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CASE REPORT

A Rare Heterozygote with a Novel IVS-II-786 (T>A) Mutation on β-Globin Gene in a Patient with Thalassemia

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SUMMARY

Background: **Thalassemia is an inherited hemolytic blood disease, whose pathogenesis is an imbalance in the expression of hemoglobin. We report a case of a rare β-globin gene intron mutation for thalassemia patient.**
Methods: **The blood routine test was performed with an automatic blood cell analyzer. Hb analysis was conducted by hemoglobin (Hb) analyzer. The common β-thalassemia and α-thalassemia gene mutations were detected by Gap-PCR and fluorescence PCR melting curve, and the rare β-thalassemia gene mutations were detected by DNA sequencing.**
Results: **A rare heterozygous mutation of β-globin gene IVS-II-786 (T>A) was found in this case. Blood routine analysis showed the following values: Hb 92 g/L, RBC 4.1 x 10^{12}/L, MCV 74.10 fL, MCH 22.4 pg, MCHC 303 g/L, HCT 0.304 L/L, and RET-He 22.7 pg. Hemoglobin analysis showed values of HbA_2 2.2% and HbF < 2% by automatic capillary electrophoresis. The results of gene analysis and DNA sequencing showed that the β-globin gene IVS-II-786 (T>A) mutation was heterozygous.**
Conclusions: **The heterozygote of β-globin gene IVS-II-786 (T>A) mutation was detected for the first time, and the clinical manifestation was moderate anemia. Hemoglobin analysis indicated that the level of HbA_2 was decreased. This mutation is relatively rare and easy to misdiagnose in clinical practice. It will provide a new type of evidence and guidance for genetic counseling and clinical treatment of beta thalassemia.**
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KEYWORDS

β-thalassemia, β-globin gene, IVS-II-786 (T>A) mutation, phenotype

CASE REPORT

Thalassemia is an inherited hemolytic blood disease, whose pathogenesis is an imbalance in the expression of hemoglobin, which occurs mainly in tropical and subtropical regions [1]. At present, patients with severe β-thalassemia are mainly treated with regular blood transfusion [2], and allogeneic hematopoietic stem cell transplantation can be used as a radical treatment, but it is limited by donor matching [3]. The CRISPR-mediated gene modification of hematopoietic stem cells has been used for research of beta-thalassemia cure in recent year

[4,5]. Mutations in the intron region of the β-globin gene may interfere with pre-mRNA transcription, resulting in reduced or absent β-globin synthesis [6]. The IVS-II-654 (C>T) mutation is relatively common, while IVS-I-130 (G>C), IVS-II-5 (G>C) and so on are relatively rare [7].

We have reported a rare β-globin gene mutation IVS-II-786 (T>A) and analyzed the effect of this mutation on β-globin thalassemia. The case of anemia of unknown cause was found in our hospital in January, 2023. The patient was a 45-year-old woman with anemia for 5 years, who had no history of blood transfusion, but showed high fever and intermittent diarrhea. Blood routine analysis showed Hb 92 g/L, RBC 4.1 x 10^{12}/L, MCV 74.10 fL, MCH 22.4 pg, MCHC 303 g/L, HCT 0.304 L/L, and RET-He 22.7 pg. Hemoglobin analysis showed HbA_2 2.2% and HbF < 2% by automatic capillary electrophoresis. Fluorescent PCR melting curve was used to detect 21 types of β-thalassemia gene mutations, which included -28 (A>G), -29 (A>G), -30 (T>C), -31 (A>C), -32 (C>A), -73 (A>T), -90 C>T), CD 41 - 42 (-TTCT), CD 26 (G>A), CD 27 - 28 (+C), CD 30 (A>G), CD 37 (G>A), CD 43 (G>T), CD 71 - 72 (+A), CD 14 - 15 (+G), CD 15 - 16 (+G), CD 17 (A>T), IVS-II-654 (C>T), IVS-I-1 (G>T), IVS-I-5 (G>C), and IVS-II-5 (G>C). None of 21 types of β-globin gene mutation were found in this case. The α-deletion thalassemia mutation analysis revealed that no common deletion thalassemia mutation was detected (Figure 1), also showed no common non-deletion thalassemia mutation using Gap-PCR including-SEA, -α3.7, -α4.2 and three rare types of -THA, -α21.9, -α27.6. The β-globin gene was amplified by polymerase chain reaction (PCR) and the PCR product was analyzed by direct nucleotide sequencing. The results of gene analysis and DNA sequencing showed that the β-globin gene IVS-II-786 (T>A) mutation was heterozygous, which was a novel and rare mutation type (Figure 2).

DISCUSSION

There are 350 million thalassemia carriers worldwide, and more than 300,000 babies are born with sickle-cell disease or thalassemia every year [8,9]. The prevalence of thalassemia in southern China is high with a carrier rate of 3 - 24%. The carrier rate of the disease is different in different areas of China, with strong genetic heterogeneity and ethnic and regional differences. Studies have shown that thalassemia mainly occur in Guangxi and Guangdong provinces, China [3]. Based on the epidemiological data, more than 130 types of mutations in alpha-thalassemia (over 40 deletions and 90 non-deletions). More than 900 types of mutations in beta-thalassemia were found all over the world. There were more than 50 mutations in the intron II region, accounting for more than half of all intron mutations (HbVar database) [10].

The β-globin gene contains 3 exons and 2 introns, and its gene mutation may lead to the decrease or deletion of β-globin synthesis [11]. Although introns are located in the non-coding region of the globin gene, mutations in this region may interfere with the pre-mRNA transcription of the globin gene, further interfering with the function of the β-globin gene and protein expression [4]. Different types of gene mutations may affect the globin gene and determine the clinical phenotype of patients with anemia. Patients with β-globin thalassemia show variations in the disease according to the degree which β-globin gene expression is suppressed. There are two main types: β+-thalassemia (reduced synthesis of β-globin) and β0-thalassemia (complete inability to synthesize β-globin) [12]. The mechanism by which β-globin expression is affected by mutations in the intron region of the β-globin gene is related to the site at which the mutation occurs.

Mutations in the IVS-II-786 (T>A) gene have not been reported in the past, and only a few mutations in the IVS-II-1 (G>C) gene have been reported so far [13]. A case of heterozygote with novel and rare mutation in IVS-II-786 (T>A) gene was found. The patient presented with moderate anemia, no history of blood transfusion, no hepatosplenomegaly, but had been anemic for 5 years. Hemoglobin analysis showed that HbA_2 was 2.2%, HbF was normal, and no thalassemia gene mutation was found. The degree of clinical anemia and the results of hemoglobin analysis in this case were similar to those of other β-globin intron gene mutations mentioned above [14,15]. Since there are no more related mutations, more cases need to be found and further analysis is needed to understand the clinical phenotype of β-thalassemia caused by this type of gene mutation. The mutation of IVS-II-786 (T>A) is rare in China and abroad, which is very important for providing a rare type of evidence and guidance for genetic counseling and clinical treatment of beta thalassemia.

CONCLUSION

The heterozygote of β-globin gene IVS-II-786 (T>A) mutation was detected for the first time, and the clinical manifestation was moderate anemia. Hemoglobin analysis indicated that the level of HbA_2 was decreased. This mutation is relatively rare and easy to misdiagnose in clinical practice. This presents new idea for clinical diagnosis and treatment for thalassemia patients.

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Ethical Approval:
This study was approved by the ethics committee of third people's hospital of Shenzhen. All procedures performed in the studies were in accordance with the ethi-

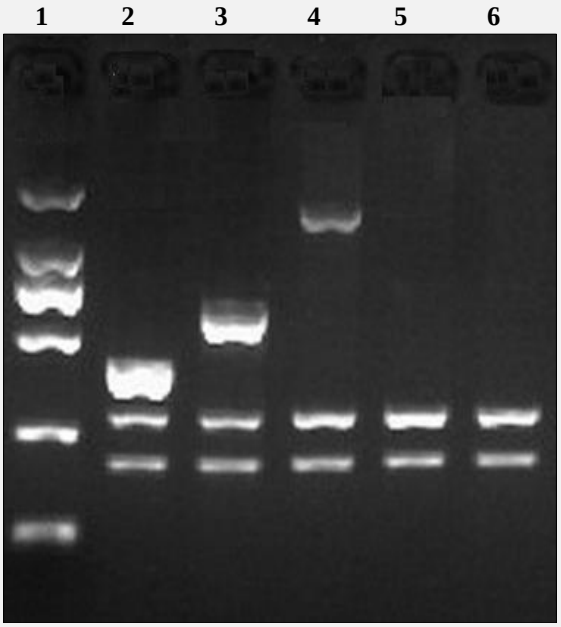

Figure 1. Electrophoresis result of the rare type alpha-thalassemia.

Line 1: Marker; Line 2: - ^{THAI}, Line 3: -α$^{21.9}$, Line 4: -α$^{27.6}$, Line 5: Negative control; Line 6: Patient.

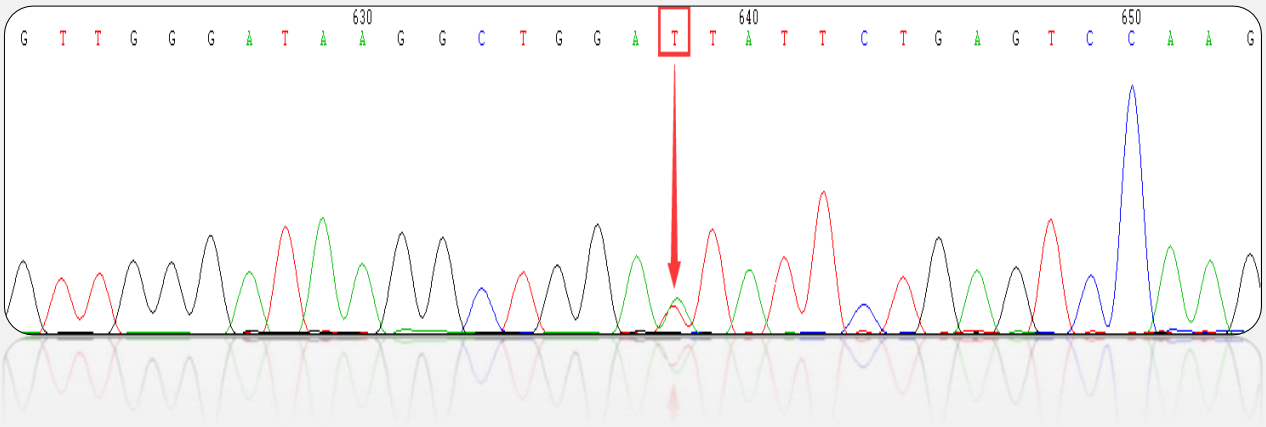

Figure 2. Sequence analysis of the β-globin chain by sequencing.

The arrow indicates the T>A substitution at position IVS-II-786.

cal standards. Informed consent was obtained.

Declaration of Interest:
The authors have no conflicts of interest to disclose.

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