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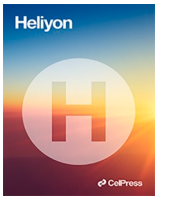

Case report

Case report: A rare heterozygous Hb CS with heterozygous HbE in a family with thalassemia in China

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A R T I C L E I N F O

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A B S T R A C T

Thalassemia is a hemoglobin disease characterized by reduced or complete absence of the production of the α/β globin gene. Currently, the detection of β-thalassemia carriers is based on differences in blood cell parameters. However, β-thalassemia carriers cannot be distinguished from α- and β-thalassemia co-inherited carriers based solely on hematological findings, and the differential diagnosis must rely on molecular diagnosis. We report a 32-year-old male from Yunnan Province, who had abnormal hemoglobin E without obvious anemia. A rare α^{CS} (CD142, TAA→CAA) combined with a βE (CD26, GAG→AAG) double heterozygous mutation was identified in the proband by PCR-reverse dot blot (PCR-RDB) and DNA sequencing. Additionally, a family lineage analysis was performed. This study complements the spectrum of rare thalassemia gene variants and is critical for clinical genetic counseling.

1. Introduction

Thalassemia is a form of hemolytic anemia that is caused by the deletion or mutation of the globin gene, leading to impaired globin chain synthesis. It is a genetic disease that poses serious threats to human health. Thalassemia is prevalent in Southeast Asia, the Middle East, the Mediterranean, Africa and the Indian subcontinent, with marked ethnic and geographic differences [1]. Thalassemia is highly prevalent in South China, such as Yunnan, Guangdong, Guizhou, Guangxi and Sichuan [2,3]. Clinical diagnosis of thalassemia mainly includes hematological screening and genetic testing. Hematological screening primarily includes routine blood tests, hemoglobin electrophoresis, and red blood cell osmotic fragility tests. Genetic diagnosis is typically used for the detection of common hotspot deletions or mutation types of thalassemia gene defects in China [4]. Currently, thalassemia gene test kits can detect the 23 most prevalent thalassemia variants in China, encompassing 3 deletional α-thalassemia variants, 3 non-deletional α-thalassemia variants, and 17 common β-thalassemia variants. Among them, α^{CS} combined with a βE double heterozygous mutation has been rarely reported in China, and its clinical characteristics require investigation. In this study, a case of Hb Constant Spring (Hb CS, HBA2: c.427, T > C) combined with a CD26 (HBB: c.79, G > A) double heterozygous mutation was detected, and family lineage was analyzed.

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2. Case description

The proband, a 32-year-old male from Yunnan Province, visited the department of neurology due to intermittent headache and dizziness in February 2023. The proband reported no obvious abnormalities in head MRI and CT, and denied having abnormal blood pressure and dyslipidemia. The doctor inquired about the medical history and learned that the proband was diagnosed with thalassemia in Yunnan; however, the specific examination was unknown. Physical examination: no pale lips, no yellow mucosa, no liver and spleen enlargement, no lymph node enlargement. Previous history: no history of blood transfusion, no history of iron therapy. Then, the proband was diagnosed as a thalassemia carrier of α^{CS} (TAA→CAA) heterozygous mutation combined with a βE (GAG→AAG) heterozygous mutation through complete blood count (CBC), hemoglobin (Hb) analysis, and genetic analysis in our hospital. With no obvious anemia, the proband did not need treatment for the time being. Then, the proband was advised to go to the department of psychology. The doctor evaluated the proband's intermittent headache and dizziness as related to anxiety and depression, and treated him with Flupentixol-melitracen.

In China, a database and literature search revealed that this genotype of the proband has rarely been reported. The parents of the proband are from Yunnan. The family members were investigated, and their CBC, Hb analysis, and genetic analysis were performed (Fig. 1A). CBC was detected with an automated hematology analyzer (Sysmex XN9100, Japan). The white blood cell (WBC) count, Hb level and platelet (PLT) count were normal for the proband (II1), father (I1), sister (II2), and daughter (III1). They showed decreased levels of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) without obvious anemia. The mother (I2) of the proband had microcytic hypochromic anemia with decreased levels of MCV (79.1 fL), MCH (25.2 pg), and Hb (105 g/L). Capillary electrophoresis (CE) (Sebia Minicap-FP; Sebia, France) were used to detected Hb analysis of the peripheral blood samples. The mother (I2) and sister (II2) had normal HbA2 levels (normal range 1.5%–3.7 %), whereas the proband (II1), father (I1), and daughter (III1) had

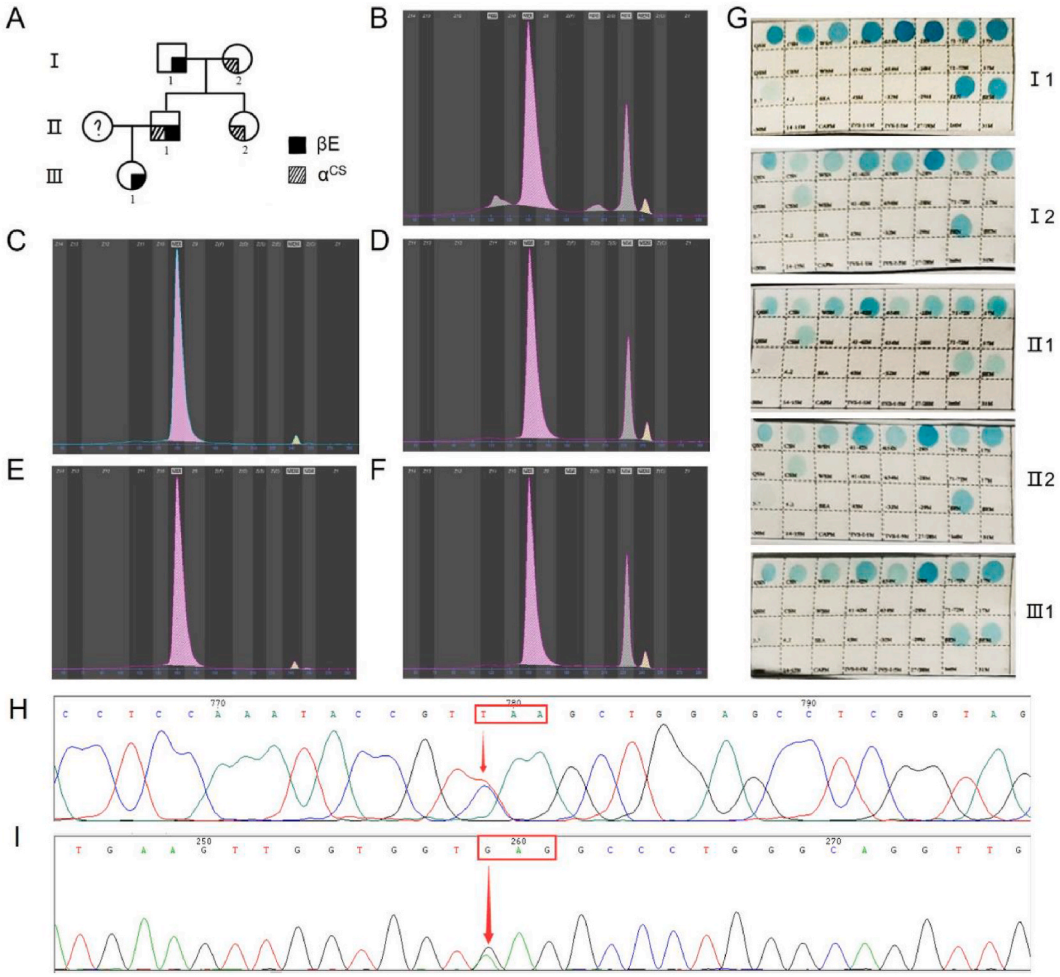

Fig. 1. Identification of the thalassemia genotype. (A) Family pedigree. Circles: female members; squares: male members; (B–F) Hb analysis via capillary electrophoresis: (B) father, (C) mother, (D) proband, (E) sister, (F) daughter of the proband; (G) Genotype confirmation via PCR-RDB for the family; (H) The DNA sequencing revealed a heterozygous T/C mutation in α2; (I) The DNA sequencing revealed a heterozygous G/A mutation in β. Arrows indicate the positions of the mutations.

abnormal hemoglobin E (HbE), suggesting the possibility of thalassemia (Table 1; Fig. 1B–F).

Then, we used PCR-RDB technology (Yaneng BIOscience, China) to confirm the genotype. We extracted genomic DNA from 200 μL venous blood samples with the DNA extraction kit (Tianlong, China), and detected 3 deletional α-thalassemia variants (–$\alpha^{3.7}$, –$\alpha^{4.2}$ and –^{SEA}), 3 non-deletional α-thalassemia variants, (α^{CS} (CD142, TAA→CAA), α^{QS} (CD125, CTG→CCG), and α^{WS} (CD122, CAC→CAG)), and 17 common β-thalassemia variants (CD17 (AAG→TAG), CD41-42 (-TTCT), CD71–72 (+A), IVS-II-54 (C→T), IVS-I-1 (G→T), −28 (A→G), βE (GAG→AAG), CD43 (GAG→TAG), −29 (A→G), CD27/28 (+C), CD14–15 (+G), IVS-I-5 (G→C), CD31 (–C), −32 (C→A), −30 (T→C), 5′UTR: +40–43 (–AAAC), and Int (ATG→AGG)). According to the PCR-RDB assay, the proband's genotype had α^{CS} (CD142, TAA→CAA) combined with a βE (CD26, GAG→AAG) double heterozygous mutation. The genotype of his father (I1) and daughter (III1) was characterized by a βE heterozygous mutation, whereas the genotype of his mother (I2) and sister (II2) featured an $\alpha^{CS}\alpha/\alpha\alpha$ heterozygous mutation (Fig. 1G). Hb analysis of the proband suggested the presence of a large number of HbE. To exclude the rare thalassemia mutations, DNA sequencing was performed by the ABI 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and analyzed by the Mutation Surveyor software (version 5.0.1). Sequence analysis (alignment to reference sequences NG_000006.1 and NG_000007.3) revealed heterozygous mutations c.427T > C in HBA2 and c.79G > A in HBB, which was consistent with the PCR-RDB analysis results (Fig. 1H and I).

3. Discussion

According to the defective globin chain, thalassemia is mainly divided into α- and β-thalassemia, caused by HBA1/2 and HBB gene mutations, respectively [5]. The pathogenesis of thalassaemia is characterised by an imbalance in globin chain production, resulting in ineffective erythropoiesis, increased hemolysis, and disrupted iron homeostasis. Depending on the severity of anemia and the need for regular RBC transfusions, thalassemia can be divided into mild, intermediate, and major thalassemia. Mild thalassemia is caused by heterozygous mutations in a single thalassemia gene. Patients with mild thalassemia are clinically asymptomatic, with or without mild anemia. Individuals with major thalassemia usually require regular RBC transfusions. Intermediate thalassemia is less severe, and patients do not require transfusions or may receive sporadic transfusions [6]. In this study, the father and daughter of the proband were β-thalassemia carriers, and the mother and sister of the proband were α-thalassemia carriers. Only the mother had slight microcytic hypochromic anemia.

The incidence of thalassemia is high in Southeast Asia. Unlike hemoglobin sickle (HbS), the most common in Africa and the Mediterranean, HbE-producing β-thalassemia is dominant in Southeast Asia. This disease is mainly caused by the mutation of GAG to AAG at codon 26 of the β-globin gene, resulting in an amino acid change from glutamic acid (Glu) to lysine (Lys), activating cryptic splicing sites that regulate mRNA splicing of the β-globin gene. The activated cryptic splicing sites can compete with normal splicing sites and reduce the production of normally spliced mRNA, resulting in reduced β-globin gene synthesis and the production of variant mRNA. The latter can direct structurally abnormal, but functional HbE synthesis. Therefore, the clinical symptoms of βE carriers are mild. However, when βE is combined with other types of β-thalassemia, the clinical symptoms are different [7,8]. Additionally, the hematological results of single α-gene deficiency combined with β-thalassemia were similar to those of β-thalassemia, indicating that the usual single α-gene inactivation did not significantly effect the manifestations of β-thalassemia. It may be due to the improvement in the α/β-globin chain imbalance [9]. The compound heterozygous Hb CS/Hb E genotype has been reported in Southeast Asia. In a study of 202 Thai individuals, the frequency of double heterozygotes for Hb E and Hb CS was 8.4 % (17/202) [10]. Among the Có-Tu ethnic minority of Vietnam, the frequency of double heterozygotes for Hb E and Hb CS was 2.4 % (7/298) [11]. Notably, the double heterozygous Hb CS/Hb E carriers were often misdiagnosed as pure Hb E carriers on routine Hb analysis because Hb CS was not identified. Thalassemia has significant ethnic and geographic differences. HbE is the most common β-globin gene mutation genotype in thalassemia patients in Yunnan, which is different from the other parts of China [12]. A study has reported that among the 38,812 reproductive age couples, heterozygous Hb CS/Hb E genotypes were found in five cases in the Guangxi-Yunnan-Guizhou province, China [13]. The frequency of double heterozygotes for Hb E and Hb CS is 0.0129 % (5/38812), which is very low and is only briefly mentioned in the epidemiological statistic without a detailed characterization of its clinical features. In this study, both the father and daughter of the proband carried a βE heterozygous mutation and had significantly increased HbE levels, but neither displayed an

Table 1
Blood screening and genetic analysis for thalassemia in enrolled participants.

Case	Sex	Age	RBC (10^{12}/L)	Hb (g/L)	MCV (fL)	MCH (pg)	RDW (fl)	HbA (%)	HbA2 (%)	HBF (%)	HbE (%)	Genotype	
												α	β
Father (I1)	M	61	5.54	139.0	74.0	25.0	40.7	68.7	2.6	0.0	22.4	αα/αα	βE/β^N
Mother (I2)	F	57	4.17	105.0	79.1	25.2	43.1	97.7	2.3	0.0	0.0	$\alpha^{CS}\alpha$/αα	β^N/β^N
Proband (II1)	M	32	5.44	144.0	80.0	26.5	38.1	73.6	3.4	0.0	23.0	$\alpha^{CS}\alpha$/αα	βE/β^N
Sister (II2)	F	34	4.89	131.0	78.6	26.8	41.5	97.6	2.0	0.0	0.0	$\alpha^{CS}\alpha$/αα	β^N/β^N
Daughter (III1)	F	5	4.58	115.0	71.0	25.1	35.0	71.4	3.3	0.4	24.9	αα/αα	βE/β^N

MCV: normal range ≥82 fL; MCH: normal range ≥27 pg.

anemia phenotype. The proband had a rare heterozygous Hb CS with a heterozygous HbE genotype. CBC analysis showed that the proband had no obvious anemia. Hb analysis showed that the proband's HbE was the same as the father and daughter, with a simple heterozygous βE mutation, validating the peptide chain balance theory of thalassemia. This study helps to further understand the manifestations of thalassemia with α-gene heterozygous mutation combined with a β-gene heterozygous mutation.

Thalassemia is an autosomal recessive disease, with normal clinical manifestations in thalassemia gene carriers. Yunnan is one of the provinces in China with a high prevalence of thalassaemia. The prevalence of thalassemia in Yunnan Province is complicated. Geographical location, ethnic distribution, population migration, and other factors impact gene frequency. A study has indicated that the frequency of thalassemia gene carriers in Yunnan was 10.71 %. Among them, the frequency of α-thalassemia gene carriers was 3.46 % and that of β-thalassemia gene carriers was 7.28 % [12]. Although the gene carriers may not show obvious symptoms, they may pass on the disease gene to the next generation, thereby increasing the risk of thalassemia in the offspring. Consequently, the implementation of pre-pregnancy screening and prenatal diagnosis in high-risk couples who are both thalassemia carriers represents an efficacious strategy for the reduction of the risk of thalassaemia at birth [14]. In addition, the proband in this study, originally from Yunnan, relocated to the north for work and residence. This case illustrates that the probability of detecting thalassemia in the northern regions has considerably increased because of changes in place of residence or intermarriage with individuals in the north. This study also suggests that the hematological parameters of β-thalassemia carriers cannot be distinguished from those of α-thalassemia and β-thalassemia co-carriers, and the differential diagnosis rely on gene analysis. Therefore, clinicians must consider the possibility of α-thalassemia combined with β-thalassemia heterozygous mutations in genetic counseling, even if the patient's hematological parameters are close to the normal range. This study has some limitations. For example, The proband sought medical treatment for intermittent headache and dizziness, not because of hematological symptoms. It was subsequently determined that the proband's symptoms were not related to thalassemia, and treatment details for the proband's initial symptoms were lacking. In addition, we failed to test the proband's wife triggering some flaws in the family pedigree.

4. Conclusions

Genetic analysis and DNA sequencing were used to identify a rare heterozygous Hb CS with heterozygous HbE in a Chinese family with thalassemia. This study adds to the data on rare thalassemia genotypes and holds important implications for clinical genetic counseling.

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Data availability

The patient data used to complete this study was not deposited into a publicly available repository. Further data will be made available from the corresponding author on reasonable request.

Statement

This study expands the spectrum of rare thalassemia gene variants by documenting a case with α^{CS} (CD142, TAA → CAA) and βE (CD26, GAG → AAG) double heterozygous mutations, which is pivotal for clinical genetic counseling. The proband exhibited abnormal hemoglobin E levels but no apparent symptoms of anemia. The program was granted ethical approval by the Human Research Ethics Committee of Peking Union Medical College Hospital (No. I-22PJ1013) and all participants provided informed consent.

CRediT authorship contribution statement

Di Wang: Writing – original draft, Formal analysis, Data curation. **Han Zhang:** Formal analysis, Data curation. **Zhuo Yang:** Methodology. **Wei Su:** Investigation. **Yaling Dou:** Writing – review & editing, Resources, Conceptualization. **Yingchun Xu:** Writing – review & editing, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B. Modell, M. Darlison, Global epidemiology of haemoglobin disorders and derived service indicators, Bull. World Health Organ. 86 (2008) 480–487, https://doi.org/10.2471/blt.06.036673.

[2] X.M. Xu, Y.Q. Zhou, G.X. Luo, C. Liao, M. Zhou, P.Y. Chen, J.P. Lu, S.Q. Jia, G.F. Xiao, X. Shen, J. Li, H.P. Chen, Y.Y. Xia, Y.X. Wen, Q.H. Mo, W.D. Li, Y.Y. Li, L. W. Zhuo, Z.Q. Wang, Y.J. Chen, C.H. Qin, M. Zhong, 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 (2004) 517–522, https://doi.org/10.1136/jcp.2003.014456.
[3] J. Zhang, J. He, X. Mao, X. Zeng, H. Chen, J. Su, B. Zhu, Haematological and electrophoretic characterisation of β-thalassaemia in yunnan province of southwestern China, BMJ Open 7 (2017) e013367, https://doi.org/10.1136/bmjopen-2016-013367.
[4] V. Brancaleoni, E. Di Pierro, I. Motta, M.D. Cappellini, Laboratory diagnosis of thalassemia, Int J Lab Hematol 38 Supplement 1 (2016) 32–40, https://doi.org/10.1111/ijlh.12527.
[5] F.B. Piel, D.J. Weatherall, The α-thalassemias, N. Engl. J. Med. 371 (2014) 1908–1916, https://doi.org/10.1056/NEJMra1404415.
[6] X. Shang, X. Xu, Update in the genetics of thalassemia: what clinicians need to know, Best Pract. Res. Clin. Obstet. Gynaecol. 39 (2017) 3–15, https://doi.org/10.1016/j.bpobgyn.2016.10.012.
[7] R.E. Hirsch, N. Sibmooh, S. Fucharoen, J.M. Friedman, HbE/β-thalassemia and oxidative stress: the key to pathophysiological mechanisms and novel therapeutics, Antioxidants Redox Signal. 26 (2017) 794–813, https://doi.org/10.1089/ars.2016.6806.
[8] D.R. Higgs, The molecular basis of α-thalassemia, Cold Spring Harb Perspect Med 3 (2013) a011718, https://doi.org/10.1101/cshperspect.a011718.
[9] A. Kattamis, J.L. Kwiatkowski, Y. Aydinok, Thalassaemia, Lancet 399 (2022) 2310–2324, https://doi.org/10.1016/S0140-6736(22)00536-0.
[10] K. Sanchaisuriya, G. Fucharoen, N. Sae-Ung, A. Jetsrisuparb, S. Fucharoen, Molecular and hematologic features of hemoglobin E heterozygotes with different forms of alpha-thalassemia in Thailand, Ann. Hematol. 82 (2003) 612–616, https://doi.org/10.1007/s00277-003-0689-y.
[11] V.H. Nguyen, K. Sanchaisuriya, K. Wongprachum, M.D. Nguyen, T.T.H. Phan, V.T. Vo, P. Sanchaisuriya, S. Fucharoen, F.P. Schelp, Hemoglobin Constant Spring is markedly high in women of an ethnic minority group in Vietnam: a community-based survey and hematologic features, Blood Cells Mol. Dis. 52 (2014) 161–165, https://doi.org/10.1016/J.BCMD.2013.12.002.
[12] B.S. Zhu, J. He, J. Zhang, X.H. Zeng, J. Su, X.H. Xu, S.Y. Li, H. Chen, Y.H. Zhang, A Study on Gene Mutation Spectrums of α- and β-thalassemias in Populations of Yunnan Province and the Prenatal Gene Diagnosis, vol. 47, Zhonghua Fu Chan Ke Za Zhi, 2012, pp. 85–89. https://pubmed.ncbi.nlm.nih.gov/22455737/. (Accessed 15 April 2024).
[13] G.D. Xu, C.F. Wang, J.L. Wang, M. Lin, Z.Y. Chang, J.H. Liang, X.H. Chen, S.M. Zhong, X.J. Nong, W.J. Wei, Y.B. Deng, Prevalence and molecular characterization of common thalassemia among people of reproductive age in the border area of Guangxi-Yunnan-Guizhou Province in Southwestern China, Hematology 27 (2022) 672–683, https://doi.org/10.1080/16078454.2022.2080427.
[14] E.P. Vichinsky, Changing patterns of thalassemia worldwide, Ann. N. Y. Acad. Sci. 1054 (2005) 18–24, https://doi.org/10.1196/annals.1345.003.