Two pregnant women with abnormal hemoglobin tests confirmed to have rare mutations for β-thalassemia: A case series study

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Abstract

Thalassemia is a well-understood genetic disorder, and routine genetic tests typically cover 95% of known genetic mutations. Discordance between the clinical phenotype and genotypes suggest that expanded genetic studies should be performed to look for rare mutations.We report two pregnant women at 17 week gestations. Routine laboratory and geneti

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Abstract

Background: Thalassemia is a well-understood genetic disorder, and routine genetic tests typically cover 95% of known genetic mutations. Discordance between the clinical phenotype and genotypes suggest that expanded genetic studies should be performed to look for rare mutations.

Methods: We report two pregnant women at 17 week gestations. Routine laboratory and genetic tests were conducted first. DNA sequencing was then performed to detect potential rare thalassemic mutations.

esults: Patient 1 was a 25-year-old pregnant woman with mild anemia (hemoglobin 98 g/L; red blood cell count 4.89×10^{12}/L; mean corpuscular volume 59.71 fL; mean corpuscular hemoglobin 20 pg). Patient 2 was a 22-year-old pregnant woman with abnormal hemoglobin electrophoresis results (HbA 53.9%; HbA_2 2.6%; and HbF 0%). Both had negative results via routine genetic screening for thalassemia. DNA sequencing identified a heterozygous *HBB* : c.43delC (P.Leu15cysfs*5) mutation in patient 1 and a heterozygous *HBB* : c.341T>A (p.V113E) mutation in patient 2.

onclusion: Two rare β-thalassemic genotypes (c. 43delC/β^N and c.341T>A/β^N) were identified in these two pregnant women. Necessary genetic tests should be expanded in patients with a high suspicion for thalassemia.

Key words: β-thalassemia; β-globin; HBB gene; rare mutation

Introduction

Thalassemia is a heterogeneous group of hemolytic diseases; it follows an autosomal- recessive inheritance pattern and is caused by a globin synthesis disorder due to an α- and/or β-globin gene mutation(s). Its clinical phenotypes range from asymptomatic to fatal hemolytic anemia or even fetal demise (Taher, Weatherall, & Cappellini, 2018). Currently in China, the routine genetic tests for thalassemia include three common deletions and three point mutations in the α-globin gene and 17 point mutations in the β-globin gene, which collectively account for over 95% of α- and β- thalassemic gene mutations. Approximately 5% of globin gene mutations that are rare or unknown are not tested for during routine screening (Taher, Otrock, Uthman, & Cappellini, 2008; Sankaran & Nathan, 2010; Rachmilewitz & Giardina, 2011; Viprakasit & Ekwattanakit, 2018). In clinical settings, discordance between the clinical phenotype and detected genotype suggest that certain genetic mutations might be missed. Therefore, further genetic tests are required to reveal rare genetic mutations in order to improve the diagnosis and treatments of thalassemia (Steinberg, 1988; Lo & Singer, 2002).

β-Thalassemia is one of the most common genetic diseases of hemoglobin synthesis worldwide. It results from diminished or absent β-globin (HBB) gene expression (Galanello & Origa, 2010; Origa, 2017). The human HBB gene is located in the chromosome 11p15.5 region. Most β-thalassemic cases are caused by a point mutation in the *HBB* gene, with only a small proportion of cases having an *HBB* deletion mutation. More than 200 *HBB* mutations have been identified; among them, more than 40 have been reported in the Chinese population (Cao & Galanello, 2010; Asadov et al., 2018).

We here in report the genetic diagnosis and phenotypes of two rare β-thalassemic mutations in pregnant women with mild anemia or hemoglobin abnormality, but with a negative result from routine reverse dot blot hybridization (RDBH) screening.

Materials and Methods

Ethical statement

This study was approved by the Medical Ethics Committee of the Women and Children Healthcare Hospital of Zhuzhou (No. 2017037). Written informed consent was obtained from the patients.

Patients

Two pregnant women at 17 weeks gestation were referred to our hospital for further thalassemia screening. Patient 1 was a 25-year-old woman who had mild anemia reported in her routine blood test. Patient 2 was a 22-year-old woman who had hemoglobin abnormality detected in her hemoglobin electrophoresis test.

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Hematologic tests

Two milliliters of peripheral venous blood was drawn for complete blood counts using an automated blood cell counter (XE-5000, Sysmex Company, Japan). Hemoglobin was analyzed using an automatic high-pressure capillary electrophoresis device (Capillarys 2 FlexPiercing, SEBIA Company, France) according to the manufacturer's instructions.

Routine genetic screening

The three common deletion types (SEA, -α3.7, and -α4.2) and three point mutations of α-thalassemia (CS, QS, and WS) were tested by Gap-polymerase chain reaction (PCR) and RDBH. We also tested the following

17 β-thalassemic point mutants: CD41-42 (-TCTT), CD43 (G-T), -28 (A-G), -29 (A-G), -30 (T-C), -32 (C-A), CD71-72 (+A), βE, CD17 (A-T), CD31 (-C), CD14-15 (+G), CD27-28 (+C), IVS- I -1 (G-T), IVS- I -5 (G-C), IVS- II -654 (C-T), CAP+1(A-C), and Int (ATG-AGG) using PCR and RDBH methods. All these tests were conducted with the α- or β-thalassemia gene-detection kit (Hybribio, Guangzhou, Guangdong, China) according to the manufacturer's instructions.

DNA-sequencing analysis

Genomic DNA was isolated using the MiniBEST Whole Blood Genomic DNA Extraction Kit (Takara, Dalian, China). We performed PCR using PrimeSTAR HS DNA Polymerase (Takara, Dalian, China) on a GeneAmp PCR System 9700 (Applied Biosystems, USA) under the following conditions: predenaturation at 94°C for 5 min; then 35 cycles of denaturation at 94°C for 1 min; annealing at 58°C for 1 min; and primer template extension at 72°C for 2 min; followed by a final extension at 72°C for 10 min. The primers that we used for PCR amplification were *HBA1*forward, 5'-TGGAGGGTGGAGACGTCCTG-3'; *HBA1* reverse, 5'-TCCATCCCCTCCTCCCGCCCCTGCCTTTTC-3'; *HBA2* forward, 5'-TGGAGGGTGGAGACGTCCTG-3'; *HBA2* reverse, 5'-CCATTGTTGGCACATTCCGG-3'; *HBB* forward, 5'TACCCTTGGACCCAGAGGTTCTTTG-3'; and *HBB* reverse, 5'-ATGGTAGCTGGATTGTAGCTG-3'. Sanger sequencing was conducted at Tsingke Biotechnology (Beijing, China).

Results

Hematologic test results

esults of the hematologic tests included red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hemoglobin electrophoresis (Table 1, Figure 1). Patient 1 had mild anemia with a significantly reduced MCV. Patient 2 had a significant reduction in the percentage of hemoglobin A.

Genetic test results

We detected no α- or β-thalassemic mutations in both pregnant women during the routine genetic screening. Sanger sequencing identified a heterozygous *HBB* : c.43delC mutation in patient 1 (Figure 2). This mutation was located at codon 15 in exon 1 of the *HBB* gene. Deletion of the coding nucleotide at position 43 caused a translational frameshift that resulted in premature termination of translation at the fifth amino acid after the deletion and yielded a largely truncated β chain. Sanger sequencing revealed a heterozygous *HBB* : c.341T>A (p.V113E) mutation in patient 2 (Figure 2).

Discussion

β-Thalassemia is a common blood disorder with autosomal recessive inheritance that is principally caused by hemoglobin beta (HBB) gene mutations. Most patients with β-thalassemia cannot produce enough healthy red blood cells. As such, they require frequent blood transfusions throughout their lifespans. Blood transfusions may cause various complications, including progressive liver failure, abnormal renal functions, endocrine dysfunctions, and growth retardation, which can seriously affect the quality of life and result in early death in adolescence (Karimi, Cohan, De Sanctis, Mallat, & Taher, 2014; He et al., 2017). Therefore, it is important to improve our understanding of the β-thalassemia etiology in order to provide early diagnosis and management.

The present study reported two pregnant women who experienced mild anemia or hemoglobin abnormality as detected by routine blood tests and hemoglobin electrophoresis. However, we did not identify any α- or β-globin gene-related deletions or point mutations in the routine thalassemic screening. To identify potentially rare or unknown thalassemic mutations, we performed further genetic tests in these two patients. Sanger sequencing revealed a rare frameshift mutation c.43delC (p.Leu15Cysfs*5) in the *HBB* gene in the 25-year-old pregnant woman. Upon database and literature searches, we found that this mutation was first reported in a one-year-old girl with significantly reduced MCV (63.4 flz) and MCH (18.2 pg). Here in, we reported a second case of the *HBB* : c.43delC variant. Our findings provided additional information regarding the

hematologic phenotype of this mutation. In the 22-year-old pregnant woman with a hemoglobin abnormality (HbA, 53.9%), we observed another rare β-thalassemic mutation, *HBB* : c.341T>A (p.V113E), also known as the Hb New York variant. Due to conflicting reports regarding hematologic disorders associated with this variant, its clinical significance remains uncertain. The hematologic phenotype of the 22-year-old pregnant woman with the *HBB* : c.341T>A variant in our study could enhance our understanding of this rare variant.

The vast majority of thalassemic patients can be screened by routine blood tests combined with hemoglobin electrophoresis. Based on the hematologic test results, further genetic tests can be performed to confirm diagnosis. However, routine thalassemia genetic screening testing was unable to detect rare thalassemic gene mutations. Therefore, for cases with a high suspicion for thalassemia, we recommend further evaluations be carried out using technologies, such as Sanger sequencing, next-generation sequencing, or exome sequencing, to provide an accurate diagnosis and facilitate future individualized gene therapy.

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Figure legends

Figure 1. Hemoglobin electrophoresis shows a significant fraction of abnormal hemoglobin in a 22-year-old pregnant woman (patient 2).

Figure 2. Diagram of the *HBB* gene structure with mutations (A). Results of Sanger sequencing for these two patients (B and C).

Table 1. Hematologic test results from the pregnant women in this study.

PMeasurements	Patient 1	Patient 2	Reference range
RBC count (10^{12}/L)	4.89	4.46	3.0–5.0
Hb (g/L)	98	117	110–150
MCV (fL)	59.7	78.7	80–100
MCH (pg)	20	26.2	27–34
HbA (%)	93.7	53.9	[?]94.5%
HbA_2 (%)	5	2.6	2.5–3.5%
HbF (%)	1.3	0	[?]2%

RBC, red blood cell; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin. HbA: human hemoglobin alpha, HbA2: human hemoglobin alpha 2, HbF: human hemoglobin F.

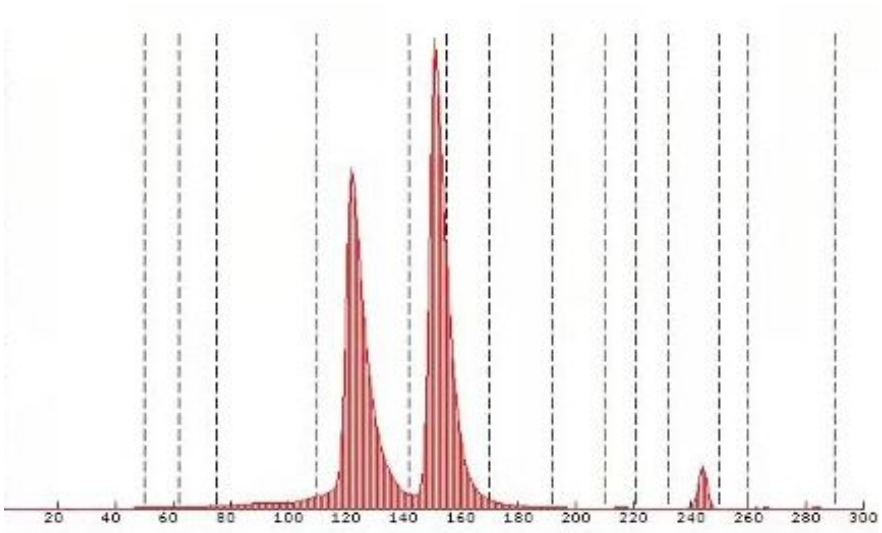


Figure 1.

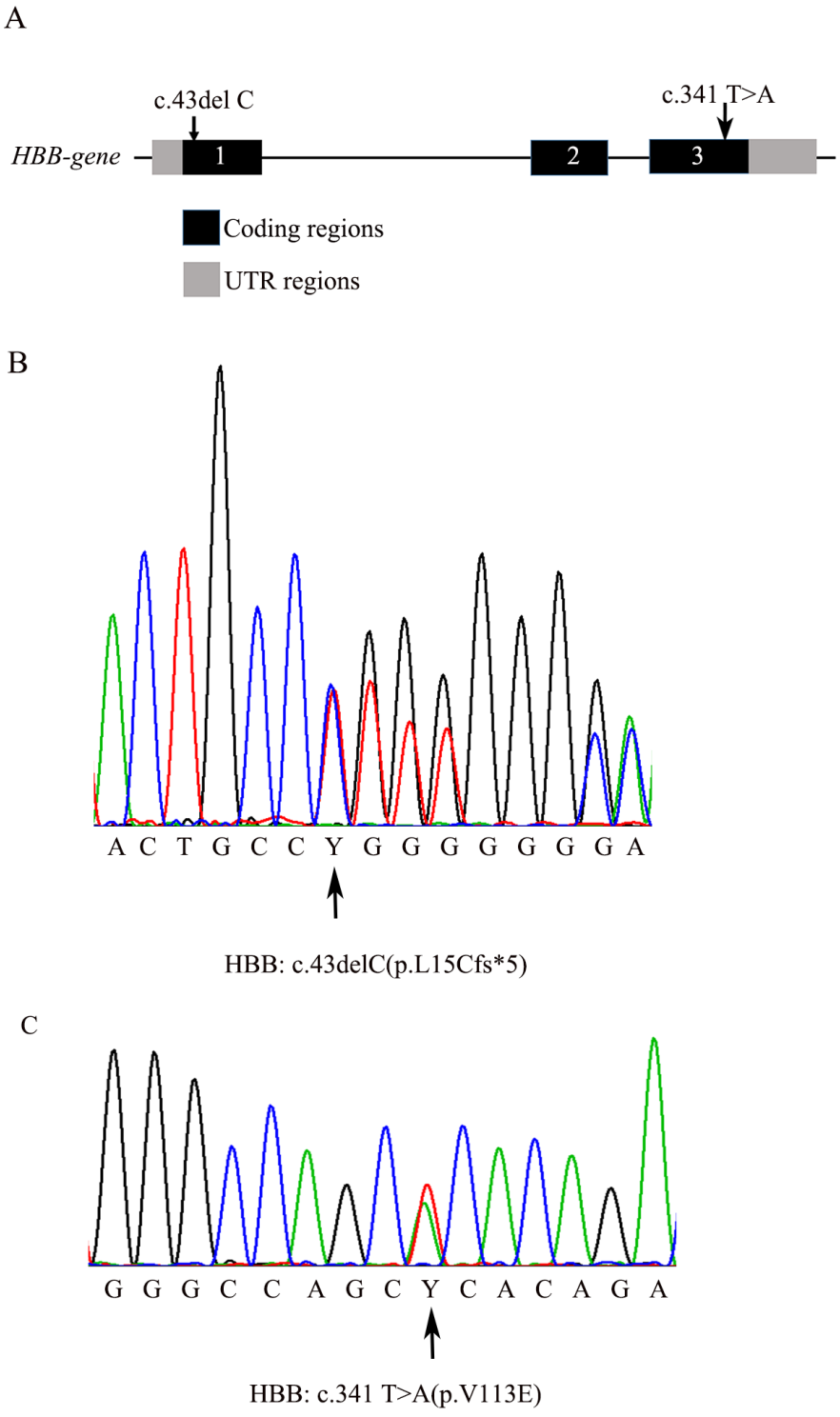
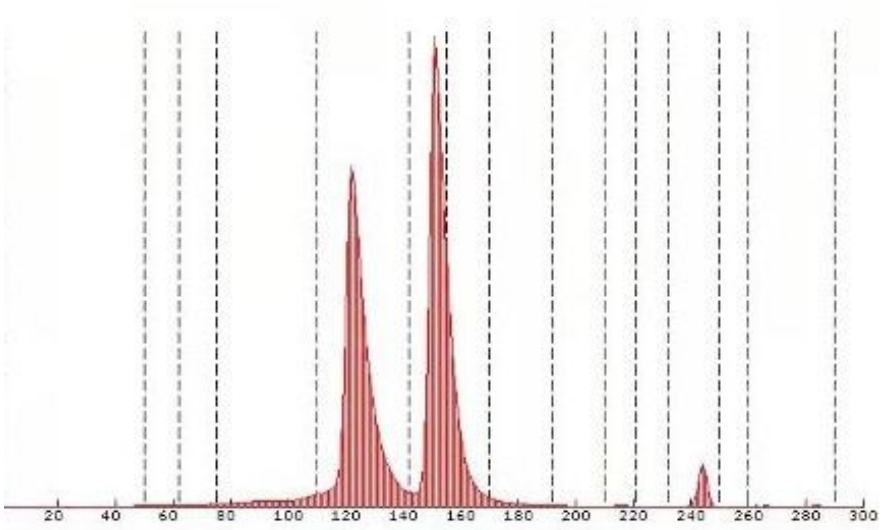
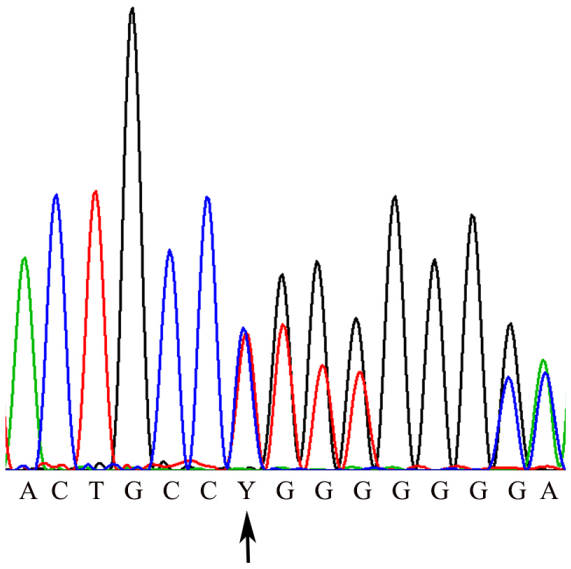


Figure 2.



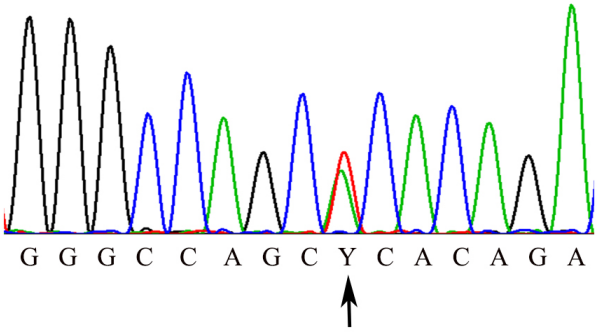
A

B



HBB: c.43delC(p.L15Cfs*5)

C



HBB: c.341 T>A(p.V113E)