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The research for various genotypes and phenotypes related to rare -90 (C>T) β-thalassemia mutation in Ganzhou city, Southern China

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Thalassemia is a heterogeneous group of genetic disorders affecting the hemoglobin genes leading to decrease synthesis of globin chains of hemoglobin and resulting in ineffective erythropoiesis. It usually contains α- and β-thalassemia, most of common mutation types of which can be detected. However, it's inclined to miss rare thalassemia mutation types. Here, we analyzed the molecular and hematological characteristics of seven cases with rare β-thalassemia -90 (C>T) (*HBB*: c.-140 C>T) mutation. Five of them carried β-90 (C>T) heterozygous mutation with a β^+ thalassemia trait. One case was αSEA/β-90 genotype with decreasing MCV and MCH obviously, and the other was a β+/β0 intermediate thalassemia patient with β-90/βCD17 genotype, presenting with moderate anemia. A pedigree of one case was analyzed subsequently. It was found that the proband's maternal grandfather and mother were carriers of α3.7/β-90 double heterozygous thalassemia, who presented that MCV and MCH were decreased normally or slightly, and HbA2 was increased. The proband and his aunt were β-90 (C>T) carriers. It's necessary to point that the MCV and MCH were much higher in carrier of α3.7/β-90 genotype compared with either αSEA/β-90 genotype or β-90 heterozygous mutation. In this study, we explore the genotypes and phenotypes of four diverse β-90、αSEA/β-90、α3.7/β-90、β-90/βCD17 thalassemia mutations, which enriches the gene profile of β-thalassemia mutation in Chinese population.

KEYWORDS

-90 (C>T), rare β -thalassemia, phenotype, genotype, China

Introduction

Thalassemia is a heterogeneous grouping of genetic disorders that results from the defect of hemoglobin synthesis (1). The most common type of hemoglobin comprises 2 α-globin and 2 β-globin subunits, which encoded by the α-globin gene on chromosome 16 and the β-globin gene on chromosome 11 respectively (2). It's known that α-globin gene

has 4 alleles to produce 2 α-globin chain and β-globin gene has 2 alleles to produce 2 β-globin chain (3). Once globin gene is defected, it can lead to the diminished or absent production for one or more of the globin chains. Later this imbalance of globin chain impairs the production of normal hemoglobin. At last This impairment causes ineffective erythropoiesis which results in thalassemia disease (4). Thalassemia is widespread in southern China (5). Ganzhou, as a southern city in Jiangxi province, also has a high incident rate of thalassemia. It has been indicated that a higher prevalence of thalassemia with the heterozygote frequency of 9.49% in Ganzhou, whereas the low frequency was found in middle (3.90%) and northern Jiangxi (2.63%) (6). Most of common thalassemia mutation types can be detected, but the rare types of thalassemia mutations are inclined to be missed. Here, we analyzed the molecular and hematological characteristics of seven cases with rare β-thalassemia -90 (C>T) (*HBB*: c.-140 C>T) mutation. And then a pedigree analysis was further performed.

Subjects and methods

Subjects

Case 1 was admitted due to "lead poisoning". Routine examination revealed microcytic hypochromic anemia, so hemoglobin electrophoresis and genetic testing were performed. Subsequently, the proband's grandfather, grandmother, aunt, mother and father were screened for thalassemia and performed for thalassemia genetic diagnosis. Case 2 was admitted due to syncope for 3 times and thrombocytosis for more than 5 months. Thalassemia genetic testing was performed because of microcytic hypochromic anemia, and the patient was also complicated with iron deficiency anemia. Case 3 was hospitalized due to pulmonary infection and combined with old tuberculosis, chronic gastritis and coercive spondylitis. After admission, the patient was found to have microcytic hypochromic anemia and underwent thalassemia genetic detection. Cases 4, 5, 6 and 7 were routinely screened for thalassemia phenotype due to pregnancy supervision or fertility testing, and genetic testing for thalassemia was performed after positive screening. In addition, case 7 underwent splenectomy in her childhood.

Methods

After obtaining the consent of subjects or their guardians, peripheral blood was extracted with EDTA anticoagulant tubes for sample collection. SysmexXN2100 instrument was used for blood cell analysis. The reference range of hemoglobin (Hb) was (120-160) g/L for men and (110-150) g/L for women. The RBC reference range was (4.3-5.8) $*10^{12}$/L for male and (3.8-5.1) $*10^{12}$/L for female. The reference range of mean erythrocyte volume (MCV) was (82-100) fL. The mean hemoglobin content (MCH) reference range was (27-34) pg. The hemoglobin electrophoresis was performed by SEBIA capillary electrophoresis. The reference range of HbA was (96.5-97.5) % and the reference range of HbA2 was (2.5-3.5) %.

The whole blood DNA extraction reagent of Tianlong Company was used to extract DNA from peripheral blood on NP968-C extraction instrument of Tianlong Company. The thalassemia genetic detection reagent was produced by Xiamen Zhishan Biology Co. Ltd with melting curve method. The detection range of reagent included three kinds of deletion α thalassemia types (-SEA, -α3.7, -α4.2), three kinds of non-deletion α thalassemia types (αCS/, αQS/, αWS/) and twenty-one β gene mutations (IVS-II-654, -28, -29, -30, -31, -32, CD14-15, CD15-16, CD17, CD30, IVS-I-5, IVS-I-1, CD26, CD27-28, CD41-42, CD43, CD37, CD71-72, IVS-II-5, -73 and -90).

Results

Hematological features, Hb electrophoresis, and genotype analysis of seven cases

Cases 1 to 5 were detected to be -90 (C>T) (*HBB*: c.-140 C>T) heterozygous mutation with a typical β-thalassemia trait, including decreased MCV, decreased MCH, and increased HbA2. Case 6 has double heterozygotic mutations of the SEA/αα deletion combined with β-thalassemia -90 (C>T) mutation showing decreased MCV and MCH evidently. Case 7 carried compound heterozygous mutation of β-90 and βCD17, with genotype of β^{-90}/β^{CD17}, which showed the characteristics of moderate anemia (Table 1).

TABLE 1 The Hematological Profile and Molecular Findings of seven cases.

Parameters	case 1	case 2	case 3	case 4	case 5	case 6	case 7
sex	male	male	male	female	female	female	female
age (year)	1	25	47	29	26	35	26
Hb (g/L)	106	93	76	107	118	123	77
RBC (10^{12}/L)	4.84	4.91	3.78	4.64	5.23	5.18	3.13
MCV (fL)	70.2	64.6	70.6	73.5	73.4	75.3	75.4

(Continued)

TABLE 1 Continued

Parameters	case 1	case 2	case 3	case 4	case 5	case 6	case 7
MCH (pg)	21.9	18.9	20.1	23.1	22.6	23.7	24.6
HbA (%)	82.6	94.3	95.3	89.7			
HbA2 (%)	4.8	5	4.7	6.3			
HbF (%)	12.6	0.7		4			
α genotype	αα/αα	αα/αα	αα/αα	αα/αα	αα/αα	SEA/αα	αα/αα
β genotype	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{CD17}

Pedigree analysis of case 1

The aunt of proband (case 1) carried β-90 heterozygous mutation, which was consistent with the proband. The hematological phenotype showed significant decreases in MCV and MCH and increases in HbA2. However, the maternal grandfather and mother of proband were α3.7/β-90 double heterozygous state of thalassemia, with normal or slightly decreased MCV, mildly reduced MCH, but HbA2 was still elevated (Table 2; Figure 1).

Discussion

The mutation of β-thalassemia -90 (C>T) (*HBB*: c.-140 C>T) is caused by the base transition at -90 position within the upstream promoter of β-globin gene, which located in the CACC box at the binding site of erythroid Kruppel-like factor (EKLF). Due to the mutation of the binding site, the binding affinity between EKLF and the CACCC box decreases, leading to a decline in the transcription level of β-globin gene (7), which is about 60% of the normal transcription level, resulting to the phenotypic characteristics of β+ thalassemia (8).

The β-thalassemia -90 (C>T) mutation was detected in all of seven cases because of microcytic anemia, and delivered in the studied family. The clinical phenotypes of the subjects were consistent with the genetic diagnosis. Simple β-90 carrier was asymptomatic with a β-thalassemia trait, characterized by decreased MCV, decreased MCH, significantly increased HbA2 (>3.5%), indicating a β+ thalassemia phenotype. In this study, the $\alpha^{3.7}/\beta^{-90}$ genotype showed normal or slightly decreased MCV, slightly decreased MCH and higher HbA2, while the α^{SEA}/β^{-90} genotype showed MCV, MCH was decreased significantly. It's consistent with the published phenotype of α^{SEA}/β^{-90} patient (9). It indicated that there was no difference of hematological characteristics between β^{-90}/β^{N} and α^{SEA}/β^{-90} genotypes. However, the MCV and MCH of $\alpha^{3.7}/\beta^{-90}$ genotype was much higher than the other two genotypes. It concluded that hematologic phenotype was different once β-90 was combined with different types of α-thalassemia deletion. It needs more evidence to prove the conclusion due to a few cases in $\alpha^{3.7}/\beta^{-90}$ and α^{SEA}/β^{-90} genotypes. Meanwhile, it should be noted that when MCV and MCH are normal or slightly decreased, it may also be double heterozygotes of αβ-thalassemia. The genotype of β^{-90}/β^{CD17} was β^{+}/β^{0} intermediate thalassemia, which presented as moderate microcytic hypochromic anemia. The patient had undergone splenectomy at the age of six, which was matched well with the clinical manifestations of moderate β-thalassemia.

TABLE 2 The Hematological Profile and Molecular Findings in Pedigree of case 1.

Parameters	proband	Father	Mother	Aunt	Grandpa	Grandma
Hb (g/L)	106	166	125	127	130	140
RBC (10^{12}/L)	4.84	5.46	4.84	5.54	4.84	4.67
MCV (fL)	70.2	91	81.8	75.1	82.6	91.2
MCH (pg)	21.9	30.4	25.8	22.9	26.9	30
HbA (%)	82.6	97.1	91.3	91.8	91.9	
HbA2 (%)	4.8	2.9	5.3	5.6	5.6	
HbF (%)	12.6		3.4	2.6	2.5	
α genotype	αα/αα	αα/αα	α3.7/αα	αα/αα	α3.7/αα	αα/αα
β genotype	β^{-90}/β^{N}	β^{N}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{-90}/β^{N}	β^{N}/β^{N}

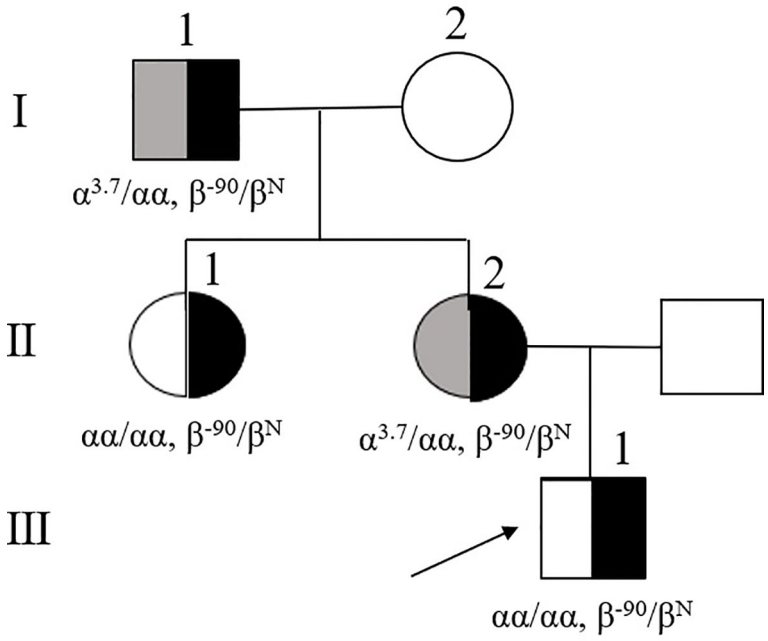

FIGURE 1
Pedigree analysis of the proband's family. The arrow indicates the proband (case1, III-1) who carried β^{-90}/β^{N} thalassemia. The corresponding globin genotype of each family member is indicated. "β^{N}" means normal beta globin gene.

β-thalassemia -90 (C>T) mutation was firstly identified in the Portuguese population (10). Later, this rare mutation was found in the India (11), Algeria (12), Pakistan (13) and Thailand (14). With the development of high-throughput second-generation sequencing technology, rare carriers of β-thalassemia -90 (C>T) mutation have been found in many southern regions in China including Guangdong, Guangxi, Hunan, Fujian and so on (15–17). It means that β-thalassemia -90 (C>T) mutation is a relatively common mutation in Chinese rare thalassemia population.

In this research, seven cases and a pedigree associated with β-90 (C>T) rare thalassemia mutation were studied elaborately in China. We found four various genotypes β^{-90}/β^{N}, $\alpha^{3.7}/\beta^{-90}$, α^{SEA}/β^{-90} and β^{-90}/β^{CD17}. This study enriches the gene profile of β-thalassemia mutation in Chinese population, which is of great significance for prevention and control of thalassemia and genetic counseling.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Clinical Ethics Committee of First Affiliated Hospital of Gannan Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

HZ, XH and DL collected and analyzed the data. HZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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