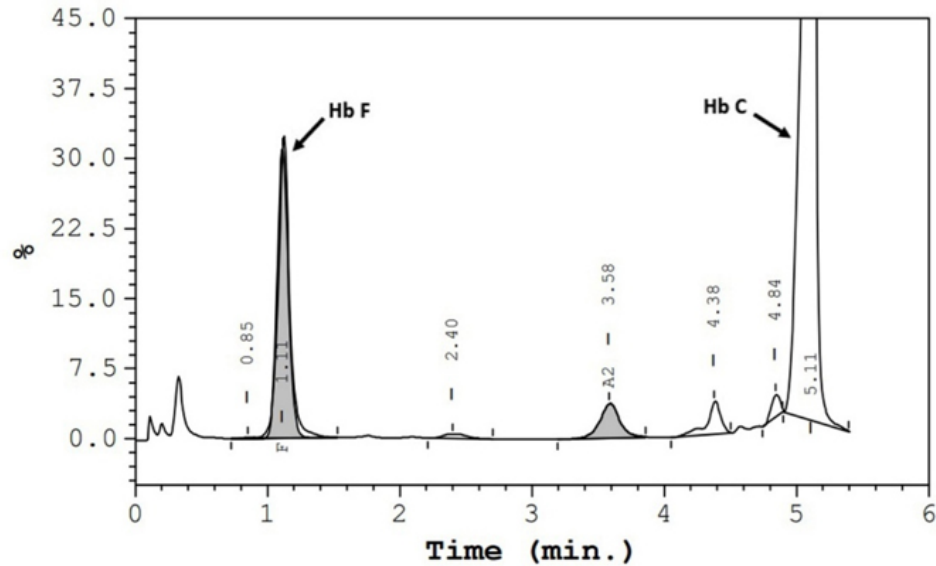


Figure 2 Patient’s chromatogram from high performance liquid chromatography

codon 8/9 (+G), codon 15 (G>A), codon 17 (A>T), codon 19 (A>G), codon 26 (G>A), codon 27/28 (+C), IVS1-5 (G>C), codon 35 (C>A), codon 41 (-C), codon 41/42 (-TCTT), codon 43 (G>T), codon 71/72 (+A) and IVS1-654 (C>T). Subsequent direct sequencing of the β -globin gene revealed a compound heterozygous state consisting of a point mutation at codon 6 (GAG>AAG), corresponding to Hb C, and a 105 bp deletion spanning nucleotide positions -74 to +31, consistent with β -thalassemia. Given the mild clinical severity, DNA testing for β -globin gene deletions was performed to evaluate potential co-inheritance of β -thalassemia, but the results were negative. In conclusion, this patient was diagnosed with compound heterozygosity for Hb C and β -thalassemia.

The patient’s mother and sister also presented with mild anemia, while other physical examinations were normal. The mother had an Hb of 11.4 g/dL and MCV of 63.2 fL, while the sister had an Hb of 10.5 g/dL and MCV of 60.0 fL. Hb typing revealed an A₂A pattern

with Hb A₂ > 3.5% (Table 1), consistent with β -thalassemia trait. To confirm the β -globin mutation, direct sequencing was performed and identified the same 105 bp deletion found in the patient. The father’s blood results were unavailable because he denied consent for testing. The patient’s pedigree is shown in Figure 3.

Discussion

The prevalence of Hb C in Thailand is uncommon, with most cases involving Hb C trait or Hb C co-inherited with Hb E.^{11-14,16} However, the combination of Hb C with β^0 -thalassemia has not been widely reported in Thailand. Compound heterozygosity for Hb C and β -thalassemia is classified as β -thalassemia intermedia, with clinical severity ranging from mild to moderate anemia (Table 2), mild splenomegaly, and no history of blood transfusion.^{2-4,7-10,16} In this case, the patient presented with mild anemia, mild splenic enlargement and no history of blood transfusion.

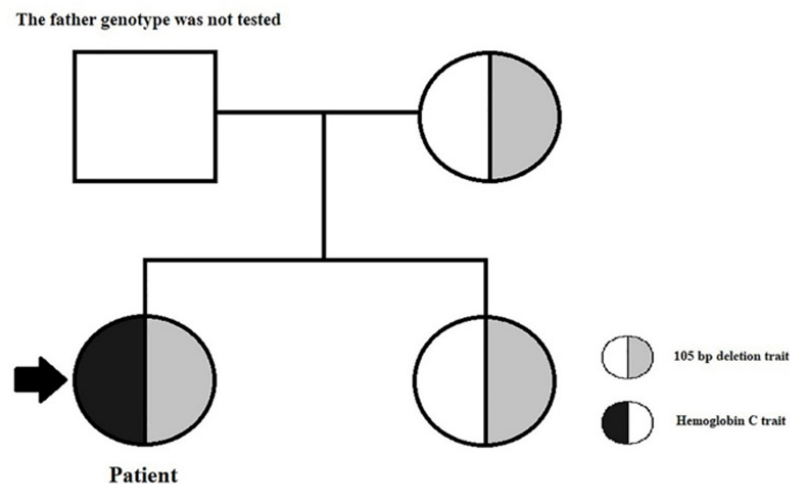


Figure 3 Patient's Pedigree

Table 2 Review of hematologic parameters and molecular studies of patients with compound heterozygote of Hb C and β^0 -thalassemia

Case	1 ⁽⁷⁾	2 ⁽⁸⁾	3 ⁽⁹⁾	Patient in this report
Sex-Age	M-22	M-45	M-53	F-30
RBC ($10^{12}/L$)	4.94	N/A	5.68	4.65
Hb (g/dL)	9.9	8.6	11.5	10.2
Hct	N/A	N/A	37.0	29.7
MCV (fL)	60.9	N/A	65	64
MCH (pg)	20.1	N/A	20	22
MCHC (g/L)	N/A	N/A	N/A	34
RDW (%)	19.3	N/A	N/A	21.1
Hb A (%)	-	N/A	N/A	-
Hb A2 (%)	5.7*	N/A	5.0*	3.8*
Hb F (%)	9.5*	14.0*	5.8*	17.6*
Hb C (%)	80.9*	78.0*	88.6*	74.4*
α Genotype	N/A	$\alpha\alpha/\alpha\alpha$	N/A	$\alpha\alpha/\alpha\alpha$
Genomic DNA analysis for β^0 -thalassemia	Hb C and CD 8/9 (+G) (β^0) [HBB: c.27_28insG]	Hb C and codon 177 (-C) (β^0) [HBB: c.354delC]	Hb C and codon 6 (-A) (β^0) [HBB: c.20delA]	Hb C and 105 bp deletion (β^0) [HBB: c.-75_31del]

* All hemoglobin typing results were obtained using high performance liquid chromatography

The PBS in Hb C cases often shows a significant number of target cells, similar to those seen in Hb E. However, in Hb C trait, only few target cells are typically observed. PBS findings in Hb C disease (homozygous Hb C) include microcytosis, spherocytes, target cells and Hb C crystals. In contrast, PBS findings for compound heterozygosity for Hb C and β -thalassemia (both β^0 and β^+) resemble those of Hb C disease but lack Hb C crystals.²⁻⁶

Hb C cannot be distinguished from Hb E using alkaline electrophoresis; however, HPLC and capillary electrophoresis (CE) techniques can effectively differentiate between Hb C and Hb E.^{14,16} Hb typing for Hb C trait typically shows Hb C levels of 30-40% and Hb A levels of 50-60%. In compound heterozygosity for Hb C and β^+ -thalassemia, Hb C ranges from 65-80%, Hb A from 10-30% and Hb F from 2-5%. For compound heterozygosity for Hb C and β^0 -thalassemia, Hb typing

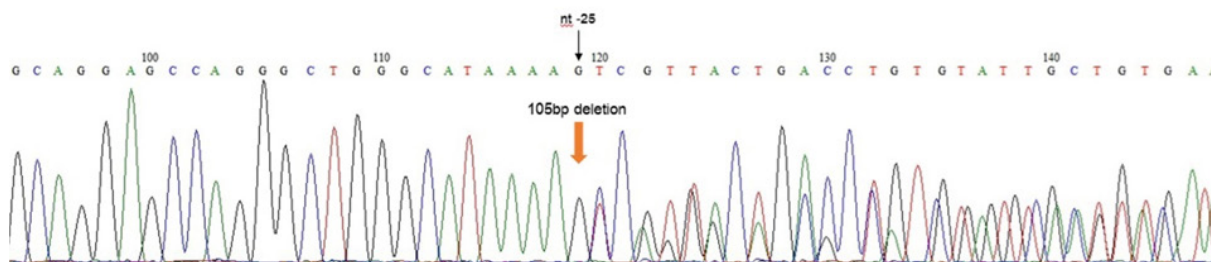


Figure 4 Sanger sequencing for deletion of 105 bp (HBB: c.-74_31del)

reveals predominantly Hb C, Hb F levels of 9.5-24.5% and no Hb A. In Hb C disease, Hb C levels are 93.0-98.2%, with a slight increased or absence of Hb F and no Hb A.^{1-3,5-10,17}

In this report, the patient's Hb typing showed only Hb C and Hb F, with elevated Hb F levels of approximately 20%. This profile is consistent with compound heterozygosity for Hb C and β^0 -thalassemia. Direct sequencing of the β -globin gene confirmed a 105 bp deletion spanning from the 5' untranslated region (UTR), 25 nucleotides upstream of the mRNA cap site, to 81 nucleotides downstream of the mRNA cap site in exon 1 (Figure 4). This frameshift deletion disrupts mRNA production and subsequent β -globin chain synthesis, leading to β^0 -thalassemia. The 105 bp deletion represents a rare form of β^0 -thalassemia, which cannot be detected using standard DNA analyses for common β -thalassemia mutations in Thailand.

Conclusion

Hb C is a relatively uncommon hemoglobinopathy, yet it is observed worldwide. The diagnosis of Hb C disease or compound heterozygosity for Hb C and β^0 -thalassemia requires differentiation through laboratory testing. On PBS, Hb C disease is characterized by the presence of Hb C crystal, which are absent in compound heterozygosity for Hb C and β^0 -thalassemia. Hb typing in both Hb C disease and compound heterozygosity for Hb C and β^0 -thalassemia predominantly shows Hb C without Hb A. However, Hb C/ β^0 -thalassemia is distinguished by elevated levels of Hb F, whereas Hb C

disease shows only slightly increased or absent Hb F. Direct sequencing of the β -globin gene is essential to confirm rare β -globin mutations, particularly in cases where standard diagnostic methods may not detect them.

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