

α -thalassemia deletion [^{-SEA} (Southeast Asian)] and a compound heterozygote for the Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}/\beta^{CD17}$ -thalassemia mutation: A case report

HOU QIAN¹, WEIFENG LI¹, XIUHUA LIN¹, JI XU¹, XIAOLI ZHANG¹, WEIHUA ZHAO²,
YIKE WU¹ and WENLAN LIU¹

¹The Center for Medical Genetics and Molecular Diagnosis and ²Prenatal Diagnosis Center,
Shenzhen Second People's Hospital/The First Affiliated Hospital of Shenzhen University Health Science Center,
Shenzhen, Guangdong 518035, P.R. China

Received October 14, 2022; Accepted March 6, 2023

DOI: 10.3892/mmr.2023.12999

Abstract. In the present study, an α -thalassemia deletion [^{-SEA} (Southeast Asian)] and a compound heterozygote for the Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}/\beta^{CD17}$ -thalassemia mutation in a 15-year-old girl was identified by gap-PCR, PCR-reverse dot-blot hybridization and multiplex ligation-dependent probe amplification. Molecular analysis indicated that the proband's father carried a hemoglobin subunit β (*HBB*) heterozygous mutation in codon 17 (CD17; c.52A>T), the mother was a double heterozygous carrier of the Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia mutation combined with an ^{-SEA} deletion, and the proband inherited both mutations from her mother and father, thus carrying the Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}/\beta^{CD17}$ -thalassemia combined with the ^{-SEA} deletion in a compound heterozygous state. The proband was diagnosed as severe thalassemia intermedia and experienced a clinical phenotype aggravation (severe anemia and splenomegaly) from no obvious clinical symptoms to being dependent on monthly blood transfusions.

Introduction

Thalassemia is a hereditary blood disease common in southern China, and the population carrier rate is 3-24%. It

is caused by a disorder in the synthesis of globin chains and can be divided into two main subcategories, α -thalassemia and β -thalassemia, and several rare categories (δ -, γ -, $\delta\beta$ - and $\epsilon\gamma\delta\beta$ -thalassemia) (1,2). Compound heterozygotes for β -thalassemia usually have a severe transfusion-dependent phenotype (3). Generally, some conditions that decrease the production of α -globin (α -thalassemia) can reduce the clinical severity (4). The present study reports a case of a 15-year-old Chinese girl carrying compound heterozygosity for Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}/\beta^{CD17}$ -thalassemia combined with an ^{-SEA} (Southeast Asian) deletion causing a thalassemia intermedia phenotype.

$\delta\beta$ -thalassemia is a rare variant that results from a deletion in both the δ and β globin genes. The deletion causes the fetal γ -globin gene to reopen and continue to be expressed into adulthood, resulting in abnormal levels of fetal hemoglobin (HbF) (5). Within the Chinese population, Chinese type, Yunnan type and Guangzhou type $\delta\beta$ thalassemia have been reported; these are all $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia, of which Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia is the most common (6-9). The deletion range of Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia is ~78.9 kb (Fig. 1B), which includes part of the $\Lambda\gamma$ globin gene, all δ - and β -globin genes, and the DNA sequences with regulatory function downstream of β -globin gene (10). This thalassemia genotype was first reported by Mann *et al* (11) in the 1970s. However, little research has been focused on its clinical features. To date, only a few cases of compound heterozygous mutations of Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia have been reported, and these cases illustrated normal, moderate or major anemia (12-15). Notably, whether the interaction between $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia and other common β -thalassemia mutations leads to moderate or major clinical phenotypes mainly depends on the type of β -thalassemia allele (β^{+} or β^{0}).

The present study provides a detailed description of clinical and molecular characteristics in a 15-year-old Chinese girl carrying a compound heterozygosity for Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}/\beta^{CD17}$ -thalassemia combined with the ^{-SEA} deletion, which represents an example of how combined types of thalassemia affect the clinical severity of Chinese $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia.

Correspondence to: Dr Wenlan Liu, The Center for Medical Genetics and Molecular Diagnosis, Shenzhen Second People's Hospital/The First Affiliated Hospital of Shenzhen University Health Science Center, 3002 Sungang West Road, Shenzhen, Guangdong 518035, P.R. China
E-mail: wlliu@szu.edu.cn

Abbreviations: MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MLPA, multiplex ligation-dependent probe amplification; PLT, platelet count; PCR-RDB, PCR-reverse dot-blot hybridization; WBC, white blood cell

Key words: α -thalassemia, heterozygous mutation, severe thalassemia intermedia, $G\gamma^{+}(\Lambda\gamma\delta\beta)^{0}$ -thalassemia

Materials and methods

Ethical statement. The present study was approved by the ethics committee of Shenzhen Second People's Hospital (Shenzhen, China; approval no. 20210707004) and written informed consent were obtained from the family.

Proband and parents. The proband was a 15-year-old girl from Yulin City, Guangxi, China. 2 ml peripheral blood from the proband and her parents (father, 44 years old; mother, 43 years old) were collected using EDTA as anticoagulant.

Analysis of hematological parameters and hemoglobin (Hb) components. Hematological parameters were analyzed using a Sysmex XN-1,000 automated blood cell counter (Sysmex Corporation). Hb components were tested using a V8 hemoglobin electrophoresis instrument with the V8 Nexus Hemoglobin Ultrascreen kit (Helena Laboratories Corporation).

DNA sample preparation. Genomic DNA was isolated from peripheral blood using an automatic nucleic acid extractor (Xiamen Kaishuo Biological Co., Ltd.) according to the manufacturer's instructions.

Gene analysis. The α -thalassemia deletions ($-\alpha^{3,7}$, $-\alpha^{4,2}$, $-\alpha^{SEA}$, $-\alpha^{THAI}$) were analyzed using conventional gap-PCR with deletion type α -thalassemia gene test kit (Shenzhen Yilifang Biological Products Co., Ltd.). The non-deletion α -thalassemia mutations (Hb Constant Spring, Hb Quong Sze and Hb Westmead) were analyzed using conventional PCR-reverse dot blot hybridization (RDB) with non-deletion α -thalassemia gene mutation test kit (Shenzhen Yilifang Biological Products Co., Ltd.). The 19 β -thalassemia mutations, including -28(A>G) (HBB: c.-78A>G), -29 (A>G) (HBB: c.-79A>G), -30 (T>C) (HBB: c.-80T>C), -32 (C>A) (HBB: c.-82C>A), -50(A>G) (HBB: c.-100G>A), codons 14/15 (+G) (HBB: c.45_46insG), codon17(A>T) (HBB: c.52A>T), codon26 (or Hb E)(G>A) (HBB:c.79G>A), codons27/28 (+C) (HBB: c.84_85insC), codon31 (-C) (HBB: c.94delC), codons 37 (G>A) (HBB: c.113G>A), codons 41/42 (-TTCT) (HBB:c.126_129delCTTT), codon43 (G>T) (HBB: c.130G>T), codons71/72 (+A) (HBB: c.216_217insA), IVS-I-1 (G>T) (HBB: c.92 + 1G>T), IVS-I-5 (G>C) (HBB: c.92 + 5G>C), IVS-II-654 (C>T) (HBB: c.316-197C>T), CAP +1 (A>C) (HBB: c.-50A>C) and initiation codon (T>G) (HBB: c.2T>G) were analyzed using conventional RDB with β -thalassemia gene mutation test kit (Shenzhen Yilifang Biological Products Co., Ltd.). Chinese $G_\gamma^{+}(A_\gamma\delta\beta)^0$ -thalassemia was analyzed by gap-PCR according to the manufacturer's instructions. The gap-PCR primer sequences are provided in Table I. Multiplex ligation-dependent probe amplification (MLPA) was performed according to the manufacturer's protocol using a SALSA MLPA Probemix P102 HBB kit (cat. no. P102; MRC-Holland) to further confirm the β -globin gene cluster deletion identified by gap-PCR. PCR reaction mixtures contained 0.1-1 μ g of genomic DNA, 0.2 mmol/l each dNTP, 0.2 mmol/l each primer, 20 mmol/l Tris-HCl (pH 8.4), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$ and 2.5 U of Platinum™ Taq polymerase (Thermo Fisher Scientific, Inc.). After initial denaturation at

95°C for 5 min, a total of 35 PCR cycles were performed under the following PCR conditions: 95°C for 45 sec, 60°C for 30 sec and 72°C for 90 sec; and a final extension at 72°C for 5 min. After amplification, 3 μ l of product was run on a 1% agarose gel in 0.5X Tris-Borate-EDTA buffer pre-stained with SYBR Safe (1:10,000; Invitrogen; Thermo Fisher Scientific, Inc.) for 30 min at 5-6 V/cm. After electrophoresis, the gel was visualized using an ultraviolet transilluminator.

DNA sequencing. Two sets of primer pairs were designed and used to amplify and sequence the hemoglobin subunit α genes (HBA1 and HBA2): HBA1, forward 5'-CAAGCATAAACC CTGGCGCGC-3', and reverse 5'-CCTGGCACGTTTGCT GAG-3'; HBA2, forward 5'-CAAGCATAAACCCCTGGCG CGC-3', and reverse 5'-ATTGTTGGCACATTCGGGA-3'. The PCR mixture comprised: 50 ng of genomic DNA; up to ddH₂O, 30 μ l; 10X LA buffer, 5.0 μ l; 2.5 mmol/l dNTPs, 3.0 μ l; 10 pmol/ μ l forward primer, 1.0 μ l; 10 pmol/ μ l reverse primer, 1.0 μ l; 5 mol/l betaine, 5.0 μ l; DMSO, 2.5 μ l; 2 U/ μ l Taq polymerase, 0.4 μ l. The C1000 Thermal Cycler (Bio-Rad Laboratories, Inc.) was used with the following thermocycling conditions: Initial denaturation step of 95°C for 5 min, denaturation at 95°C for 40 sec, annealing at 66°C for 30 sec and extension at 72°C for 70 sec, for a total of 33 cycles. DNA sequencing was performed using the Sanger sequencing method, with the reference sequence NC_000011.10. The PCR products were sequenced using an ABI PRISM™ 3,130xl Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sequencing results are shown in Figs. S1-S5.

Case report

The proband, a 15-year-old girl from Yulin City, Guangxi, China was born normally and without any observable development delay. The proband was diagnosed with thalassemia ($-\alpha^{SEA}/\alpha\alpha$; $\beta^{CD17}/\beta^{CD17}$:homozygote) in October 2002 at Yulin People's Hospital of Guangxi Province, China at 6 years of age and did not initially receive regular blood transfusion. By 10 years of age, she was admitted to a local hospital due to cold symptoms and received a blood transfusion for the first time. At 13 years and 8 months old, she had a Hb level of 55 g/l and received a transfusion of 4 units of leukocyte-poor red blood cells (LPRC), her second blood transfusion, and was subsequently discharged from the hospital. At 13 years and 9 months old, she presented at Shenzhen Second People's Hospital (Shenzhen, China), experiencing headaches and dizziness without obvious cause. She had a Hb level of 51 g/l and was admitted to hospital. At the time of admission, the proband weighed 33.9 kg, below the 3rd percentile. She exhibited facial characteristics of thalassemia (the skull was enlarged, the cheekbones prominent, the eye distance widened and the bridge of the nose was low and flat.), marked paleness and fatigue, but was not jaundiced. The proband's liver was 4 cm below the midclavicular line of the right costal margin, the spleen was 8 cm below the left costal margin and she had grade III ejection systolic murmurs at the apex.

On the day of her admission, routine hematological evaluation revealed a Hb level of 51 g/l, a mean corpuscular volume (MCV) of 69 fl, a mean corpuscular Hb (MCH) of 21.3 pg,

Table I. Primer sequences.

Primer sequence (5'-3')	GenBank ID: Nucleotides
F, CAAGGGCACCTTTGCCAGCT	NC_000011.10:g.5249437_5249417
R, CTAGAATTCTTCTGGTCCTCCCT	NC_000011.10:g.5169215_5169237

F, forward; R, reverse.

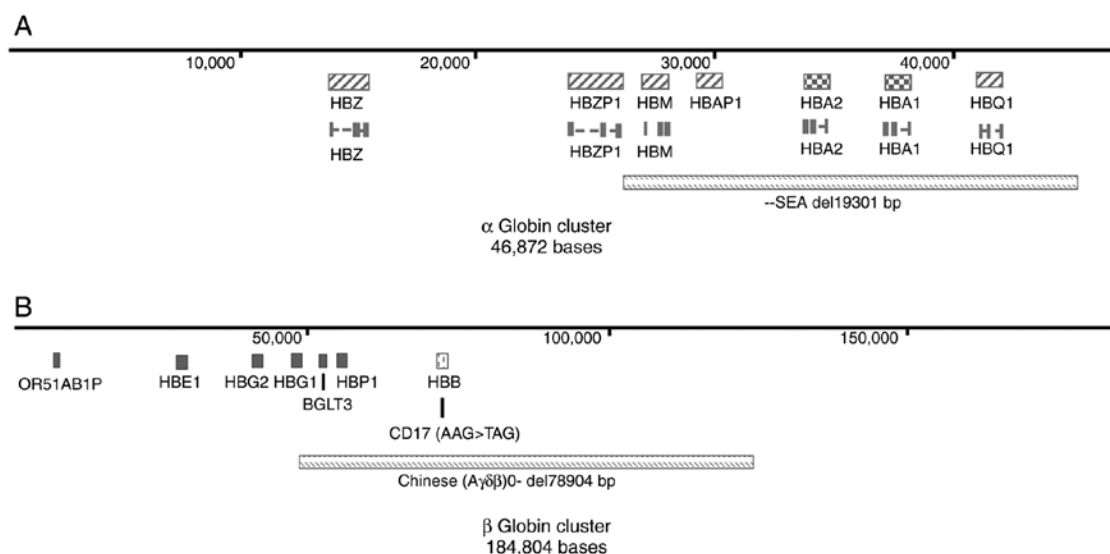


Figure 1. α and β globin clusters. (A) The α globin cluster with the region of the 19,301 bp-^{SEA}/ α deletion indicated with the yellow box. (B) The β globin cluster with the region of the 78,904 bp Chinese $\gamma^+(\gamma\delta\beta)^0$ deletion indicated with the yellow box; the CD17 mutation site of β^{CD17}/β^N , with base mutation (AAG>TAG). CD17, codon 17; del, deletion; SEA, Southeast Asian.

a white blood cell (WBC) count of $8.83 \times 10^9/l$ and a platelet count of $143 \times 10^9/l$. On day 2, her Hb further dropped to 37 g/l and she subsequently received a transfusion of 2 units of LPRC. Three days later, she remained fatigued and dizzy and continued to exhibit grade III ejection systolic murmurs at the apex. An echocardiogram showed that in the resting state, there was no obvious abnormality in the intracardiac structure and blood flow. As the proband's Hb level was 52 g/l, she received another transfusion of 2 units of LPRC. Five days after admission, she complained of dizziness in the morning with a Hb level of 66 g/l and received a third transfusion of 2 units of LPRC. Six days after admission, her Hb level recovered to 85 g/l and she received a fourth transfusion of 2 units of LPRC. Blood tests for total bilirubin, unconjugated bilirubin, aspartate aminotransferase, alanine aminotransferase, serum ferritin (FER), human growth hormone, triiodothyronine, total thyroid hormone, free triiodothyronine, free thyroid hormone, serum thyroid stimulating hormone, human chorionic gonadotropin, progesterone, estradiol and follicle-stimulating hormone were performed (Table II). Seven days after admission, T2*-weighted MRI analysis revealed moderate iron overload in the patients' liver. Notably, Hb electrophoresis showed changes of HbF fraction (normal range, <2.3%) and HbA2 fraction (normal range, 2.5-3.5%). The father showed an increased HbA2 fraction, with HbA (92.79%; lane 6), HbF (1.32%; lane 7) and HbA2 (5.89%; lane 11). The proband's mother showed increased

HbF compared to the father with HbA (89.89%; lane 6), HbF (7.54%; lane 7) and HbA2 (2.56 %; lane 11), and the proband exhibited HbA (82.45%; lane 6), HbF (14.78%; lane 7) and HbA2 (2.78%; lane 11) (Fig. 2). Hematological data of the family showed changes of Hb (normal range, 110-150 g/l), MCV (normal range, 80-100 fl) and MCH (normal range, 27-34 pg) The proband showed data on fluctuations. The father and mother showed both MCV and MCH decreased (Table III).

Since several studies have reported that Chinese $\gamma^+(\gamma\delta\beta)^0$ -thalassemia is clinically manifested as no anemia or mild anemia, with increased HbF (10-18%), and normal Hb A2 (2.5-3.5%) (16,17), it was suspected that the proband may carry Chinese $\gamma^+(\gamma\delta\beta)^0$ -thalassemia, and therefore the genotypes of the girl and her parents were analyzed using gap-PCR, PCR-RDB and MLPA (Figs. S6-S7). The common-^{SEA} deletion and Chinese $\gamma^+(\gamma\delta\beta)^0$ -thalassemia were detected using gap-PCR and the codon17(A>T) (HBB: c.52A>T) mutation was detected by PCR-RDB. The data showed that the father was heterozygous for the codon17(A>T)(HBB: c.52A>T; Fig. 3C), the mother carried the Chinese $\gamma^+(\gamma\delta\beta)^0$ -thalassemia combined with the-^{SEA} deletion in a double heterozygous state and no point mutations of HBB were detected by PCR-RDB (Fig. 3A and B, respectively; Fig. S8, and their daughter inherited both mutations from her mother and father, thus carrying the Chinese $\gamma^+(\gamma\delta\beta)^0/\beta^{CD17}$ -thalassemia combined with

Table II. Relevant biochemical indicators during hospitalization of proband.

Parameter	Results	Normal range (range in different periods)
TBIL, $\mu\text{mol/l}$	43.40	3.00-22.00
UCB, $\mu\text{mol/l}$	35.50	0.00-19.00
AST, U/l	95.00	14.00-36.00
ALT, U/l	51.00	9.00-52.00
FER, ng/ml	1,381.90	10.00-291.00
hGH, ng/ml	1.90	<10.00
TT3, ng/ml	0.72	0.60-1.81
TT4, ng/ml	51.50	45.00-133.00
FT3, pmol/l	4.81	3.50-6.59
FT4, pmol/l	15.16	11.50-22.70
TSH, mIU/l	4.98	0.35-5.50
HCG, mIU/ml	2.00	0.00-10.00
P4, ng/ml	0.21	0.15-1.40 (follicular phase)
E2, pg/ml	32.47	19.5-144.20 (follicular phase)
FSH, mIU/ml	3.06	2.5-10.20 (follicular phase)

TBIL, total bilirubin; UCB, unconjugated bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FER, serum ferritin; hGH, human growth hormone; TT3, triiodothyronine; TT4, total thyroid hormone; FT3, free triiodothyronine; FT4, free thyroid hormone; TSH, serum thyroid stimulating hormone; HCG, human chorionic gonadotropin; P4, progesterone; E2, estradiol; FSH, follicle-stimulating hormone.

the-^{SEA} deletion in a compound heterozygous state (Fig. 3; Table IV). Ten days later, the proband had occasional dizziness. Her Hb level was 96 g/l and hematological indexes were significantly improved. The proband received a total of eight LPRC transfusions over 10 days, with Hb fluctuating between 37-96 g/l. The patient's hematological indexes are summarized in Table IV.

Since the diagnosis of the Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0/\beta^{CD17}$ -thalassemia combined with the-^{SEA} deletion at 13 years and 10 months old, the proband became transfusion dependent. She has since received four units per month of LPRCs in view of her low Hb level and persistent symptoms. Her Hb level remained a steady 90-105 g/l throughout the following 18 months, prior to follow-up. FER levels were regularly screened during follow-up and iron-chelating therapy, desferrioxamine mesylate, was administered when FER was >1,000 ng/ml. Liver and kidney function was monitored periodically by clinical biochemical analysis. The proband, now 15 years and 3 months old is still under regular follow-up at the Department of Hematology of Shenzhen Second People's Hospital (Shenzhen, China), and is progressing well. Towards the start of patient follow-up, the Hb level dropped to 62 g/l, and stabilized between 90-105 g/l in the middle to late stages. WBCs were consistently maintained within the biological reference interval, whereas MCV and MCH were consistently below the biological reference interval. RBC gradually rises towards the biological reference range, indicates that the anemia symptoms are not further aggravated. These results during follow-up are displayed in Fig. 4. A timeline of the probands disease progression is shown in Fig. 5.

Discussion

Thalassemia is a hereditary hemolytic disease that is prevalent in Southeast Asia, the Indian subcontinent, and Africa. In China, there is a high incidence of α -thalassemia in Guangdong is 8.53% and β -thalassemia is 2.54% (18). The large population base and high carrying rate result in various types of thalassemia. In 1972, Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0$ -thalassemia was first reported by Mann *et al* (11), the deletion range of which involves $\Lambda\gamma$, $\psi\beta$, δ , β -alleles and 3'-HS-1 regions of globin gene cluster, covering ~100 kb (7). This mutation is generated through non-homologous recombination (5). Although a number of previous studies have reported that the clinical manifestations of Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0$ -thalassemia heterozygotes show significantly reduced MCV and MCH levels, it is a typical characteristic of mild thalassemia with microcytic hypochromic anemia (12-14,19,20). However, when Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0$ -thalassemia coexists with other common β -thalassemia gene mutations, the patients often exhibit moderate or severe anemia with increased HbF levels (12-14).

To date, there are few studies reporting a compound heterozygous mutation of Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0/\beta^N$ -thalassemia combined with α -thalassemia (21). In a previous study, the Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0/\beta^{CD41-42}$ -thalassemia combined with the- $\alpha^{3.7}$ deletions was reported to be transfusion independent (13). In the present report, the proband had no obvious disease symptoms before 13 years of age in 2020. After a severe, unexplained headache and dizziness, hepatosplenomegaly and marked paleness and fatigue were discovered at 13 years and 9 months old and the hematological results (Hb, 51 g/l; MCV,

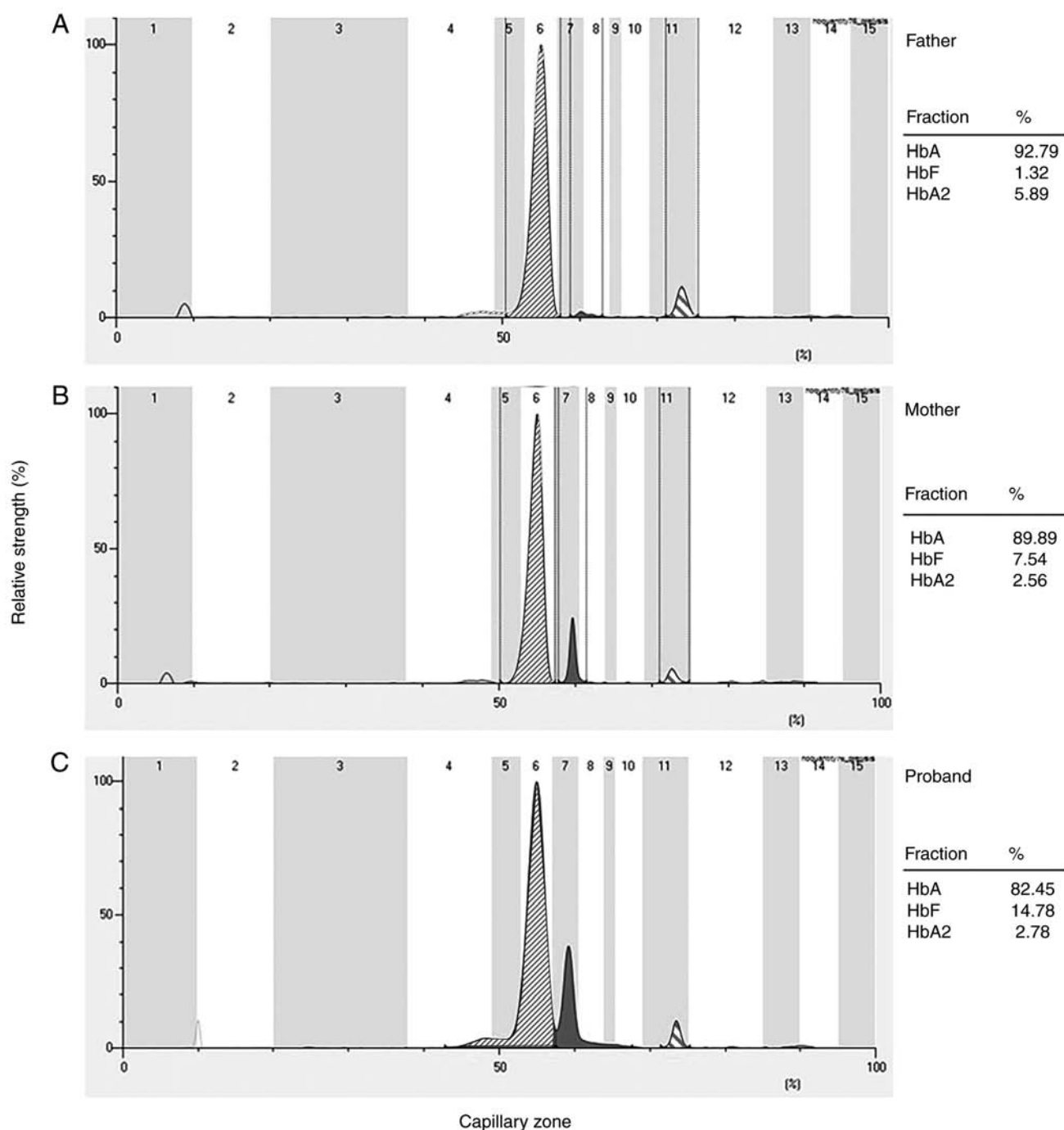


Figure 2. Hb electrophoresis analysis. The three most common Hb bands, HbA (lane 6), HbF (lane 7) and HbA2 (lane 11), were analyzed by electrophoresis, and the fractions of each in the (A) father, (B) mother and (C) proband (post-blood transfusion) are shown. Hb, hemoglobin; HbF, fetal hemoglobin.

69 fl; MCH, 21.3 pg) lead to the diagnosis of β -thalassemia intermedia. As headache and dizziness cannot be relieved by clinical treatment, Hb has been reduced to 37 g/l, which can only be treated by regular blood transfusion. The Hb level returned to normal, increased to 90-105 g/l and remained stable for ~18 months.

Intermediate thalassemia patients vary in degree of anemia, the severity of onset is closely related to the amount of β -chain synthesis, most symptoms in childhood, the clinical manifestations of moderate anemia between mild and severe, mild or moderate spleen enlargement, may have jaundice, varying degrees of skeletal changes,

sexual development delay. There is a wide range of variation among individuals with different intermediate forms of β -thalassemia: in mild cases, clinical symptoms are not significant, and physical examination reveals small cell hypothermic anemia and moderate hemoglobin reduction. In severe cases, similar to severe beta-thalassemia, the liver and spleen are enlarged and require irregular blood transfusion to maintain life (21-23). The present study highlighted a case of Chinese $G_{\gamma^+}(\Delta\gamma\delta\beta)^0/\beta^{CD17}$ -thalassemia combined with an $-SEA$ deletion, an uncommon β -thalassemia state (transitioning to severe β -thalassemia state with age) caused by a rare β -globin genotype.

Table III. Hematological and genetic diagnosis data of the proband and their mother and father.

Parameter	Proband	Mother	Father
Age, years	15	43	44
Hb, g/l	3.20-12.00 ^a	11.80	11.50
MCV, fl	65.30-78.80 ^a	73.60	64.00
MCH, pg	20.80-27.70 ^a	25.20	20.10
HbA, %	82.45 ^a	89.89	92.79
HbF, %	14.78 ^a	7.54	1.32
HA2, %	2.78 ^a	2.56	5.89
<i>HBA</i> genotype	$\text{SEA}/\alpha\alpha$	$\text{SEA}/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$
<i>HBB</i> genotype	$G_{\gamma^+}(\Lambda\gamma\delta\beta)^0/\beta^{CD17}$	$G_{\gamma^+}(\Lambda\gamma\delta\beta)^0/\beta^{CD17}$	β^{CD17}/β^N

^aFluctuations caused by the administration of blood transfusions. Hb, hemoglobin; *HBA*, hemoglobin subunit α ; *HBB*, hemoglobin subunit β ; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; SEA, southeast Asian

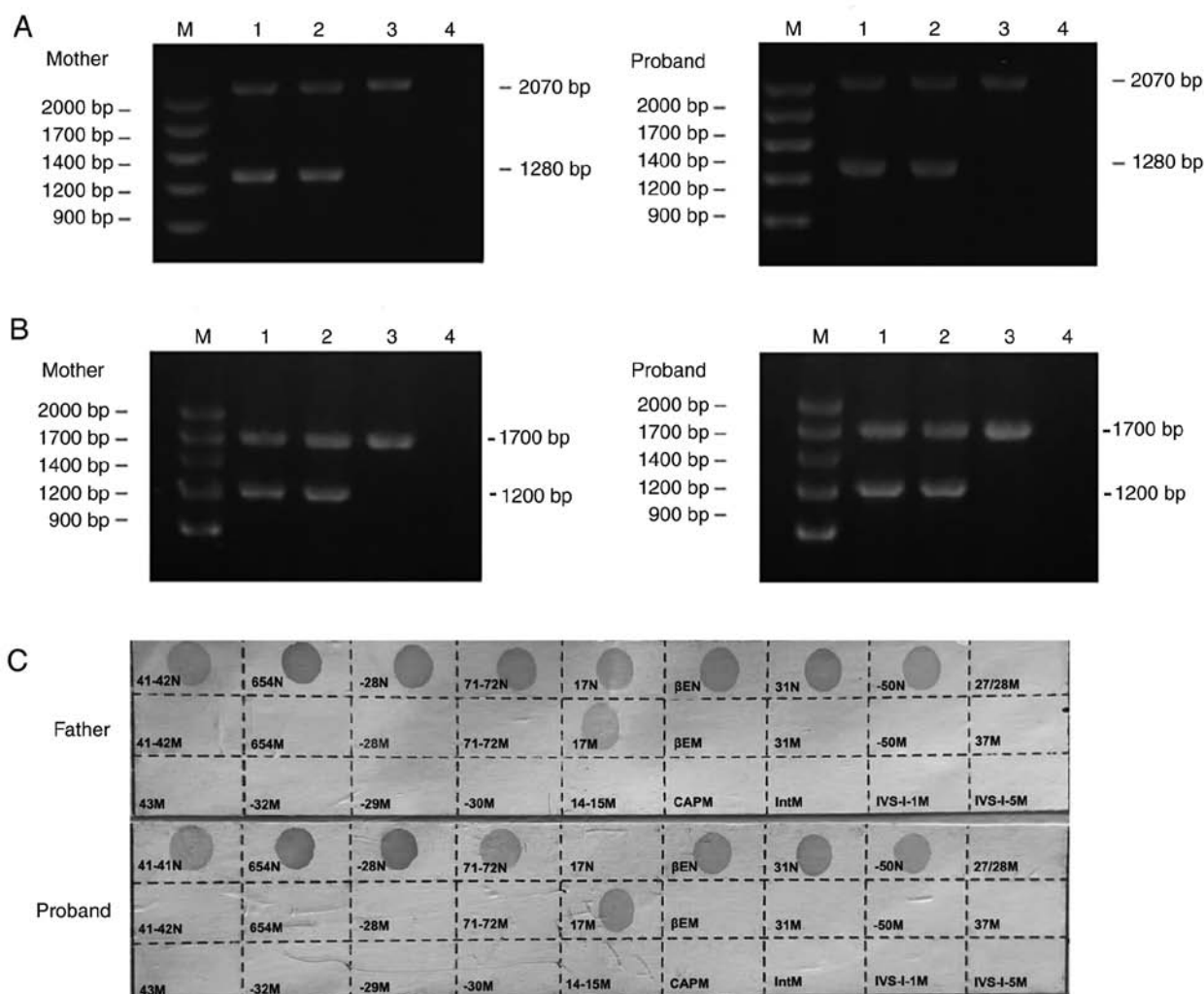


Figure 3. Gene mutation analysis results of thalassemia in the family. (A) Gap-PCR agarose gel electrophoresis results of the Chinese $G_{\gamma^+}(\Lambda\gamma\delta\beta)^0$ mutation in the mother and proband. (B) Common thalassemia genotype result of $\text{SEA}/\alpha\alpha$ in the mother and proband. M, marker; lane 1, $\text{SEA}/\alpha\alpha$; lane 2, positive control; lane 3, negative control; lane 4, blank control. (C) PCR-RDB results of β gene mutation in father and proband. SEA, southeast Asian; PCR-RDB, PCR-reverse dot blot.

In the present report, analysis of the proband's peripheral blood samples revealed β^{CD17} locus mutation homozygous thalassemia combined with the SEA deletion. Her father

presents with β^{CD17} locus mutation thalassemia, and the mother presents with SEA deletion mutation thalassemia. As the proband's HbF value of 14.78% was significantly elevated,

Table IV. Changes in hematological data during the proband's hospitalization.

Date	Normal range	11/06/2020	12/06/2020	14/06/2020	16/06/2020	17/06/2020	22/06/2020
Transfusion, units	n/a	n/a	n/a	2	2	2	2
WBC, $10^9/l$	5-15	8.38	5.09	5.20	6.12	5.49	7.91
RBC, $10^{12}/l$	3.5-5.5	n/a	1.78	2.35	2.78	3.43	3.67
Hb, g/l	110-150	51	37	52	66	85	96
MCV, fl	80-100	69	66.9	69.4	74.5	76.4	78.5
MCH, pg	27-34	21.3	20.8	22.1	23.7	24.8	26.2
PLT, $10^9/l$	100-300	143	131	114	146	154	120

Hb, hemoglobin; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; n/a, not applicable; PLT, platelet count; RBC, red blood cells; WBC, white blood cells.

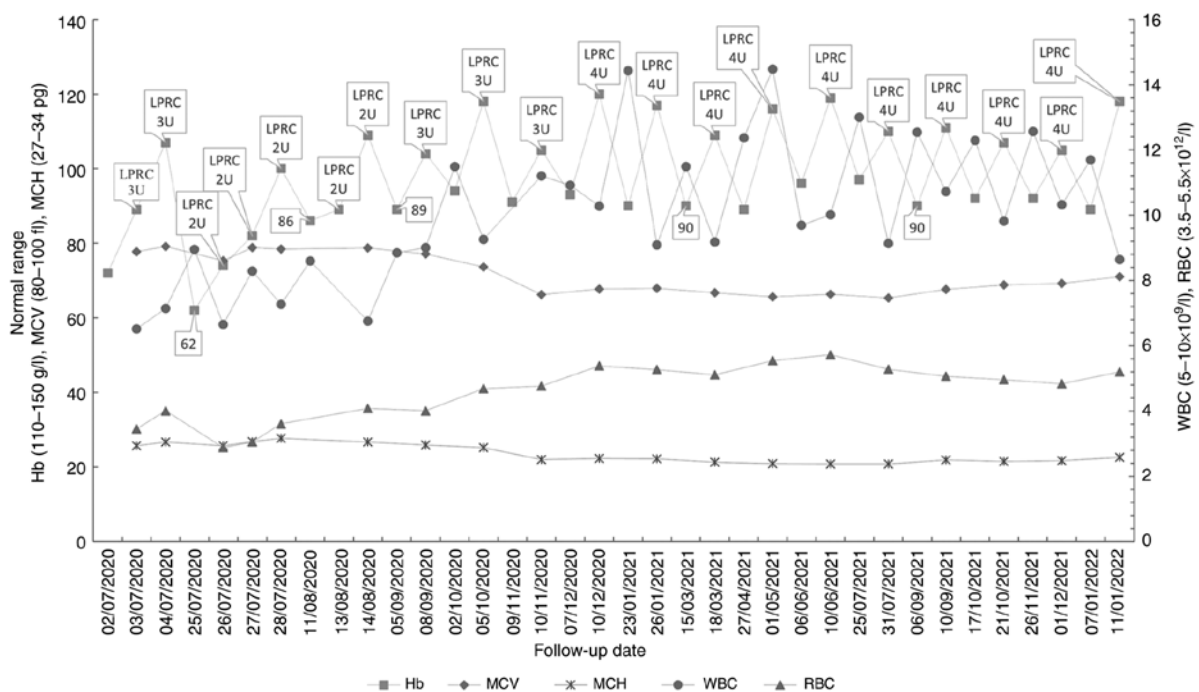


Figure 4. Analysis of changes in Hb, MCV, MCH, WBC and RBC during the proband's follow-up period. The label on the Hb data shows dates of transfusion and dosage (e.g., 1 unit for LPRC prepared from 200 ml whole blood) and intermittent Hb levels. Hb, hemoglobin; LPRC, leukocyte-poor red blood cells; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; RBC, red blood cells; U, unit; WBC, white blood cells.

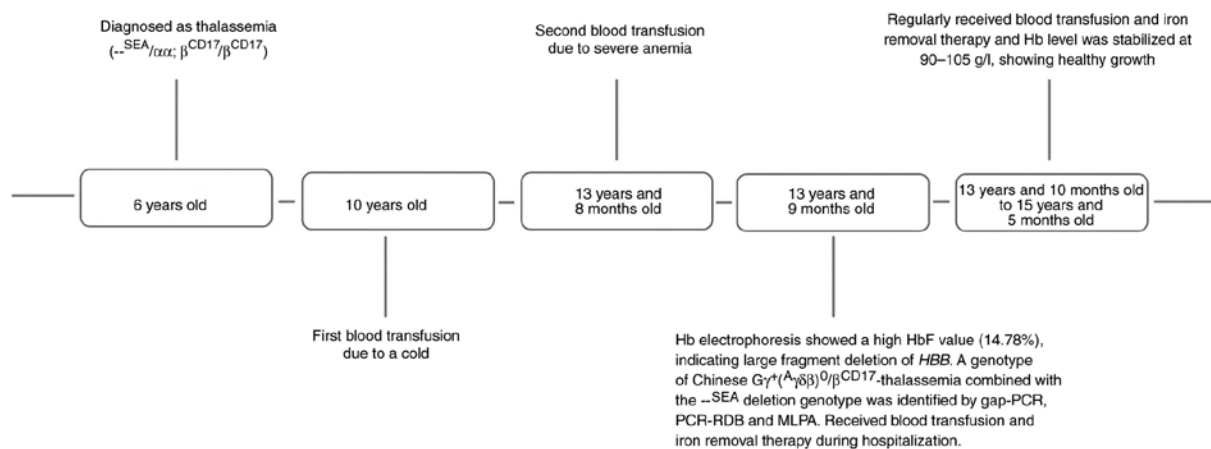


Figure 5. Timeline of the progression of the patient's clinical presentation. Progression of the disease from 6-15 years of age, including all transfusions. Hb, hemoglobin; *HBB*, hemoglobin subunit β ; MLPA, multiplex ligation-dependent probe amplification; PCR-RDB, PCR-reverse dot blot; SEA, southeast Asian.

she and her mother were subjected to further genetic tests and were found to carry the Chinese $G_{\gamma^+}(\Delta\gamma\delta\beta)^0$ -thalassemia mutation. The present findings suggested that when genetic tests of thalassemia are found to be homozygous and further pedigree analysis does not support this result, the possibility of the presence of a Chinese $G_{\gamma^+}(\Delta\gamma\delta\beta)^0$ -thalassemia deletion gene should be considered; that is, the impression of 'homozygosity' in the presence of a large segment of another chromosome gene deletion results in wrong judgments. Within the Chinese population, $G_{\gamma^+}(\Delta\gamma\delta\beta)^0$ -thalassemia and South-East Asian-type hereditary persistent fetal hemoglobinemia are the most common causes of significantly increased HbF levels ($\geq 10\%$) (16). Therefore, for cases with increased HbF levels, the aforementioned deletions should be analyzed first, and MLPA can be used to detect other types of β -globin gene deletions when necessary. If the conventional thalassemia gene tests reveal a homozygous state for the mutation site and this result does not match clinical symptoms or thalassemia screening results, deletion of the β -globin gene cluster should be considered. In the present case, the proband was accurately diagnosed with the rare Chinese $G_{\gamma^+}(\Delta\gamma\delta\beta)^0/\beta^{CD17}$ and the SEA deletion genotype. In clinical practice, the diagnosis of a thalassemia gene is based on the diagnostic principle of combining phenotype and genotype. When the hematologic phenotype of the subject is inconsistent with the genotype of thalassemia detected by routine tests, rare or undiscovered mutations may exist, which should be further tested and analyzed for confirmation. The results of this case also confirmed the importance and practicability of this genetic diagnosis principle for thalassemia.

The present case report is a textbook model for β -thalassemia, outlining both the clinical and the genetical heterogeneity of the disease, as well as the importance of age-related complications. Complications of β -thalassemia intermedia are associated with ineffective hematopoietic bone marrow, chronic anemia and progressive iron overload. Symptoms usually start between 6-10 years of age and increase in severity with age. Timely blood transfusion and iron removal treatment can delay and reduce the occurrence of childhood complications.

The molecular diagnosis of β -thalassemia in light of the clinical progression of the disease should be carefully discussed. The differences between β -thalassemia intermedia and β -thalassemia major are usually difficult to distinguish; however, according to the proband's blood transfusion requirements outlined in the present report (average pre-transfusion Hb level < 60 g/l) the most reasonable diagnosis is severe thalassemia intermedia.

Acknowledgements

Not applicable.

Funding

The work was supported by Shenzhen High-level Hospital Construction Fund and by a grant from the Shenzhen Science and Technology Innovation Commission (grant. no. JCYJ20170817172241688).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HQ, WLL and WFL designed the present study. XHL, XLZ, JX, WHZ and YW performed the experiments and analyzed the data. HQ wrote the manuscript. HQ and WLL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study protocol was conducted in line with The Declaration of Helsinki, and was approved by the Ethics Committee of Shenzhen Second People's Hospital (Shenzhen, China; approval no. 20210707004); written informed consent was obtained from the parents for themselves and on behalf of the patient.

Patient consent for publication

Written informed consent was obtained from the parents of the patient.

Competing interests

The authors declare that they have no competing interests.

References

1. Martin A and Thompson AA: Thalassemias. *Pediatr Clin North Am* 60: 1383-1391, 2013.
2. He S, Li J, Li DM, Yi S, Lu X, Luo Y, Liang Y, Feng C, Chen B, Zheng C and Qiu X: Molecular characterization of α - and β -thalassemia in the Yulin region of Southern China. *Gene* 655: 61-64, 2018.
3. Cao A and Galanello R: Beta-thalassemia. *Genet Med* 12: 61-76, 2010.
4. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, Barella S, Uda M, Usala G, Abecasis GR and Cao A: Amelioration of Sardinian beta0 thalassemia by genetic modifiers. *Blood* 114: 3935-3937, 2009.
5. Panyasai S, Fucharoen S, Surapot S, Fucharoen G and Sanchaisuriya K: Molecular basis and hematologic characterization of deltabeta-thalassemia and hereditary persistence of fetal hemoglobin in Thailand. *Haematologica* 89: 777-781, 2004.
6. Zeng YT, Huang SZ, Chen B, Liang YC, Chang ZM, Harano T and Huisman TH: Hereditary persistence of fetal hemoglobin or (delta beta)0-thalassemia: three types observed in South-Chinese families. *Blood* 66: 1430-1435, 1985.
7. Chen W, Zhang X, Shang X, Cai R, Li L, Zhou T, Sun M, Xiong F and Xu X: The molecular basis of beta-thalassemia intermedia in southern China: Genotypic heterogeneity and phenotypic diversity. *BMC Med Genet* 11: 31, 2010.
8. So CC, So AC, Chan AY, Tsang ST, Ma ES and Chan LC: Detection and characterisation of beta-globin gene cluster deletions in Chinese using multiplex ligation-dependent probe amplification. *J Clin Pathol* 62: 1107-1111, 2009.
9. Zhang JW, Song WF, Zhao YJ, Wu GY, Qiu ZM, Wang FN, Chen SS and Stamatoyannopoulos G: Molecular characterization of a novel form of (A gamma delta beta)zero thalassemia deletion in a Chinese family. *Blood* 81: 1624-1629, 1993.
10. Zhuang J, Jiang Y, Wang Y, Zheng Y, Zhuang Q, Wang J and Zeng S: Molecular analysis of α -thalassemia and β -thalassemia in Quanzhou region Southeast China. *J Clin Pathol* 73: 278-282, 2020.

11. Mann JR, MacNeish AS, Bannister D, Clegg JB, Wood WG and Weatherall DJ: $\delta\beta$ -thalassemia in a chinese family. *Br J Haematol* 23: 393-402, 1972.
12. Peng CT, Liu SC, Chiou SS, Kuo PL, Shih MC, Chang JY and Chang JG: Molecular characterization of deletional forms of beta-thalassemia in Taiwan. *Ann Hematol* 82: 33-36, 2003.
13. Tan Jin Ai MA, Yap SF, Tan KL, Wong YC, Wee YC and Kok JL: Mild beta-thalassemia intermedia caused by compound heterozygosity for (G)gamma((A)gammadeltabeta)(o)/beta-thalassemia and molecular characterization of the defect in four Chinese families. *Acta Haematol* 109: 169-175, 2003.
14. Wu Y, Yao Q, Zhong M, Wu J, Xie L, Su L and Yu F: Genetic research and clinical analysis of deletional Chinese $\gamma^{+}(\delta\beta)^{0}$ -thalassemia and Southeast Asian HPFH in South China. *Ann Hematol* 99: 2747-2753, 2020.
15. He S, Wei Y, Lin L, Chen Q, Yi S, Zuo Y, Wei H, Zheng C, Chen B and Qiu X: The prevalence and molecular characterization of $(\delta\beta)^{0}$ -thalassemia and hereditary persistence of fetal hemoglobin in the Chinese Zhuang population. *J Clin Lab Anal* 32: e22304, 2018..
16. Cai WJ, Li J, Xie XM and Li DZ: Screening for common β -globin gene cluster deletions in Chinese individuals with increased hemoglobin F. *Int J Lab Hematol* 37: 752-757, 2015.
17. Craig JE, Barnetson RA, Prior J, Raven JL and Thein SL: Rapid detection of deletions causing delta beta thalassemia and hereditary persistence of fetal hemoglobin by enzymatic amplification. *Blood* 83: 1673-1682, 1994.
18. Xu XM, Zhou YQ, Luo GX, Liao C, Zhou M, Chen PY, Lu JP, Jia SQ, Xiao GF, Shen X, *et al*: The prevalence and spectrum of alpha and beta thalassaemia in Guangdong Province: Implications for the future health burden and population screening. *J Clin Pathol* 57: 517-522, 2004.
19. Du L, QD, Wang JC, *et al*: Analysis and prenatal diagnosis of 5 families with 813-thalassemia. *J Mol Diagn Ther* 7: 27-32, 2015.
20. Chen M, Zhang M, Chen L, Lin N, Wang Y, Xu L and Huang H: Genetic research and clinical analysis of β -globin gene cluster deletions in the Chinese population of Fujian province: A 14-year single-center experience. *J Clin Lab Anal* 36: e24181, 2022.
21. Liang HF, Liang WM, Xie WG, Lin F, Liu LL, Li LJ, Ge YY, Lu M, Liao YW, Zeng GK, *et al*: The gene spectrum of thalassaemia in Yangjiang of western Guangdong Province. *Front Genet* 14: 1126099, 2023.
22. Taher AT, Radwan A and Viprakasit V: When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. *Vox Sang* 108: 1-10, 2015.
23. Weatherall DJ: The definition and epidemiology of non-transfusion-dependent thalassemia. *Blood Rev* 26 (Suppl 1): S3-S6, 2012.