

RESEARCH ARTICLE

The prevalence and molecular characterization of $(\delta\beta)^0$ -thalassemia and hereditary persistence of fetal hemoglobin in the Chinese Zhuang population

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Objective: To reveal the prevalence and molecular characterization of $(\delta\beta)^0$ -thalassemia [$(\delta\beta)^0$ -thal] and hereditary persistence of fetal hemoglobin (HPFH) in the Chinese Zhuang population.

Methods: A total of 105 subjects with fetal hemoglobin (Hb F) level $\geq 5\%$ from 14 204 unrelated ones were selected for the study. Multiplex ligation dependent probe amplification was firstly used to analyze dosage changes of the β -globin gene cluster for associated with $(\delta\beta)^0$ -thal and HPFH mutations. The gap polymerase chain reaction was then performed to identify the deletions using the respective flanking primers. Hematologic data were recorded and correlated with the molecular findings.

Results: Twenty-one (0.15%) subjects were diagnosed with Chinese $\text{C}_\gamma(\text{A}_\gamma\delta\beta)^0$ -thal. Nine (0.06%) were diagnosed with Southeast Asia HPFH (SEA-HPFH) deletion. Seventy-five (0.53%) cases remained uncharacterized. Three genotypes for Chinese $\text{C}_\gamma(\text{A}_\gamma\delta\beta)^0$ -thal and SEA-HPFH deletion were identified, respectively. The genotype-phenotype relationships were discussed.

Conclusion: Our study for the first time demonstrated that $(\delta\beta)^0$ and HPFH were not rare events, and molecular characterized $\text{C}_\gamma(\text{A}_\gamma\delta\beta)^0$ -thal and HPFH mutations in the Chinese Zhuang population. The findings in our study will be useful in genetic counseling and prenatal diagnostic service of β -thalassemia in this populations.

KEYWORDS

$(\delta\beta)^0$ -thalassemia, β -globin cluster, fetal hemoglobin, hereditary persistence of fetal hemoglobin, prevalence

1 | INTRODUCTION

The thalassemias (thals) are a group of inherited hemoglobin disorders resulting from defects in the synthesis of one or more of the hemoglobin chains. According to the type of globin involved, thalassemia can be divided into α -, β -, $\delta\beta$ -thal and hereditary persistence of fetal hemoglobin (HPFH).¹ Two types of the determinants for $\delta\beta$ -thal or HPFH, namely, the deletional and nondeletional types, have been

classified on the basis of extensive molecular studies.^{2,3} $(\delta\beta)^0$ -thal and HPFH are caused by large deletions in the β -globin cluster involving δ - and β -globin genes, with or without A_γ -globin genes.⁴ These mutations are characterized by high fetal haemoglobin (Hb F) levels in adult. Heterozygotes for $\delta\beta$ -thal have hypochromic microcytic red cells with the levels of Hb F ranging from 5% to 20%, and, in contrast, HPFH heterozygotes have normal blood indices with higher Hb F (15%-30%).⁵ Homozygotes for $(\delta\beta)^0$ -thal and compound heterozygotes for $(\delta\beta)^0$ -thal

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with β -thal usually lead to a clinical phenotype of β -thal intermedia or major. Though, HPFH homozygotes are clinically asymptomatic, compound heterozygotes for HPFH with β -thal express the phenotype β -thal intermedia.^{6,7} Furthermore, there are different types of $(\delta\beta)^0$ -thal or HPFH deletions have been reported in different ethnic groups and different regions.⁸ Therefore, well-known the prevalence and molecular characterization of these mutations in those populations or regions with high prevalence of β -thal has a great effect on genetic counseling, public education, and prenatal diagnosis for β -thal control.

China is a multiethnic country, comprised of 56 ethnic groups. Each ethnic group has its own characteristics, including various components of the body.⁹ The Zhuang group is the second largest group with 44.9 million populations and 95% lived in Guangxi Zhuang Autonomous Region, Southern China, where the highest prevalence of β -thal has been found among all the high-risk regions of China.¹⁰⁻¹² The carrier frequency is 17.55% for α -thal and 6.43% for β -thal in Guangxi region.¹⁰ However, there is no comprehensive data on $(\delta\beta)^0$ -thal and HPFH mutations reported in the Chinese Zhuang population. In this study, we aim to reveal the prevalence and molecular characterization of $(\delta\beta)^0$ -thal and HPFH with a large-scale survey in the Chinese Zhuang population.

2 | MATERIALS AND METHODS

2.1 | Human subjects

Between January 1, 2010 and June 30, 2015, a total of 14 204 unrelated subjects who were all of Zhuang descents attended the hemoglobinopathy screening program at Guangxi Zhuang Autonomous Women and Children Care Hospital. Only participants with increased HbF levels ($\geq 5\%$) were included in this study. Information sheets regarding nationality, gender, age, dialect, natives and written consent forms were available in Chinese to ensure comprehensive understanding of the study objectives. Informed consents were signed or thumb printed by the participants. All studies were approved by the Ethic Committee of Guangxi Zhuang Autonomous Women and Children Care Hospital.

2.2 | Hematological parameters and red cell indices analysis

Peripheral venous blood samples of 2.5 mL volume were collected from all subjects, with an Ethylenediaminetetraacetic acid anti-coagulated tube. Peripheral blood counts and red blood cell incidences were determined using the SYSMEX XE800i automatic blood cell analyzer (Sysmex Corporation, Kobe, Japan). Quantitative assessment of hemoglobin HbF, HbA, and HbA₂ were performed using automated capillary electrophoresis system (CapillaryS 2, software version 6.2; Sebia, Paris, France).

2.3 | Genetic analysis

Genomic DNA were extracted from peripheral blood leukocytes using DNA blood extraction kits (Tiangen Bio-Tech Co. Ltd., Beijing, China). Molecular study for common alpha and beta defects in Chinese population were performed as previously described.^{13,14} Multiplex

ligation-dependent probe amplification (MLPA) was firstly used to analyze dosage changes of the β -globin gene cluster for associated with $(\delta\beta)^0$ -thal and HPFH mutations. Probes and reaction mixture for ligation and PCR were purchased from MRC-Holland (SALSA MLPA kit P102 HBB; MRC-Holland, Amsterdam, the Netherlands). The gap polymerase chain reaction (Gap-PCR) and sequence analysis were then performed to identify the deletions using the respective flanking primers (Table 1).

3 | RESULTS

A total of 105 individuals with Hb F ($\geq 5\%$) from 14 204 unrelated subjects were enrolled to characterize the molecular basis of $(\delta\beta)^0$ -thal and HPFH in our study. There were 59 men and 46 women, aged 1 to 42 years. Data indicated that 21 subjects were identified with Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thal mutation and nine were identified with SEA-HPFH deletion. The carrier rate of Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thal and SEA-HPFH mutations in the Chinese Zhuang population were 0.15% and 0.06% respectively. Moreover, 75 (0.53%) subjects remained uncharacterized. Among the 21 Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thal mutations, there were 16 heterozygotes, two compound heterozygotes for Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ and Southern Asian α -thal deletion ($-^{\text{SEA}}/\alpha\alpha$), and three compound heterozygotes for Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ and β -thal. Out of the nine SEA-HPFH mutations, seven were heterozygous, two were compound heterozygous for SEA-HPFH and β -thal mutation.

The results of hematological data of each cases were showed in Table 2. All cases with heterozygotes for Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thal had hypochromia and microcytosis, whereas cases with SEA-HPFH deletion had normal, hypochromic or borderline red blood cell indices. Cases with compound heterozygous for Chinese $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thal or SEA-HPFH deletion with β -thal mutation showed a range of phenotype severity from mild to severe anemia.

4 | DISCUSSION

Hereditary persistence of fetal hemoglobin (HPFH) and $(\delta\beta)^0$ -thal are caused by deletions within the β -globin gene (*HBB*) cluster that remove

TABLE 1 Primer sequences and reference

PCR primer	Primer sequences (5'→3')	References
For $\text{C}_\gamma(\text{A}^\gamma\delta\beta)^0$ -thalassemia		
Fwd	GGCATATATTGGCTCAGTC	3,6
Rev-1	CTTGCGAGAATAAAGCCTATC	
Rev-2	TCAACAATTATCAACATTACACC	
For SEA-HPFH deletion		
Fwd	TGGTATCTGCAGCAGTTGCC	3,7
Rev-1	AGCCTTCAGGTAGCAGAATC	
Rev-2	ATTGTTGAGTTGCAGGATCG	
For Taiwanese deletion		
Fwd	TTCCCA AAACCTAATAAGTAAC	3
Rev	TGG TGC AAA GAG GCA TGA TAC	

Fwd, Forward primer; Rev, Reverse primer.

TABLE 2 The hematological data of 30 cases with Chinese $G_{\gamma}(\Delta\gamma\delta\beta)^0$ -thal and SEA-HPFH deletion

Cases	Sex	Age (y)	Hb (g/dL)	MCV (fL)	MCH (pg)	Hb A (%)	Hb A ₂ (%)	Hb F (%)	α -Genotype	β -Genotype
1	F	3	12.4	75.2	27.3	77.7	2.5	19.8	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
2	M	24	13.9	78.0	24.2	77.0	2.6	20.4	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
3	F	2	10.6	68.4	23.0	84.5	2.8	12.7	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
4	F	2	12.2	69.5	25.3	81.0	2.6	16.4	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
5	M	2	13.7	69.8	24.0	82.9	2.4	14.7	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
6	M	5	10.5	60.6	20.0	75.2	2.5	22.3	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
7	M	18	13.3	69.4	23.1	84.7	2.9	12.4	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
8	M	2	13.4	67.9	21.7	83.8	2.7	13.5	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
9	M	13	12.2	75.8	23.9	83.2	2.8	14.0	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
10	M	3	13.2	64.1	21.8	86.9	2.3	10.8	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
11	F	40	12.8	78.0	23.0	84.9	2.5	12.6	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
12	M	19	12.3	79.1	25.7	87.3	2.7	10.0	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
13	M	42	14.6	67.9	22.1	84.3	2.4	13.3	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
14	F	6	10.4	72.0	23.8	79.4	2.6	18.0	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
15	F	3	10.9	72.6	24.5	79.0	2.8	18.2	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
16	F	25	10.6	76.0	24.5	76.8	2.5	20.7	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
17	F	24	12.7	70.6	23.9	81.7	2.5	15.8	- _{SEA} / $\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
18	M	26	13.6	71.5	24.2	85.9	2.8	11.3	- _{SEA} / $\alpha\alpha$	$\beta^N/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}$
19	F	20	9.4	65.5	20.3	8.5	3.7	87.8	$\alpha\alpha/\alpha\alpha$	$\beta^{\text{CD41-42(-TCCT)}/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}}$
20	F	4	5.7 ^a	61.9 ^a	20.1 ^a	61.5 ^a	3.7 ^a	34.8 ^a	$\alpha\alpha/\alpha\alpha$	$\beta^{\text{CD41-42(-TCCT)}/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}}$
21	M	6	6.0 ^a	59.5 ^a	21.1 ^a	65.1 ^a	3.8 ^a	31.1 ^a	$\alpha\alpha/\alpha\alpha$	$\beta^{\text{CD41-42(-TCCT)}/\beta^{\text{Chinese } G_{\gamma}(\Delta\gamma\delta\beta)^0}}$
22	M	27	1.4	76.1	23.9	77.4	3.1	19.5	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
23	F	23	12.4	77.1	26.5	73.7	2.8	23.5	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
24	M	12	13.3	79.9	25.7	72.4	3.0	24.6	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
25	M	33	13.1	80.6	25.8	75.0	2.7	22.3	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
26	F	24	12.6	78.9	23.3	76.5	2.9	20.6	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
27	F	28	12.5	73.5	24.2	74.3	2.5	23.2	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
28	F	33	11.8	72.6	25.3	75.4	2.8	21.8	$\alpha\alpha/\alpha\alpha$	$\beta^N/\beta^{\text{SEA-HPFH}}$
29	F	22	11.2	64.7	22.4	0.9	3.9	95.2	$\alpha\alpha/\alpha\alpha$	$\beta^{\text{IVS-II-654}/\beta^{\text{SEA-HPFH}}}$
30	M	6	10.4	63.0	22.0	0.5	3.8	95.7	$\alpha\alpha/\alpha\alpha$	$\beta^{\text{CD41-42(-TCCT)}/\beta^{\text{SEA-HPFH}}}$

Hb, haemoglobin; RBC, red blood cell counts; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; Thal, thalassemia.

^aPatients receiving regular red cell transfusion therapy.

elements that affect the expression of the γ -globin genes.¹⁵ So far, more than 40 types of $(\delta\beta)^0$ -thal and HPFH deletions have been reported in the world.⁸ And at least 12 have been described in Southeast Asian population.¹⁶ Over three types of $(\delta\beta)^0$ -thal or HPFH have been characterized in different regions of China, which include Chinese $G_{\gamma}(\Delta\gamma\delta\beta)^0$ -thal,^{17,18} Yunnanese $(\Delta\gamma\delta\beta)^0$ -thal,¹⁹ Cantonese $(\Delta\gamma\delta\beta)^0$ -thal, (18) and SEA-HPFH.²⁰ The structural differences among them are the deletional length and the varying 5' and 3' breakpoints (Figure 1). In this study, only Chinese $G_{\gamma}(\Delta\gamma\delta\beta)^0$ -thal, and the SEA-HPFH have been

identified in the Chinese Zhuang population. There were not evidences for other types of $(\delta\beta)^0$ -thal and HPFH. Data showed that the carrier rate of $(\delta\beta)^0$ -thal was 0.15% and SEA-HPFH thal was 0.06%, in which both were lower than that in Thailand population and the local population in Guangdong Province,^{7,18} but they were higher than that in Chinese Hakka population and Taiwan population.^{3,21} Moreover, the carrier rate of SEA-HPFH was lower than that in Karachi group of Pakistan.²² The comparison of carrier rate here indicated that there may be major differences in the prevalence of $(\delta\beta)^0$ -thal and HPFH

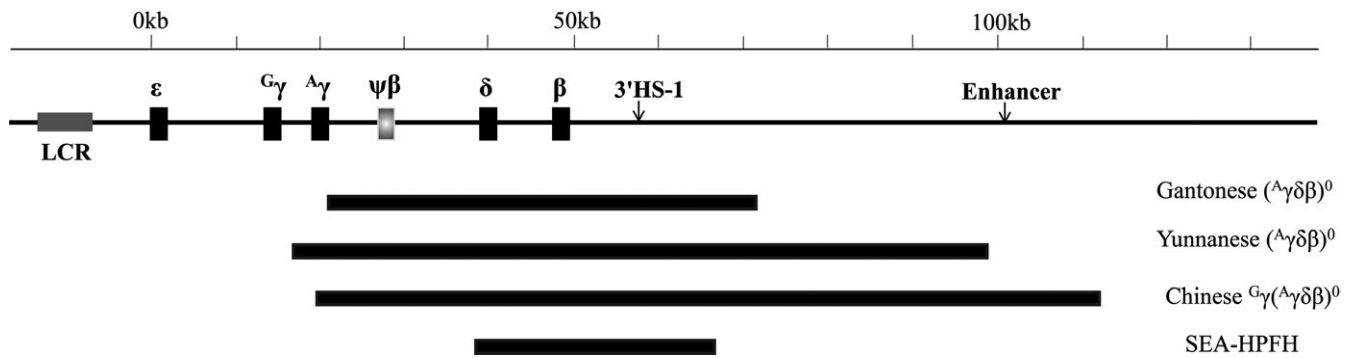


FIGURE 1 Scheme of β -globin gene cluster and the four deletions identified.^{17–20,32} LCR, locus control region; 3'HS-1, 3' DNase hypersensitive site 1

that among different ethnic groups and the Chinese $G\gamma(A\gamma\delta\beta)$ ⁰ and SEA-HPFH are the most common β -globin cluster deletions in the Chinese Zhuang population.

The Chinese $G\gamma(A\gamma\delta\beta)$ ⁰-thal is an about 80 kb deletion that starts with its 5' breakpoint in intron II of $A\gamma$ -globin gene and the 3' breakpoint far downstream to the β -globin gene, including an enhancer element 53 kb 3' of the β -globin gene (Figures 1 and 2A).^{23–27} This mutation was first identified from Chinese population in 1972 by Mann et al.²³ Since then, it has been described in different populations.^{17,28,29} In this study, we performed a survey of the incidence and

molecular characteristics of thalassaemias with a large-scale survey in Chinese Zhuang people. Subjects carry the heterozygotes for Chinese $G\gamma(A\gamma\delta\beta)$ ⁰-thal in our study had high Hb F levels (10%–20%) and hypochromic microcytic red cell indices, which are consistent with previous report.³⁰ While, carriers had a wider size of mean corpuscular volume (MCV 60.6–78.0 fl) and mean corpuscular hemoglobin (MCH 20.0–27.3 pg) (Table 2). Moreover, combination of Chinese $G\gamma(A\gamma\delta\beta)$ ⁰-thal and β^0 -thal can produce varying phenotypes.³⁰ The IVS-II-654(C>T) mutation, which creates an additional 5' donor splice site at position 652 resulting in a frame shift and a stop codon, is usually designated

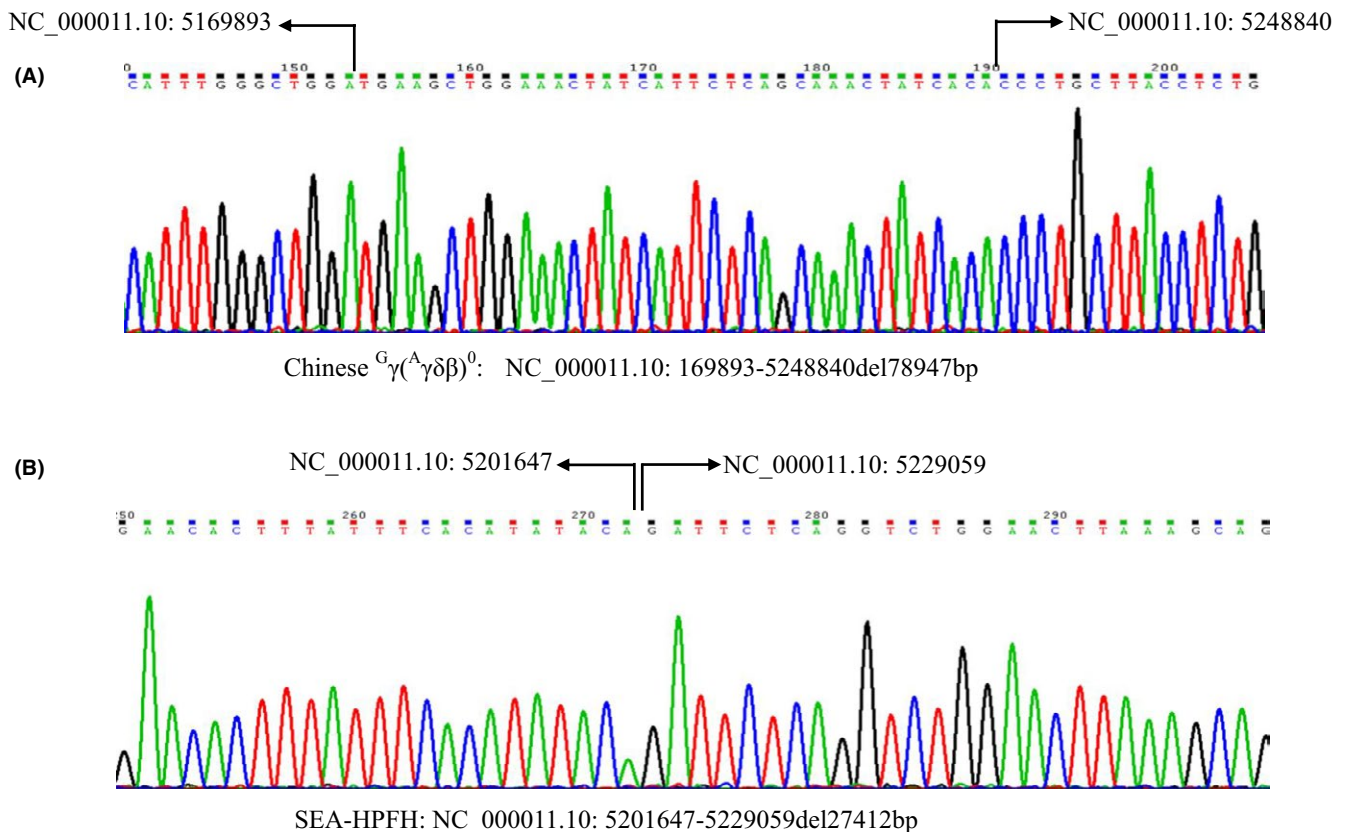


FIGURE 2 Characterization of the breakpoints of Chinese $G\gamma(A\gamma\delta\beta)$ ⁰-thalassaemia and SEA-HPFH deletion by direct DNA sequencing. The GenBank coordinates of the two nucleotides flanking each deletion are indicated. NCBI Refseq nomenclature is indicated below. (A) Chinese $G\gamma(A\gamma\delta\beta)$ ⁰-thal; (B) SEA-HPFH deletion

as a β^+ -thal. As a result, patients with the same $\beta^{IVS-II-654(C>T)}/\beta^{\text{Chinese } G_\gamma(A\gamma\delta\beta)^0}$ genotype can present from only mild anemia to severe transfusion dependent anemia.³⁰ Similarly, our three patients with compound heterozygosity for Chinese $G_\gamma(A\gamma\delta\beta)^0$ -thal and $\beta^{\text{CD41-42}(-TTCT)}$ showed β -thal major syndrome, while the other one showed only thal intermedia. These findings may suggest that the genetic defects on β -globin genes are not the sole determinant of clinical outcome of this disease and other nonglobin gene-related elements or external environmental factors can modify the phenotype when β -globin chain production is critically low. The phenotypic variability within the same genetic defects and uncertain prognosis make genetic and prenatal counseling difficult.

The SEA-HPFH is about 27 kb deletion mutation, in which the 5' breakpoint is located between the δ - and β -globin genes, and the 3' breakpoint is located approximately 2.3 kb downstream from the 3' HS-1 locus control region (LCR) of the β -globin gene (Figures 1 and 2B). This mutation was first found in two unrelated individuals of Vietnamese background by Motum PI in 1993.³¹ In China, it was first identified by Xu et al. in 2000 from two cases of Cantonese.³² Herein, we are the first to comprehensively describe this mutation in Zhuang population and obtained a set of hematological data from individuals with different forms of this mutation. Nine (0.06%) subjects had SEA-HPFH deletion in our study. Seven individuals with heterozygotes for SEA-HPFH showed similar features to previously reported,^{18,32} and all had normal Hb levels and slightly decreased MCV and MCH levels. While, two patients who were compound heterozygous for SEA-HPFH and β^0 -thal mutation presented as β -thal intermedia phenotypes (Table 2). This findings indicated that the SEA-HPFH deletion is more akin to a β -thal phenotype with raised Hb F as seen in all seven heterozygous subjects in the present study.

The distinction between HPFH and $\delta\beta$ -thal is subtle and is made on clinical and hematologic grounds.⁷ The SEA-HPFH and Chinese $G_\gamma(A\gamma\delta\beta)^0$ -thal deletion both have raised Hb F level, while the Hb A₂ level in heterozygous state for SEA-HPFH deletion was higher than that in heterozygotes for Chinese $G_\gamma(A\gamma\delta\beta)^0$ -thal in our study (Table 2). That may be due to the reduced quantity of δ chain synthesis in Chinese $G_\gamma(A\gamma\delta\beta)^0$ -thal. The expression of HbF is associated with γ -globin gene and can be influenced by many factors, such as quantitative trait loci (QTL) inside or outside the β -globin gene cluster.^{33,34} In this study, we only detected the deletional type of $\delta\beta$ -thal and HPFH, and other genetic factors associated with high Hb F levels were not determined and awaits further study in the future.

In summary, our study for the first demonstrated ($\delta\beta$)⁰ and HPFH were not rare events in the Chinese Zhuang population. As co-inheritance of deletional $\delta\beta$ -thal or HPFH with β -thal can result in variable clinical phenotypes, a differential diagnosis of these conditions is therefore important for providing appropriate treatment and genetic counseling to these cases.¹⁸ Since the Chinese $G_\gamma(A\gamma\delta\beta)^0$ -thal and SEA-HPFH deletion are the most common large deletions of β -globin gene cluster in the Chinese Zhuang population, Gap-PCR for the detection of these two deletions should be used in thalassemia screening program.

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CONFLICTS OF INTERESTS

The authors report no conflicts of interest.

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