

RESEARCH ARTICLE

Detection of Hb H disease caused by a novel mutation and --^{SEA} deletion using capillary electrophoresis

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Abstract

Background: Hb H disease is a serious type of α -thalassemia which cause moderate anemia while misdiagnosis by routine genetic analysis in a rare or novel Hb H disease.

Methods: The study was done on three patients and one fetus in a suspected Hb H disease family. Hb analysis was carried out using capillary electrophoresis (CE), and hematological analysis was conducted with an automated cell counter. Common α - and β -thalassemia mutations were detected by routine genetic analysis (gap-PCR and RDB-PCR). Novel mutation diagnostic methods were based on DNA sequencing.

Results: Capillary electrophoresis revealed clinical feature of classic Hb H disease in the proband, and hematology analysis showed moderate anemia (Hb 87 g/L). But routine genetic analysis was found that it was only a heterozygote for the --^{SEA} deletion. DNA sequencing of α -globin genes ($\alpha 1$ and $\alpha 2$) identified the breakpoints between nts 34162 and 34171 at $\alpha 2$ gene, named CD 90-93 (-AGCTTCGG) mutation. The genotype of proband and fetus was the same --^{SEA}/ α ^{CD90-93}. His father was homozygous for the novel mutation ($-\alpha$ ^{CD90-93}/ $-\alpha$ ^{CD90-93}), and his mother was heterozygote for the --^{SEA} deletion.

Conclusions: Our study for the first time described the novel mutation CD 90-93 (-AGCTTCGG). CE is a way to avoid misdiagnosis of rare or novel Hb H disease.

KEYWORDS

capillary electrophoresis, Hb H disease, novel mutation, thalassemia

1 | INTRODUCTION

α -Thalassemia is prevalent in the southern regions of China, and especially in Guangxi Zhuang Autonomous Region where the carrier rate of population is up to 17.55%.¹ The more serious types of α -thalassemia are Hb H disease and Hb Bart's hydrops fetalis which cause moderate or severe anemia or fetal abortion. The main genotype that causes Hb H disease is α^0/α^+ ,² but Hb Bart's hydrops fetalis is α^0/α^0 .³

Hb H disease is caused by deletion of both the *HBA1* ($\alpha 1$) and *HBA2* ($\alpha 2$) genes on one chromosome 16 and either a complete

deletion or a point mutation on *HBA1* or *HBA2* on the other homologue.⁴ In the southern Chinese population, the main of the two-gene deletion in cis is --^{SEA} (α^0) and --^{Thai} (α^0). Single-gene deletions $-\alpha^{3,7}$ (α^+) or $-\alpha^{4,2}$ (α^+) are majority of deletional Hb H disease, while Hb Constant Spring (Hb CS) (α^+) or Hb Quong Sze (Hb QS) (α^+) predominates in the non-deletional form. In addition, there are a few rare mutations reported in Chinese patients with α -thalassemia.⁴⁻⁷ Here, we describe a novel mutation CD 90-93 (-AGCTTCGG) in the $\alpha 2$ gene combined deletion --^{SEA} causing Hb H disease in a Chinese patient.

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Names	Primers	Production length
$\alpha 1$ gene		(bp)
$\alpha 1$ forward	TGGAGGGTGGAGACGTCCTG	1181
$\alpha 1$ reverse	TCCATCCCCTCCTCCCGCCCTGCCTT TTC	
$\alpha 2$ gene		
$\alpha 2$ forward	TGGAGGGTGGAGACGTCCTG	
$\alpha 2$ reverse	CCATTGTTGGCACATTCCGG	1085

TABLE 1 Names and sequences of PCR primers for novel mutation in this study

2 | MATERIALS AND METHODS

2.1 | Patients and clinical presentation

The proband was a 2-year-old boy who came from Baise City, Guangxi Zhuang Autonomous Region of southern China. There was no obvious retardation of growth during pregnancy and no history of jaundice at birth. He was recommended to our hospital for further diagnosis because of the clinical phenotype of moderate anemia diagnosed in a local hospital. Blood samples of him and his parents were collected using EDTA as anticoagulant after informed consent was obtained.

2.2 | Hematological analysis

Hematological parameters were determined by an automated cell counter (Sysmex X-1000, Sysmex), and hemoglobin (Hb) analysis was conducted with a capillary electrophoresis (CE) device (Capillarys 2, Sebia).

2.3 | Routine genetic analysis

Genomic DNA was extracted from peripheral blood using thalassemia detection kit (Yishengtang). Identification of common α -thalassemia in China including α -thalassemia 1 ($--^{SEA}$ and $--^{Thai}$ deletions) and α -thalassemia 2 ($-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions) was

performed routinely in our laboratory using the gap-PCR method (Yishengtang). The 17 known β -thalassemia mutations including -28 (A-G), -29 (A-G), -30(T-C), -32 (C-A), CDs 14/15 (+G), CD17 (A-T), CD 26 (G-A), CDs 27/28 (+C), CD31(-C), CDs41/42 (-TTCT), CD43 (G-T), CDs71/72 (+A), IVSI-1(G-T), IVSI-5 (G-C), IVSII-654 (C-T), CAP + 1(A-C), and initiation codon (ATG-AGG) were detected by PCR and reverse dot-blot assay (PCR-RDB). Three common non-deletions of α -thalassemia including Hb CS, Hb QS, and Hb Westmead (Hb WS) were carried out by PCR-RDB.

2.4 | DNA sequencing

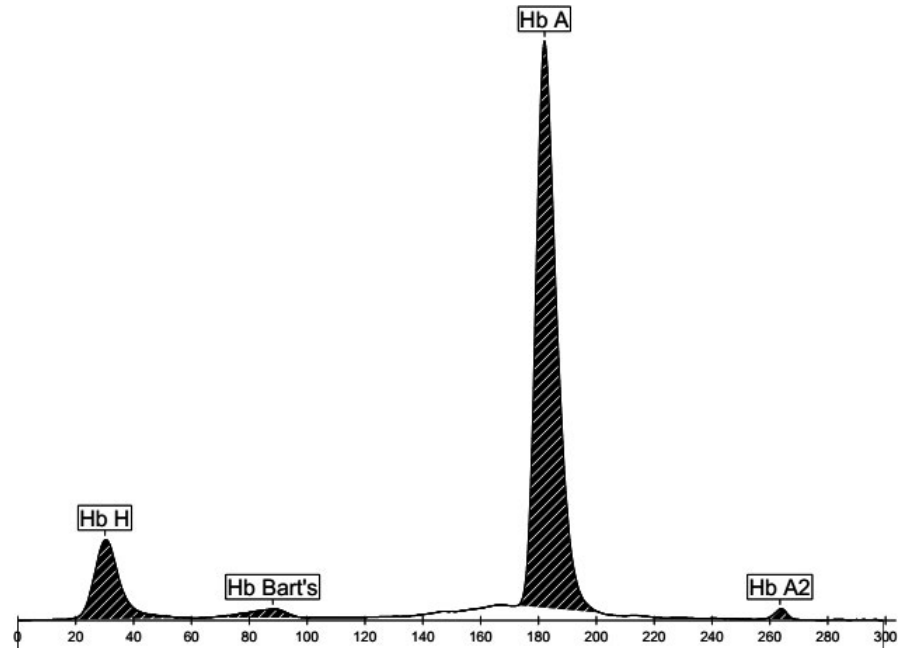
Two sets of primer pairs were designated and used to amplify and sequence the α -globin gene ($\alpha 1$ and $\alpha 2$) as shown in Table 1. The amplification was performed using 50 ng of genomic DNA, and 20 pmol of forward and reverse primers, on PCR machine (C-1000, Bio-Rad). The 47 μ L PCR reaction mixture contained 5.0 μ L of $10 \times$ LA Buffer (TaKaRa), 4.0 μ L of 2.5 mmol/L dNTPs, 2.0 μ L of 10 pmol/L forward and reverse primers, 5.0 μ L of 5 mol/L Betaine, 2.5 μ L of DMSO, 0.5 μ L of 5 μ L LA Taq enzyme, 2 μ L of DNA, and 24 μ L of DNA-free H_2O . A total of 32 PCR cycles after initial heating at 95°C for 5 minutes were performed under the following PCR condition: 97°C for 45 seconds, 66°C for 30 seconds, and 72°C for 2 minutes and a final extension at 72°C for 10 minutes. The PCR products were sequenced by the 3130 automated sequencer (Applied Biosystems).

Parameters	Proband	Mother	Father	Fetus
Age(years)	2	27	30	18 (week)
Hb (g/L)	87	106	128	N
MCV (fL)	61.1	70.9	70.4	N
MCH (pg)	16.4	20.2	20.4	N
Hb A (%)	82.5	97.4	97.9	N
Hb F (%)	0	0	0	N
Hb A ₂ (%)	0.8	2.6	2.1	N
Hb H (%)	14.3	0	0	N
Hb Bart's (%)	2.4	0	0	N
α -Globin genotype	$--^{SEA}/-\alpha^{CD90-93}$	$--^{SEA}/\alpha\alpha$	$-\alpha^{CD90-93}/-\alpha^{CD90-93}$	$--^{SEA}/-\alpha^{CD90-93}$
β -Globin genotype	β^N/β^N	β^N/β^N	β^N/β^N	β^N/β^N

TABLE 2 Hematological data and globin genotype of patients

N, none.

FIGURE 1 Result of the proband by capillary electrophoresis



3 | RESULTS

3.1 | Hematological characteristics and Hb analysis

Hematological characteristics and Hb analysis of three samples are summarized in Table 2. As shown in Table 2, there was a decrease in Hb (87 g/L, normal reference range 120-160 g/L) of the proband obviously. In addition, Hb analysis using capillary electrophoresis system showed Hb H peak, Hb Bart's peak, and decreased Hb A₂ (Figure 1). The hematological phenotype of his father and mother was low erythrocyte mean corpuscular volume (MCV, normal reference range 80-100 fL) and mean corpuscular hemoglobin (MCH, normal reference range 27-34 pg).

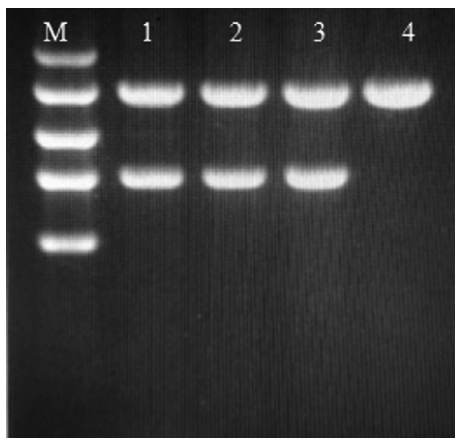


FIGURE 2 The figure of gap-PCR about this family. M = Marker 2000. Lanes 1-3 were the proband, mother, and fetus. All genotypes of them were found heterozygous of deletion α^{-SEA} (α^{-SEA}/α). Lane 4 was the father, and the genotypes were $\alpha^{-CD90-93}/\alpha^{CD90-93}$, but the result of electrophoresis showed the same as normal sample.

3.2 | Routine genetic analysis and DNA sequencing

The multiplex gap-PCR for the most common deletions gave a result of α^{-SEA} /heterozygote deletion, suggesting a discrepancy between genotype and phenotype, and a second mutation/deletion was considered (Figure 2). No mutations of three common non-deletions of α -thalassemia and common β -thalassemia were detected by PCR-RDB. The data of α -globin gene DNA sequencing revealed that a novel mutation was identified at codon 90-93 (-AGCTTCGG) on the $\alpha 2$ gene, and the breakpoints were located between nts 34162 and 34171 (Figure 3).

4 | CONCLUSION

Now, in laboratories of Chinese hospitals, the genotypes of usually used α -thalassemia genetic analysis kits were deletion genotype including α^{-SEA} , α^{-Thai} , $\alpha^{-3.7}$, and $\alpha^{-4.2}$, and non-deletion containing $\alpha^{CS}\alpha$, $\alpha^{WS}\alpha$, and $\alpha^{QS}\alpha$. They are the major α -thalassemia genotypes in the Chinese population, but not all the genotypes.⁸ In other words, patients with novel genotypes may be missed diagnosis using routine genetic analysis kits. It is important for identification and clinical genetics analysis of novel mutation in the prevalent region.

Here, we report for the first time, in Chinese patient, a novel deletion α -globin gene detected in a case of Hb H disease. The proband came to our hospital because of moderate anemia. Hematology analysis and Hb electrophoresis were proposed for screening thalassemia. Hematology analysis showed that he was a suspected thalassemia patient with Hb 87 g/L, MCV 61.1 fL, and MCH 16.4 pg (Table 2). The result of Hb electrophoresis by CE was 14.3% Hb H, 2.4% Hb Bart's, 82.5% Hb A, and 0.8% Hb A₂ (Figure 1). It was a typical picture of Hb H disease. Subsequently, he was advised to

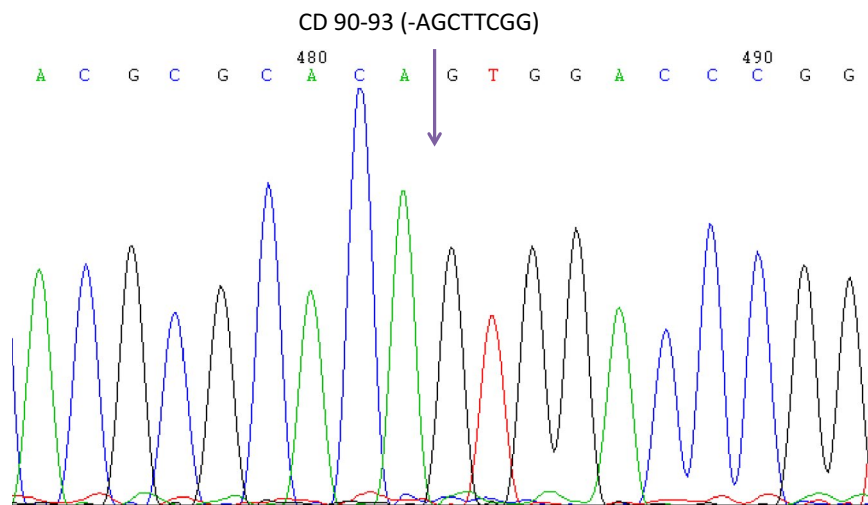


FIGURE 3 DNA sequencing analysis showing the mutation of CD90-93 (-AGCTTCGG) at the $\alpha 2$ gene. The breakpoints are present at nts 34162 and 34171

continue genetic analysis. However, only heterozygote deletion $--^{SEA}s$ was detected after 3 days (Figure 2). According to our experiences, it suggested that there was a rare or novel mutation.

Thus, his mother and father were also recommended to the same detection. Finally, DNA sequencing showed that the novel mutation was a mutation at codon 90-93 (-AGCTTCGG) on the $\alpha 2$ gene. It was obtained the registration number (KJ400031) of National Center for Biotechnology Information (NCBI) GenBank. The father was a homogenous codon 90-93 (-AGCTTCGG) (genotyped: $-\alpha^{CD90-93}/-\alpha^{CD90-93}$), and the proband was codon 90-93 (-AGCTTCGG) combining deletion $--^{SEA}$ (genotyped: $--^{SEA}/-\alpha^{CD90-93}$). As the phenotype of homogenous codon 90-93 (-AGCTTCGG) is similar to heterozygous of deletion $--^{SEA}$, we presumed that the individual heterozygous of codon 90-93 (-AGCTTCGG) (α^+) may be generally normal and similar to heterozygous of $-\alpha^{3.7}$. Surprisingly, fetus, a second baby, was detected by the same genotype as the proband by amniocentesis after a year.

In this study, firstly, we noticed that there was a Hb H disease from the result of CE, and the genetic analysis data in turn confirmed. Almost all of the Hb H disease can be separated out of the H band, so the result of CE may be used as a proof for Hb H disease whether it is caused by the well-known genotypes or the novel mutation genotypes. Relative to the low specificity of anemia in hematological analysis, CE is a good method for Hb H disease detection. As in this case, it was likely to cause misdiagnosis if there was no result of CE.

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