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CASE REPORT

An Unexpected Finding of a Novel 21.9 kb Deletion (Heyuan deletion, $\beta^{21.9kb}$) β-Thalassemia During HbA_{1c} Measurements

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SUMMARY

Background: **β-thalassemia is predominantly caused by point mutations in the β-globin gene, whereas large deletions occur less frequently. Here, we described a novel 21.9 kb deletion found in a patient with β-thalassemia during HbA_{1c} measurements.**
Methods: **The proband was a 25-year-old female who came to the hospital with her husband for routine prenatal examinations. The hemoglobin A_{1c}(HbA_{1c}) was measured by high-performance liquid chromatography (HPLC). Hb analysis was performed by capillary electrophoresis (CE). Routine genetic analysis was carried out by PCR and reverse dot-blot (PCR-RDB) and Gap-PCR. Multiplex ligation-dependent probe amplification (MLPA) was used to screen the deletion in the β-globin chain. Based on the MLPA results, the break location of the deletion was determined by third-generation sequencing (TGS). Sanger sequencing verified the breakpoint in the Gap-PCR amplification products of TGS.**
Results: **HbA_{1c} measurements suggested an elevated HbF value (> 5%) by HPLC, and a retest of the Hb analysis showed an HbF value of 27.9%, and an Hb A_2 value of 1.7% using CE. No mutations were detected by Gap-PCR and PCR-RDB. However, MLPA demostrated the presence of large fragment deletion in the β-globin chain. The positions of the deletion were located between 5,225,669 and 5,247,554 on chromosome 11 (chr11: 5,225,669-5,247,554; NG_000007.3:g.50,063-71,947 del) using TGS, spanning the length of 21,886 bp (21.9 kb deletion).**
Conclusions: **This is the first report of the 21.9 kb deletion, so we named it Heyuan deletion for the place of origin of the proband. It presented with normal hematological parameters but an elevated HbF value.**
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KEYWORDS

21.9 kb deletion, Heyuan deletion, thalassemia, third-generation sequencing (TGS), β-globin chain

INTRODUCTION

β-thalassemia is a common monogenic recessive hereditary disease [1]. It occurs when point mutations or deletions exist in the β-globin gene or regulatory elements. β-thalassemia caused by point mutations presents an elevated HbA_2value, whereas those caused by deletions often exhibit elevated HbF values [2]. Glycated hemoglobin (HbA_{1c}) is used as a marker for the long-

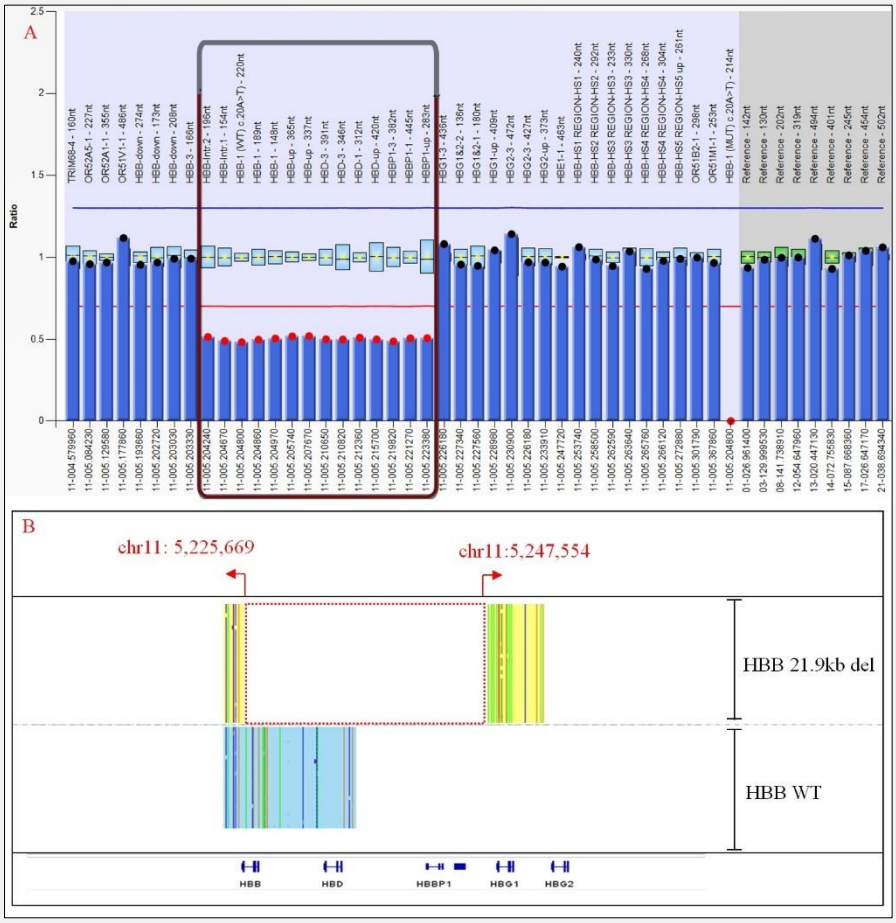

Figure 1. (A) MLPA of the proband revealed a heterozygous deletion in the β-globin gene cluster. (B) The deletion region extended from probe 196 ntto probe 283 nt (black frame).

TGS identified the deletion location between 5,225,669 and 5,247,554 on chromosome 11 (chr11: 5,225,669-5,247,554).

term monitoring of glucose control in patients with diabetes mellitus [3]. The accidental detection of Hb variants during HbA_{1c} measurements has been reported [4]. Here, we identified a novel large deletion in the β-globin gene cluster during HbA_{1c} measurements. The patient showed an elevated HbF value but had normal hematological parameters.

CASE PRESENTATION

The proband was a 25-year-old Chinese female who came from Heyuan City, Guangdong Province. She was referred for conception examination and thalassemia screening with his husband. She underwent several tests, including a complete blood count (CBC), blood glucose, HbA_{1c} measurement, urine routine analysis, liver function, kidney function, and ferritin. The hematological data for this female were normal as follows: Hb level of 11.7 g/dL (reference: 11.5 - 15.0 g/dL), mean corpuscular volume (MCV) 87.5 fL (reference: 82.0 - 100 fL), and mean corpuscular Hb (MCH) 29.2 pg (reference: 27 - 34 pg). Her fasting blood sugar was 4.34 mmol/L (reference: 3.90 - 6.10 mmol/L). The results of the other tests showed normal, except for the HbA_{1c} measurements. HPLC chromatogram suggested an elevated HbF value (26.21%) and 4.42% of HbA_1c (reference: 4.0 - 6.0%) (D-100; Bio-Rad, Hercules, California, USA). Quantification of hemoglobin fractions was performed by capillary electrophoresis (CE) (Capillary S2 Flex Piercing; Sebia, Lisses, Paris, France). CE showed similar finding to HbA_{1c} measurements with 70.4% Hb A, 27.9% HbF, and 1.7% HbA_2. This implies that the female might be a carrier of α-thalassemia,

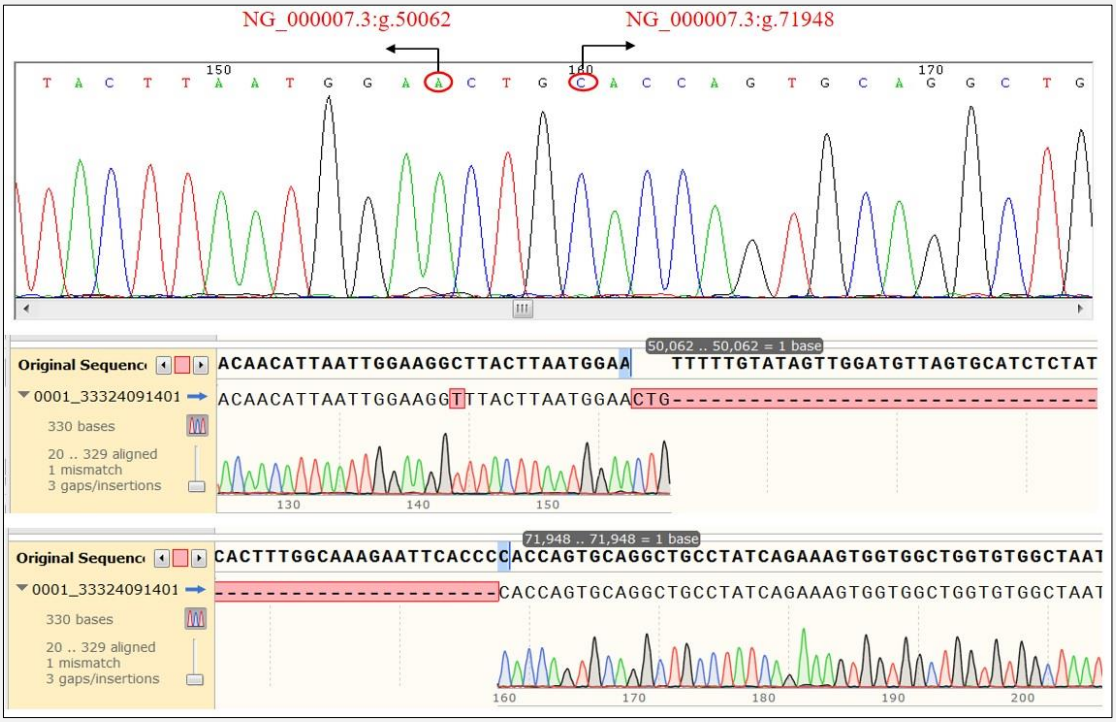

Figure 2. Sanger sequencing confirmed the breakpoints identified by TGS.

Additionally, Sanger sequencing also detected an insertion of CTG between the two breakpoints (NG_000007.3:g.50,063-71,947 del; insCTG).

β-thalassemia, or hereditary persistence of fetal hemoglobin (HPFH).

To exclude the existence of α-thalassemia, β-thalassemia, and HPFH, routine genetic analysis and multiplex ligation-dependent probe amplification (MLPA) of *HBB* gene were implemented. Gap-PCR was used to detect the four common deletions α-thalassemia in the Chinese population, including $--^{SEA}$/, $--^{THAI}$/, $-\alpha^{4.2}$/, and $-\alpha^{3.7}$/ (Yaneng Ltd., Shenzhen, China). Hb Quong Sze (Hb QS, *HBA2*:c.377T>C), Hb Constant Spring (Hb CS, *HBA2*:c.427T>C), and Hb Westmead (Hb WS, *HBA2*: c.369C>G) were detected by PCR and reverse dot-blot (PCR-RDB) (Yaneng Ltd., Shenzhen, China). No mutations were detected by Gap-PCR and PCR-RDB. However, a deletion larger than 21.8 kb in the β-globin gene cluster spanning from upstream of *HBB*-Intron 2 (probe 196 nt) to downstream of *HBBP1*-up (probe 283 nt) was identified by MLPA.

To determine the location of the deletion, we used third-generation sequencing (TGS) for identification. The TGS indicated a deletion of 21,886 bp on chromosome 11(chr11: 5,225,669-5,247,554; GRCh38). To confirm the results from TGS, we designed a specific probe for Gap-PCR and then performed Sanger sequencing on the amplified products. The forward primer used was: 5'-TTCAAGGGAGAGACCTCATTGTAAGACT-'3 and the reverse primer was: 5'-ATCCAGATGCTCAAGGC CCTTCATAATA-3'. Amplification started with denaturation for 10 minutes at 95℃, followed by 35 cycles of 1 minute at 94℃, 30 seconds at 62℃, and 1 minute at 72℃; with a final ex-tension of 5 minutes at 72℃. Sanger sequencing con-firmed the breakpoints identified by TGS. Additionally, Sanger sequencing also detected an insertion of CTG between the two breakpoints (NG_000007.3:g.50,063-71,947 del; insCTG).

DISCUSSION

In the β-globin gene cluster, there are more than 400 point mutations and less than 35 deletion associated with β-thalassemia.

In Chinese clinical laboratories, on-ly 17 point mutations are detected by routine genetic analysis, and three types of deletion are additionally tested if the sample is suspected to involve a deletion [5,6]. These deletions include Southeast Asian-hereditary persistence of fetal Hb (SEA-HPFH), Chinese $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia, and Tai-

wanese deletion. Here, our study identified a novel 21,886 bp deletion (21.9 kb deletion) in the β-globin gene cluster using MLPA and TGS. To the best of our knowledge, this deletion was reported for the first time, and we named it Heyuan deletion based on the residence of the proband.

In this study, different methods were used to screen and diagnose for thalassemia. Screening results for thalassemia were negative by CBC, so genetic analysis was not performed in the female. Routine testing for thalassemia genes can only detect 24 common mutations. MLPA can screen for large deletions but cannot determine the precise breakpoints of these deletions [7]. TGS, as the most advanced detection technology currently available, can identify large deletions, duplications, and complex structural mutations [8]. This study utilized TGS to confirm the upstream and downstream breakpoints of the deletion, presenting an effective method for identifying rare types of thalassemia.

Most cases of β-thalassemia exhibit altered hematological parameters, with the exception of a small percentage of silent β-thalassemia. The 21.9 kb deletion in our study displayed normal hematological parameters, despite its location affecting the *HBB* gene. We hypothesized that it represented silent β-thalassemia. Several mutations were discovered in our clinical practice with normal hematological indicators, but the CE screening came back positive [9]. The deletion was accidentally discovered during HbA_{1c} measurements. Heyuan deletion caused an elevated HbF value (26.21%), and fortunately the elevated HbF value had not yet reached the limiting value (30%) that interferes with HbA_{1c} measurements. This finding suggests that laboratory technicians should pay more attention to the profiles of HbA_{1c} measurements, especially in regions where hemoglobinopathies are prevalent.

CONCLUSION

In conclusion, we have identified for the first time a novel deletion in β-thalassemia with normal hematological parameters and a deletion length of 21 kb (also known as the Heyuan deletion).

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Declaration of Interest:
The authors report no conflicts of interest.

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