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CLINICAL REPORT

Hyperhaemolysis in a pregnant woman with a homozygous β^0-thalassemia mutation and two genetic modifiers

Lou Jiwu[1] | **Sun Manna**[2] | **Meixiang Lai**[3] | **Zhao Ying**[1] | **Liu Yanhui**[1]

[1]Prenatal Diagnostic Center, Dongguan Maternal & Children Health Hospital, Dongguan, China

[2]Department of Obstetrics & Gynecology, Dongguan Maternal and Children Hospital, Dongguan, China

[3]Department of Obstetrics & Gynecology, Dongguan Gaobu Hospital, Dongguan, China

Correspondence
Liu Yanhui, Department of Prenatal Diagnosis Center, Dongguan Maternal and Child Health Hospital, Dongguan 523112, Guangdong, China.
Email: liuliang71215@163.com

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Abstract

Introduction: Patients with a homozygous β^0-thalassemia mutation usually have a transfusion-dependent β-thalassemia major phenotype. However, some β-thalassemia patients present with a relatively mild and even normal phenotype and always have a high level of Hb F induced by genetic modifiers.

Methods: In this study, we identified a homozygous β^0-thalassemia mutation (*HBB*: c.126_129delCTTT) in a 36-year-old pregnant woman. She had not presented any clinical symptoms of β-thalassemia since birth. To investigate her unexpected mild phenotype, known genetic modifiers that ameliorate the severity of β-thalassemia were analysed. Besides, we described the haematological changes during pregnancy.

Results: Two genetic modifiers (a heterozygous *KLF1*: c.519_525dup mutation; and two homozygous *HBS1L-MYB* locus SNP variants: rs7776054 and rs9399137) were identified. However, she showed a gradually decreased level of Hb during pregnancy, and serious transfusion complication of hyperhaemolysis was induced and complicated the pregnancy.

Conclusion: This report is in accordance with previous findings that genetic modifiers can ameliorate the clinical severity of β-thalassemia, even without obvious clinical symptoms in a prolonged steady state. However, the steady state can be disrupted during pregnancy. In addition, raising awareness of hyperhaemolysis among clinicians treating patients with thalassemia is necessary.

KEYWORDS

foetal haemoglobin, *HBS1L-MYB*, hyperhaemolysis, *KLF1*, β-thalassemia

1 | INTRODUCTION

In southern China, there is a high prevalence of β-thalassemia (β-tha; Xiong et al., 2010; Xu et al., 2004; Yin et al., 2014). Homozygotes and compound heterozygotes for β-tha usually have a severe transfusion-dependent phenotype. However, some conditions that decrease the production of α-globin (α-thalassemia) or increase the production of γ-globin can improve the clinical severity (Galanello et al., 2009).

Several genetic modifiers that upregulate the expression of γ-globin have been identified (Chen et al., 2017; Razak et al., 2018). Deletions involving the β-globin locus (e.g. $\delta\beta^0$-thalassemia) usually have higher levels of γ-globin; two single-nucleotide polymorphisms (SNPs), rs7482144 (Xmn1

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polymorphism) in *HBG2* (OMIM: 142250) and rs368698783 in *HBG1* (OMIM: 142200), have also been shown to increase γ-globin production. In addition to these SNPs in the β-globin locus, some modifiers involved genes or polymorphisms that were located on other chromosomes, including SNPs in the *HBS1L-MYB* intergenic region on chromosome 6q23 and the *BCL11A* gene (OMIM:606557) on chromosome 2p16, and mutations in the *KLF1* gene (OMIM:600599) on chromosome 19p13.

However, the application of these modifiers in clinical practice, especially in prenatal diagnosis and genetic counselling of thalassemia, requires a more complete observation of phenotypic alterations in specific cases. Here, we provide a detailed description of clinical and molecular characteristics in a pregnant Chinese woman with a homozygous β-thal mutation, which could provide a straightforward example to determine the effect of genetic modifiers on the clinical severity of β-thal. In addition, the diagnosis and treatment of hyperhaemolysis in this patient indicated that raising awareness of such serious blood transfusion complications among clinicians treating patients with thalassemia is necessary.

2 | CLINICAL PRESENTATION

The proband was a 36-year-old, gravida 4, para 3, woman from Beiliu City, Guangxi Province in Southern China. She was born normally and had an unremarkable childhood and adolescence similar to her peers, without any noticeable development delay, jaundice, paleness, fatigue, dark-coloured urine and splenomegaly or hepatomegaly. During childhood and adolescence, she had never visited a doctor for anaemia.

Her first pregnancy was normal, and her second pregnancy was terminated early for social reasons. However, during her third pregnancy, she had a haemoglobin (Hb) level of 6.8 g/dl after postpartum blood loss of 490 ml; she did not receive prenatal thalassemia examination because her husband had a negative result. However, new-born thalassemia screening at a local hospital suggested that the first and third pregnancies, a daughter and a son currently 9 and 5 years old, respectively, were carriers for β-thal.

At 18^{+1} weeks of gestation of the current (fourth) pregnancy, routine haematological evaluation revealed an Hb level of 10.1 g/dl, a mean cell volume (MCV) of 69 fL, a mean cell Hb (MCH) of 22.7 pg, an Hb A_2 level of 2.0% and an Hb F level of 98%. At 22^{+1} weeks of gestation, she was referred to us for amniocentesis due to a high risk for trisomy 21 by noninvasive prenatal screening. The Hb level was 9.0 g/dl, and clinical chemistry analysis revealed a total bilirubin level of 23 μmol/L (TBIL, normal range 0–21) and a direct bilirubin level of 7.9 μmol/L (DBIL, normal range 0–5). Genotyping for thalassemia indicated that she was homozygote for a common β-thalassemia major mutation (*HBB*: c.126_129delCTTT) [Figure 1a–c]. Physical examination was unremarkable, without signs of anaemia, such as jaundice, paleness, splenomegaly and hepatomegaly. To investigate her unexpected mild phenotype, known genetic modifiers that ameliorate the severity of β-thalassemia were analysed (Fanis et al., 2019; Lai et al., 2016; Razak et al., 2018). A heterozygous *KLF1* mutation (c.519_525dup) and two homozygous SNP variants, rs7776054 and rs9399137, in the *HBS1L-MYB* locus were identified [Figure 1d].

Genetic analysis of amniotic fluid excluded trisomy 21 and identified an expected heterozygous *HBB*: c.126_129delCTTT mutation in the foetus. The pregnancy continued. At 28^{+1} weeks of gestation, she was admitted to a local hospital for vaginal bleeding induced by central placenta previa. Examination on admission showed an Hb level of 8.1 g/dl, a TBIL level of 21.9 μmol/L and a DBIL level of 7.8 μmol/L. She was treated with general measures. At 31^{+3} weeks of gestation, she had an Hb level of 7.2 g/dl and received a transfusion of 2 units of leukocyte filtration erythrocytes, which was her first transfusion since birth. The transfusion process was normal.

At 34^{+1} weeks of gestation, she was referred for termination of pregnancy. Examination on admission showed an Hb level of 9.9 g/dl, a TBIL level of 26.9 μmol/L and a lactate dehydrogenase level of 341 U/L (LDH, normal range 120–250). Urine was negative for Hb and erythrocytes. In view of her condition and the need for a caesarean section at any time, a second transfusion of 1.5 units of leukocyte filtration erythrocyte was administered to keep Hb >10.0 g/dl. However, posttransfusion Hb on the second day was 9.4 g/dl with TBIL 33.9 μmol/L. A third transfusion of 1.5 units of leukocyte filtration erythrocyte was administered, and similar lower posttransfusion Hb and higher bilirubin levels were observed. In addition, she presented dark brown urine and increased levels of urobilinogen (UBG, 140 μmol/L, normal range 3–16). Serologic analysis showed a positive antiglobulin test with anticomplement (C3d). Hyperhaemolysis was considered.

Five days later, her Hb level was 7.4 g/dl. In view of the falling Hb level and completion of urging foetal lung maturation with dexamethasone, caesarean section was performed to terminate pregnancy. Intraoperative blood loss was approximately 500 ml, and the fourth transfusion with 3 units of leukocyte filtration erythrocyte was administered. Posttransfusion Hb was 7.8 g/dl. She was then admitted to the intensive care unit, and methylprednisolone was started.

Unfortunately, her Hb further dropped to 4.0 g/dl on day 3. A fifth transfusion of 4 units of leukocyte filtration erythrocyte was given. Similar to previous transfusion, her Hb gradually decreased with persistently elevated levels of bilirubin and LDH. Three days later, Hb dropped to 4.7 g/dl, with TBIL 20.3 μmol/L, DBIL 9.2 μmol/L and LDH 1456 U/L. In

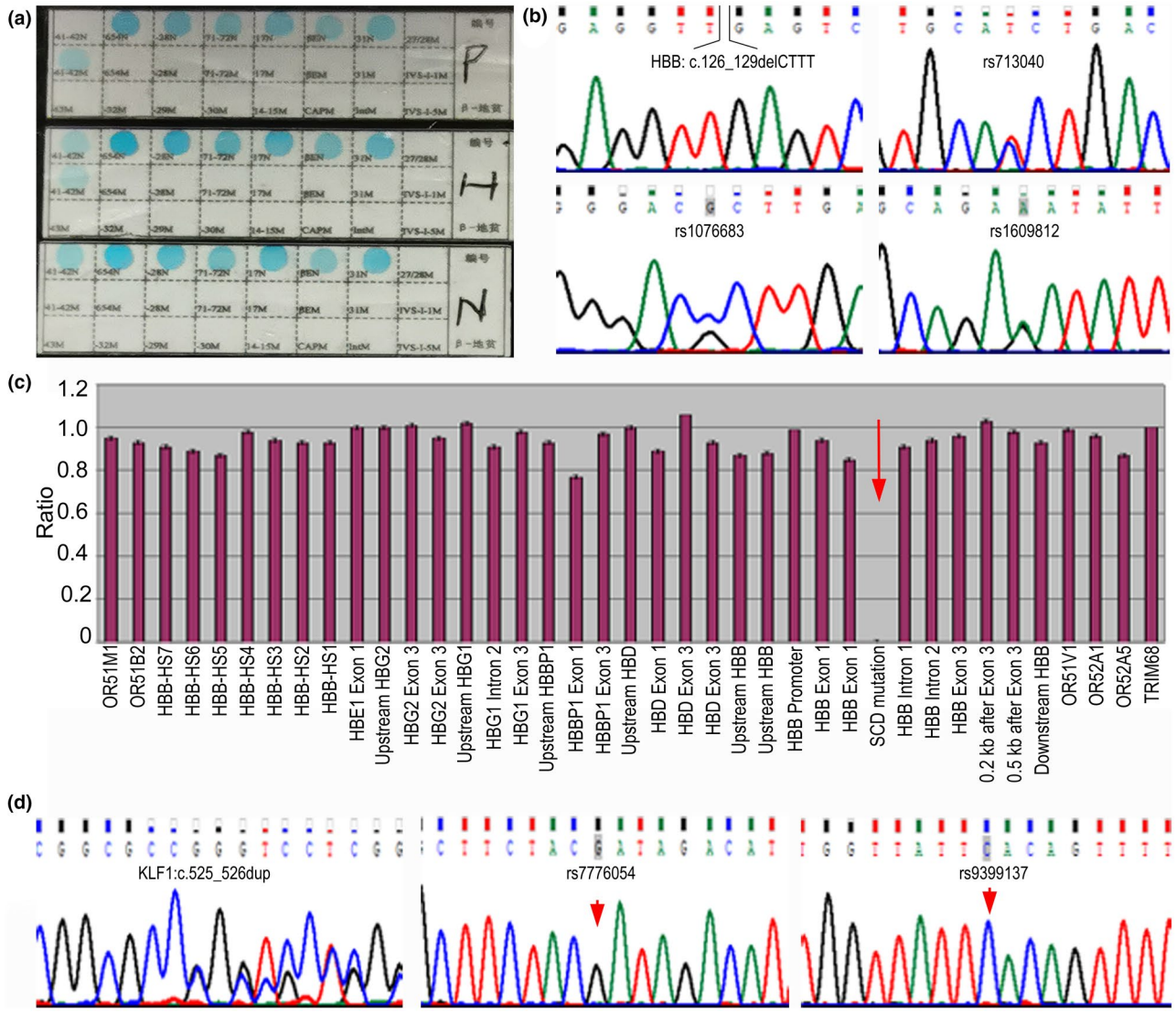

FIGURE 1 Summary of genetic analysis in the pregnant woman. (a) The homozygous *HBB*: c.126_129delCTTT mutation was first identified by a routine reverse dot blot (RDB) method. From right, the first dots at the middle and the top of each filter strip are the *HBB*: c.126_129delCTTT probe and its control probe respectively. The subjects tested are shown on the right. P: the pregnant women, H: heterozygote for *HBB*: c.126_129delCTTT mutation, N: normal control. (b) Due to lack of evidence from parents' genotypes, we further performed DNA sequencing and MLPA analysis. DNA sequencing confirmed the RDB result and identified some heterozygous benign SNPs in *HBB* gene, which suggest that there is no deletion in *HBB* gene. (c) The MLPA with HBB P102-C1 kit (MRC-Holland, Amsterdam, The Netherlands) detected no deletion and duplication, the relative position of the probes in the β-globin gene cluster is indicated in the horizontal axis. The red downward arrows indicate the probe targeting sickle cell mutation. (d) DNA sequencing indicated the heterozygous *KLF1:c.519_525dup* variant and two homozygous SNP variants, rs7776054 and rs9399137, in the *HBS1L-MYB* locus

addition, IgM anti-P1 and IgG anti-Jk^a antibodies were identified after the last transfusion. In view of her condition, she was transferred to a superior hospital for further assessment and treatment (IV immunoglobulins were given). A month later, by telephone follow-up, she said she had recovered after 2 weeks of hospitalization. Haematological and clinical chemical indexes are summarized in Table 1.

The ethics committee of Dongguan Maternal and Children Hospital approved the study. Informed consent was obtained from participants (husband and wife).

3 | DISCUSSION

HBB: c.126_129delCTTT is a common β^0-thalassemia mutation in Chinese individuals (Xu et al., 2004), for which most homozygous patients have a classic transfusion-dependent β-thalassemia major phenotype, with an average onset age of younger than 2 years old. However, the patient in our study had led a normal life since birth, without developmental delay, jaundice, paleness, fatigue, dark-coloured urine and splenomegaly or hepatomegaly.

TABLE 1 Changes of haematological and clinical chemical indexes during pregnancy and after transfusion

Gestation/ postpartum day	Day after transfusion	WBC (g/L)	RBC (T/L)	Hb (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	PLT (g/L)	TBIL (umol/L)	DBIL (umol/L)	LDH (U/L)
Normal range		5–15	3.5–5.5	11–15	31–34	80–100	27–34	100–300	0–21	0–5	120–250
18^{+1}		13.61	4.45	10.1	30.7	69.0	22.7	234			
22^{+1}		12.66	3.92	9.0	26.8	68.4	23.0	187			
24^{+3}		12.07	3.81	8.6	26.8	70.3	22.6	169	23	7.9	
28^{+1}		11.81	3.49	8.1	25.4	72.8	23.2	186	21.9	7.8	
31^{+3}		11.51	3.1	7.2	22.4	72.0	23.0	176			
Transfusion (2 units)											
32^{+6}	10	12.58	3.82	9.0	28.5	74.6	24.0	178			
34^{+1}	19	14.03	3.92	9.9	27.8	70.9	25.3	189	26.9	8.7	341
Transfusion (1.5 units)											
34^{+2}	1	13.08	4	9.7	29.1	72.8	24.3	154			
34^{+3}	2	13.05	3.81	9.4	28.3	74.3	24.7	148	33.9	11.2	
Transfusion (1.5 units)											
34^{+3}	1	13.52	3.94	9.9	28.4	72.1	25.1	148			
34^{+4}	2	10.56	3.44	8.7	24.9	72.4	25.3	150	39.2	15.5	
34^{+6}	4	10.84	3.37	8.2	23.9	70.9	24.3	153	40.4	17.8	770
34^{+1}	5	10.61	3.26	7.9	22.9	70.2	24.2	125	36.9	18	865
35^{+2}	6	11.71	3.1	7.4	21.9	70.6	23.9	173	33.3	15.2	807
Transfusion (3 units)[a]											
0	1	16.14	3.08	7.8	22.5	73.1	25.3	164	66.1	31	929
1	2	13.38	2.38	5.8	17.2	72.3	24.4	148	39.6	24.7	951
2	3	8.7	1.71	4.0	12.3	71.9	23.4	149	27.1	14.9	764
Transfusion (4 units)											
2	1	12.74	2.86	7.4	21.6	75.5	25.9	161	70.9	28.7	
3	2	11.25	2.5	6.3	18.8	75.2	25.2	153	49	23	1188
4	3	6.95	2.01	4.9	14.7	73.1	24.4	140	39	19.2	
5	4	8.78	2.04	4.7	15.1	74	23	151	20.3	9.2	1456

Abbreviations: DBIL, direct bilirubin level; Hb, haemoglobin; HCT, Haematocrit; LDH, lactate dehydrogenase; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; PLT, Platelet; RBC, red blood cell; TBIL, total bilirubin level; WBC, white blood cell.

[a]Caesarean section was performed to terminate pregnancy; transfusion was given during operation.

The identification of homozygous mutations was made incidentally on routine haematological examination during pregnancy.

The mild phenotype was due to the high level of Hb F, which compensated for the loss of Hb A and ameliorated anaemia and disease severity. The *KLF1* mutation is one of the genetic modifiers that upregulates the expression of the γ-globin gene. In 2014, Liu et al. found that the presence of *KLF1* mutations in β-thalassemia patients could lead to a dramatic improvement in clinical severity, resulting in a thalassemia intermedia phenotype, with reduced transfusion requirements and Hb levels in the range 6.5–9.2 g/dl (Liu et al., 2014). The patient in our study had a *KLF1*:c.519_525dup mutation, similar to most patients in the study by Liu et al. However, our patient was older and had a milder phenotype with transfusion independence. Given a usually relatively decreased Hb level during pregnancy, the nonpregnancy Hb level in our patient should not be lower than that of 10.1 g/dl at 17 weeks of gestation of the fourth pregnancy. The milder phenotype in our patient was due to homozygous *HBS1L-MYB* variants beyond the *KLF1* mutation. The linked rs7776054 and rs9399137 SNPs have been shown to be associated with Hb F levels in Chinese β-thalassemia patients (Farrell et al., 2011; Lai et al., 2016). Our patient was similar to two Cypriot patients reported recently by Fanis et al. (2019). The Cypriot patients were homozygous for the β-thalassemia major *HBB*: c.93–21G>A mutation and heterozygous for the *KLF1*: c.968C>T mutation. They were phenotypically healthy, with

near-normal levels of total haemoglobin (12.7–13.5 g/dl) and extremely high Hb F levels (63%–66.2%). However, the milder phenotype in Cypriot patients may be due to allelic heterogeneity: the *KLF1* missense mutation (c.968C>T) in Cypriot patients probably upregulates the expression of the γ-globin gene by way different from the loss-of-function allele (*KLF1*: c.519_525dup). Of course, the possibility of other unknown modifiers ameliorating severity cannot be ruled out in Cypriot patients.

However, the steady state can be disturbed under certain conditions, as indicated by our patient. The low level of postpartum Hb at 6.8 g/dl after blood loss of 490 ml indicated that haemolysis should have been aggravated during the third pregnancy. During the fourth pregnancy, the Hb level obviously gradually decreased from 10.1 g/dl at 17 weeks of gestation to 7.2 g/dl at 31 weeks of gestation. Ultimately, blood transfusion was necessary for her condition. Unfortunately, a serious transfusion complication of hyperhaemolysis was induced.

Hyperhaemolysis is characterized by the destruction of both host and donor red blood cells via an unknown mechanism, which was diagnosed mainly based on (1) a posttransfusion Hb level that was lower than the pretransfusion Hb level, (2) the development of an elevated LDH or TBIL above the patient's pretransfusion level and/or acute-onset haemoglobinuria and (3) the onset of symptoms between 2 and 21 days following RBC transfusion (Merrill et al., 2019). According to these criteria, hyperhaemolysis in our patient should have been triggered by the second transfusion. However, the diagnosis of hyperhaemolysis was made after the third transfusion because hyperhaemolysis has been mostly described in sickle cell disease, and we first attributed the lower Hb level after the second transfusion to suspicious bleeding induced by central placenta previa. Treatment of hyperhaemolysis is challenging. It is important to avoid further transfusion as much as possible and start immunosuppressive treatment (Danaee et al., 2015). Further transfusion probably worsens haemolysis, causing more serious anaemia, which was seen in our patient. However, further transfusions are necessary for clinical reasons. In our patient, immunosuppressive treatment with methylprednisolone was unsuccessful, and she was transferred to a superior hospital for further assessment and treatment due to our unfamiliarity with hyperhaemolysis treatment.

The pathophysiology of hyperhaemolysis is unknown. Although the *KLF1:c.519_525dup* variant had been reported to cause an apparent dominantly inherited Lu(a-b-) phenotype and anti-Lu^a or anti-Lu^b could cause delayed haemolytic transfusion reactions (Fraser et al., 2019; Thornton & Grimsley, 2019), it was impossible that the hyperhaemolysis in the pregnant woman was due to transfusion of mismatched Lutheran-typed red cells. Unlike the true Lu_{null} phenotype, the Lu (a-b-) phenotype caused by *KLF1* mutations is due to a great reduction, but not loss, in Lu antigens. So the pregnant woman should not be at risk of development of anti-Lu in presence of weaken Lu antigens. However, serologic analysis showed presence of complement C3 after the third transfusion and IgM anti-P1 and IgG anti-Jka after the last transfusion. Similar conditions were noted to coincide with hyperhaemolysis in some previous descriptive reports Merrill et al. (2019). It may be that complement and alloimmunization played a role in initiation and development of the hyperhaemolysis.

In conclusion, this report agrees with previous findings that genetic modifiers can ameliorate the clinical severity of β-thalassemia, even without obvious clinical symptoms in a prolonged steady state, which should be considered during prenatal diagnosis and genetic counselling of thalassemia. However, the steady state can be disrupted during pregnancy. In addition, raising awareness of hyperhaemolysis among clinicians treating patients with thalassemia is necessary.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Lou Jiwu analysed the clinical and genetic data and wrote the manuscript. Sun Manna and Lai Meixiang collected clinical data. Zhao Ying performed the Sanger sequencing. Liu Yanhui designed the study plan and critically reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Lou Jiwu https://orcid.org/0000-0001-8250-1498

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