International Journal of Hematology (2021) 114:307–318
https://doi.org/10.1007/s12185-021-03173-z

ORIGINAL ARTICLE

# Prevalence and molecular spectrum of α- and β-globin gene mutations in Hainan, China

Zhen Wang[1,2,3,4] · Wenye Sun[1] · Huaye Chen[1] · Yongfang Zhang[1] · Fei Wang[1] · Hongjian Chen[1] · Yao Zhou[1] · Yanhua Huang[1] · XiXi Zhou[1] · Qi Li[1,2,3,4] · Yanlin Ma[1,2,3,4]

Received: 21 January 2021 / Revised: 6 June 2021 / Accepted: 9 June 2021 / Published online: 30 June 2021


## Abstract

This study investigated prenatal diagnosis of α-thalassemia and β-thalassemia in 3049 families in 18 regions of Hainan Province. Molecular diagnosis was performed in 3049 couples with thalassemia in Hainan Province. Genomic DNA was extracted from peripheral blood of the couples and villus, amniotic fluid, or cord blood of fetuses. DNA-based diagnosis was performed using polymerase chain reaction. The most commonly detected mutation for α-thalassemia was− SEA/αα (31.53%), followed by − α4.2/αα (11.15%) and − α3.7/αα (11.02%). The most common mutation for β-thalassemia was CD41/42 (30.27%), followed by − 28 (2.56%). Prevalence was highest in the coastal regions and lowest in the Wenchang, Lingao, and Ding'an regions. We also found that the most common gene mutations in Han people and other minority groups were not homogeneous. Prenatal diagnosis showed 556 normal fetuses, 116 with α-thalassemia hydrops, and 134 with β-thalassemia major. Our findings provide important information for clinical genetic counseling regarding prenatal diagnosis for thalassemia major in Hainan Province.

**Keywords** Thalassemia · Gene mutation · Prenatal diagnosis · Genetic diagnosis · Hainan Province

## Introduction

Thalassemia, also referred to as Mediterranean anemia, is an autosomal inherited defect caused by the mutations in the α- or β-globin gene. Thalassemia is categorized into two major types, α- and β-thalassemia according to the mutations that occur in these globin genes, either of which can be further subdivided into another two forms, the $\alpha^0$ and $\alpha^+$ thalassemia and $\beta^0$ and $\beta^+$ thalassemia, respectively. The heterozygous form of $\alpha^+$ thalassemia or $\alpha^0$ thalassemia may not have symptoms of anemia, while the compound heterozygous form for $\alpha^+$ thalassemia and $\alpha^0$ thalassemia usually causes hemoglobin H. The homozygous state for $\alpha^0$ thalassemia causes Hemoglobin Bart's, which is lethal in utero or soon after birth. The heterozygous form of β thalassemia, which is called β thalassemia minor, usually shows asymptomatic microcellular anemia, while others are silent carriers. In contrast, the homozygous or compound heterozygous form of β mutations, also named the β-thalassemia major and intermediate respectively, can cause severe anemia and patients carrying these mutations need transfusion for life, which poses financial burden to their families and society.

Zhen Wang and Wenye Sun authors contributed equally.

✉ Qi Li
liqi1970@hotmail.com

✉ Yanlin Ma
mayanlinma@hotmail.com

1 Hainan Provincial Key Laboratory for Human Reproductive Medicine and Genetic Research, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University, No. 3 Xueyuan Road, Haikou, Hainan, China

2 Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Medical University, Haikou, Hainan, China

3 Hainan Provincial Clinical Research Center for Thalassemia, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou, Hainan, China

4 Haikou Key Laboratory for Preservation of Human Genetic Resource, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou, Hainan, China

The high frequency of inherited hemoglobin variants is present in tropical and sub-tropical areas such as Mediterranean countries and Southeast Asia [1]. In China, the prevalence of α-thalassemia, β-thalassemia, and α- and β-thalassemia ranges from 1.20 ~ 19.87%, 0.53 ~ 6.84%, and 0.08 ~ 1.22%, respectively [2]. Southern China including Guangxi, Guangdong, Fujian, and Hainan Provinces are the high incidence areas of the disease, and the spectrum of the mutations of these areas have been examined previously [3–5]. In addition, the spectrum of thalassemia gene mutations appears to be region- and ethnic-dependent.

As thalassemia is an inherited autosomal recessive disease and has serious impacts on the quality of patient's life, couples who are both heterozygous for thalassemia gene have 25% probability to have a child with thalassemia major. Presently, prevention programs like molecular diagnostics, genetic counselling, and prenatal diagnosis have achieved great success in preventing the occurrence of thalassemia major, as demonstrated by a decline in the birth rate of thalassemia major in some countries such as Iran, Pakistan, Thailand, and China [6–8]. A previous study has provided important information about prenatal diagnosis of thalassemia in Han and Li people in Hainan [9]. However, few studies have shown geographical distribution and family-based prenatal diagnosis of thalassemia gene mutations in Hainan Province. Furthermore, the main aim of a thalassemia prevention and control program is to prevent the birth of infants carrying thalassemia major. Hence, it is necessary to enroll pregnant women and their husbands in the study cohort.

In this study, we analyzed α- and β-thalassemia genotypes in the fetal specimen collected from 3049 pregnant women using molecular prenatal diagnosis and conducted a large-scale familial investigation in eighteen regions of Hainan Province. The aims of this study were to reveal the detailed geographical and ethnic distribution and familial prevalence of thalassemia, and provide scientific basis for thalassemia prevention and control in the province, and construct a detailed frequency map of the spectrum of thalassemia mutations in Hainan. This study will provide comprehensive data of thalassemia's prevalence in Hainan, which will significantly contribute to its control and management, genetic counseling and prenatal diagnosis.

## Materials and methods

### Subjects

This study included pregnant women and their husbands who registered for a program of diagnosis of thalassemia at The First Affiliated Hospital of Hainan Medical University in Hainan Province between January 2004 and March 2020. All couples had resided in Hainan Province, and came from eighteen regions of Hainan Province and all subjects signed the informed consent. This study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliated Hospital of Hainan Medical University.

### Samples collection

Depending on the gestational age of the fetus, chorionic villus sampling (CVS) at 10–12 weeks, amniotic fluid at 16–21 weeks, and cord blood at 18–28 weeks of gestation were collected. All procedures were performed under the guidance of ultrasonography. Peripheral blood from parents and umbilical cord blood from the fetus were collected via venipuncture of an antecubital vein using Ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. The villus samples were isolated under a dissecting microscope. Amniocentesis fluid cells were isolated by centrifugation at 2000 rpm for 2 min. Genomic DNA of peripheral blood of both parents, chorionic villus, amniotic fluid, and umbilical cord blood were extracted by Tiangen DNA extract kit (Tiangen Biotech CO., LTD., China) following the manufacturer's instructions.

### Molecular diagnosis of α- and β-thalassemia

A thalassemia gene diagnostic kit (Shenzhen Yaneng Biotechnology Co., Ltd., China) combining GAP-PCR and hybridization technology was used to test 17 known β-globin mutations including codon 41/42(− CTTT), IVS-II-654 (C → T), − 28 (A → G), codon 71/72 (+ A), codon 17 (A → T), codon 26 (G → A), codon 31(–C), codon 27/28 (+ C), IVS-I-1 (G → T), codon 43 (G → T), − 32 (C → A), − 29 (A → G), − 30 (T → C), codon 14/15 (+ G), Cap (− AAAC), Int codon (ATG → AGG) and IVS-I-5 (G → C). The amplified products were analyzed by 1.5% agarose gel electrophoresis. Predenaturation at 94 °C for 10 min, denaturation at 94 °C for 1 min, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, at 35 cycles of amplification, and extension at 72 °C 5 min. The amplified products were reverse hybridized with the reverse dot-blot membrane of the specific labeled probe. After washing, the signal on the membrane was determined.

The same kit was also used to detect the three common α-globin gene deletions, (−SEA/αα, $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$). PCR for deletion of α-thalassemia was performed according to the following protocol: initial denaturation at 94 °C for 10 min, 96 °C for 5 min, 98 °C for 45 s, 65 °C for 90 s, 72 °C for 3 min at 10 cycles of amplification, 98 °C for 30 s, 65 °C for 45 s, 72 °C for 3 min at 25 cycles of amplification and then 72 °C 10 min. The amplified products were analyzed

by 1% agarose gel electrophoresis. All mutations identified are presented in Table S1.

## Result

### Prevalence of α- and β-globin gene mutations among couples in Hainan Province

We first examined 3049 couples of Hainan Province and diagnosed (1) 4923 cases as carrying α-thalassemia, accounting for 80.73%, with the mutation rates of 31.53% for –SEA/αα deletions, 11.15% for $-\alpha^{4.2}$/αα deletions and 11.02% for $-\alpha^{3.7}$/αα deletions, respectively (Table S2); (2) 2225 cases as carrying β-thalassemia, accounting for 34.69% of the total number of patients, with the mutation rates of 30.27% for CD41/42, 2.56% and 1.02% for − 28 and IVS-II-654, respectively; (3) 1322 cases as carrying compound α- and β-thalassemia, accounting for 20.99% of the total number of patients; and (4) 1322 cases as carrying both α and β-globin gene mutations, and among them, the five most frequent types were $-\alpha^{3.7}$/αα, $-\alpha^{4.2}$/αα, $-\alpha^{WS}$/αα, $-\alpha^{3.7}/-\alpha^{4.2}$ and–SEA/αα, with all accompanied by CD41/42, accounting for 4.49, 4.25, 2.35, 1.67, and 1.53% of all the subjects, respectively.

### Geographical distribution of α- and β-thalassemia gene mutations in 18 regions of Hainan

Characterization of thalassemia mutations in the risk couples had been performed before the fetal samples were collected. During the 16-year period (2004–2020), a total of 3049 subjects were screened for thalassemia at Prenatal Diagnosis Center of The First Affiliated Hospital of Hainan Medical University. The rates of α-thalassemia carrier in 3049 couples of Hainan varied between 1.38 and 13.63% in eighteen regions. The rate was higher in coastal region (Haikou, Sanya, Lingshui, Danzhou, Ledong, Fig. 1A). The β-thalassemia carrier showed less variation, ranging from 0.67 to 4.39% in eighteen regions, which was higher in the coastal regions (Sanya, Haikou, Lingshui, Dongfang, and Ledong, Fig. 1B). The rate of α- and β-thalassemia carrier presented less variation in eighteen regions, ranging from 0.23 to 3.1%. The distributed status was similar to that of β-thalassemia carriers (Fig. 1C). We then analyzed the prevalence of α- and β-thalassemia mutations in eighteen regions of Hainan Province. The geographical distribution of α-thalassemia mutations is shown in Table 1. A significantly higher frequency of the –SEA/αα mutation was found in the general population of Hainan Province. Twenty-four

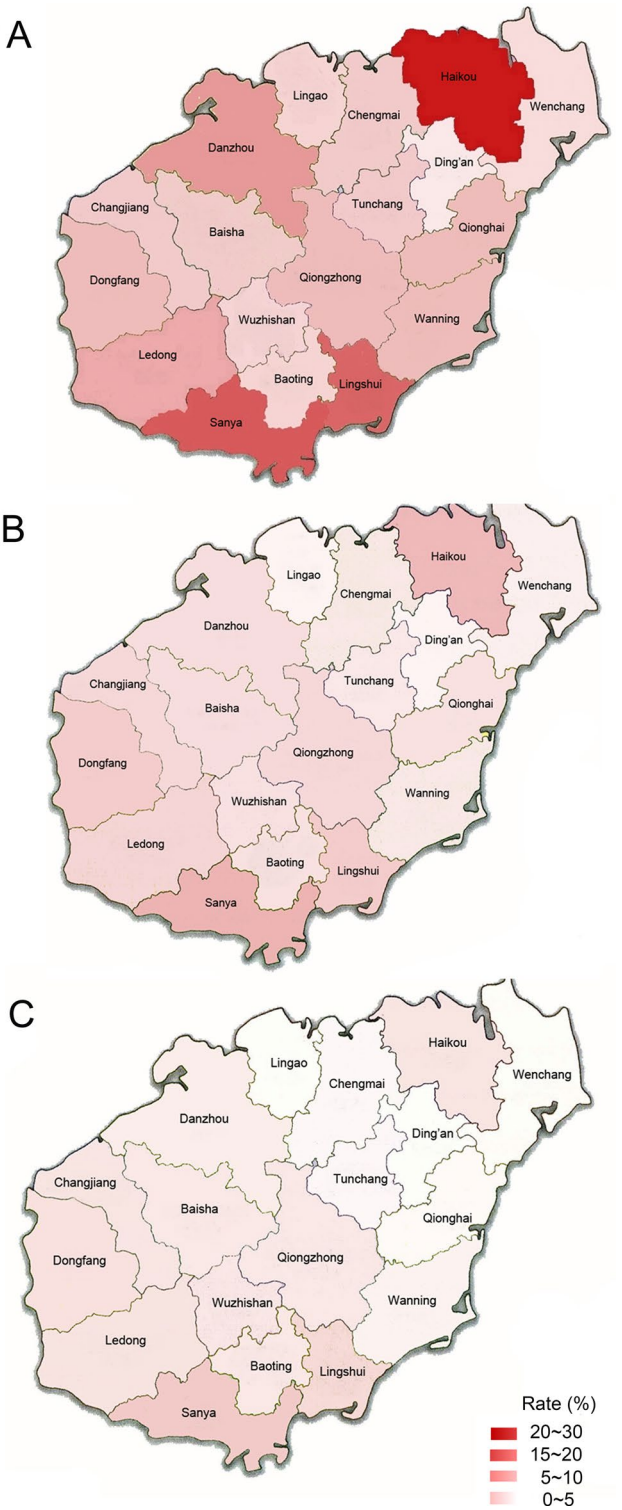


**Fig. 1** Frequencies of carrier status among couples in the eighteen regions of Hainan Province, China. **A** α-thalassemia only, **B** β-thalassemia, **C** α- and β-thalassemia

**Table 1** Distribution of α-thalassemia mutations in Hainan Province

| Genotype | Haikou | Wenchang | Wuzhishan | Lingao | Ding'an | Sanya | Danzhou | Qionghai | Dongfang | Wanning | Tunchang | Chengmai | Changjiang | Qiongzhong | Baisha | Baoting | Lingshui | Ledong |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) |
| – SEA/αα | 55.60 | 64.57 | 17.73 | 53.85 | 45.24 | 25.04 | 43.21 | 63.85 | 27.43 | 39.83 | 55.79 | 62.87 | 22.34 | 23.36 | 21.59 | 17.42 | 35.30 | 25.86 |
| – $\alpha^{4.2}$/αα | 12.64 | 6.30 | 14.18 | 13.08 | 19.05 | 16.70 | 16.90 | 10.33 | 19.47 | 14.94 | 7.89 | 7.19 | 17.55 | 13.52 | 10.23 | 18.71 | 13.12 | 13.08 |
| – $\alpha^{3.7}$/αα | 14.32 | 11.81 | 19.86 | 11.54 | 10.71 | 13.63 | 9.70 | 6.57 | 19.47 | 10.37 | 12.11 | 13.17 | 19.68 | 12.70 | 22.16 | 20.00 | 9.24 | 17.13 |
| – $\alpha^{WS}$/αα | 5.17 | 6.30 | 12.06 | 6.92 | 8.33 | 5.79 | 5.82 | 6.57 | 7.08 | 11.20 | 4.21 | 4.19 | 3.19 | 13.52 | 10.80 | 6.45 | 6.65 | 5.92 |
| – $\alpha^{3.7}$/– $\alpha^{4.2}$ | 2.05 | 2.36 | 6.38 | 0.77 | – | 7.50 | 4.16 | 0.94 | 4.87 | 1.24 | 1.05 | 4.79 | 9.04 | 4.10 | 9.09 | 10.97 | 4.62 | 9.66 |
| – $\alpha^{3.7}$/–SEA | 1.56 | 1.57 | 0.71 | 0.77 | 4.76 | 4.43 | 3.88 | 3.76 | 1.77 | 1.66 | 0.53 | 3.59 | 1.06 | 4.10 | 1.70 | 3.23 | 4.25 | 4.36 |
| – $\alpha^{3.7}$/– $\alpha^{WS}$ | 0.48 | 3.94 | 6.38 | 1.54 | – | 2.04 | 1.39 | 0.94 | 3.54 | 3.32 | 1.05 | 0.60 | 6.38 | 6.97 | 2.84 | 0.65 | 3.70 | 4.05 |
| – $\alpha^{3.7}$/– $\alpha^{3.7}$ | 0.84 | 0.79 | 2.13 | 0.77 | 2.38 | 4.26 | 1.11 | 0.94 | 3.54 | 1.66 | 1.58 | 0.60 | 6.38 | 3.28 | 5.68 | 6.45 | 3.51 | 1.56 |
| – $\alpha^{4.2}$/– $\alpha^{WS}$ | 0.60 | 0.79 | 5.67 | – | 2.38 | 4.60 | 0.28 | 0.47 | 3.10 | 2.49 | 1.05 | 0.60 | 2.66 | 1.64 | 7.95 | 3.87 | 2.77 | 3.74 |
| – $\alpha^{4.2}$/–SEA | 0.84 | – | 1.42 | 2.31 | – | 3.41 | 4.43 | – | 2.65 | 2.90 | 4.74 | — | 2.13 | 2.46 | 0.57 | 2.58 | 3.70 | 3.43 |
| – $\alpha^{QS}$/αα | 2.53 | 0.79 | 2.84 | 3.08 | 2.38 | 1.87 | 4.43 | 1.41 | – | 3.73 | 2.63 | 1.80 | 1.06 | 2.87 | 1.70 | 1.29 | 1.48 | 3.43 |
| – $\alpha^{4.2}$/– $\alpha^{4.2}$ | 0.96 | 0.79 | – | 3.08 | 1.19 | 4.77 | 1.66 | 1.88 | 2.21 | 0.41 | 0.53 | — | 2.66 | 0.82 | 3.41 | 3.23 | 4.07 | 2.18 |
| – $\alpha^{WS}$/–SEA | 0.12 | – | 0.71 | 0.77 | 2.38 | 1.02 | 1.11 | 0.94 | 0.88 | 1.66 | 1.58 | 0.60 | 1.06 | 3.28 | 1.14 | 2.58 | 2.59 | 0.93 |
| – $\alpha^{3.7}$/– $\alpha^{QS}$ | – | – | 0.71 | 0.77 | 1.19 | 1.87 | 0.28 | – | 0.44 | 0.83 | 1.05 | – | 2.66 | 2.87 | – | 0.65 | 1.48 | 1.56 |
| – $\alpha^{4.2}$/– $\alpha^{QS}$ | 0.24 | – | 4.96 | 0.77 | – | 1.19 | 0.83 | 0.47 | – | 1.66 | 1.58 | – | – | 0.82 | 0.57 | – | 1.11 | 1.25 |
| – $\alpha^{WS}$/– $\alpha^{WS}$ | 0.36 | – | 2.84 | – | – | 0.17 | 0.55 | – | 2.21 | 1.24 | 0.53 | – | 2.13 | 0.82 | – | 0.65 | 1.11 | – |
| – $\alpha^{CS}$/αα | 1.44 | – | – | – | – | 0.51 | 0.28 | – | 0.44 | 0.41 | 1.58 | – | – | 0.82 | – | – | 0.74 | – |
| – $\alpha^{QS}$/– $\alpha^{WS}$ | 0.12 | – | 0.71 | – | – | 1.19 | – | – | 0.88 | – | – | – | – | 1.23 | 0.57 | – | 0.18 | 1.25 |
| – $\alpha^{QS}$/–SEA | 0.12 | – | – | – | – | – | – | 0.94 | – | – | – | – | – | 0.41 | – | 1.29 | 0.37 | – |

**Table 1** (continued)

| Genotype | Haikou | Wenchang | Wuzhishan | Lingao | Ding'an | Sanya | Danzhou | Qionghai | Dongfang | Wanning | Tunchang | Chengmai | Changjiang | Qiongzhong | Baisha | Baoting | Lingshui | Ledong |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) |
| $-\alpha^{QS}/-\alpha^{QS}$ | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.41 | – | – | – | 0.31 |
| $-\alpha^{CS}/-\alpha^{WS}$ | – | – | – | – | – | – | – | – | – | 0.41 | – | – | – | – | – | – | – | – |
| $-\alpha^{4.2}/-\alpha^{CS}$ | – | – | 0.71 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| $-\alpha^{CS}/$— SEA | – | – | – | – | – | – | – | – | – | – | 0.53 | – | – | – | – | – | – | – |
| $-\alpha^{3.7}/-\alpha^{CS}$ | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.31 |
| Total | 831 | 127 | 141 | 130 | 84 | 587 | 361 | 213 | 226 | 241 | 190 | 167 | 188 | 244 | 176 | 155 | 541 | 321 |

types of α-thalassemia were identified in eighteen regions of Hainan Province. –SEA/αα, $-\alpha^{4.2}$/αα and $-\alpha^{3.7}$/αα are the most frequent α-thalassemia types in Haikou, Wenchang, Wuzhishan, Lingao, Ding'an, Sanya, Danzhou, Qionghai, Dongfang, Tunchang, Chengmai, Changjiang, Baisha, Baoting, Lingshui, and Dongfang, while the most common genotypes were − SEA/αα, $-\alpha^{4.2}$/αα and $-\alpha^{WS}$/αα in Wanning and Qiongzhong, five mutations ($-\alpha^{QS}/-\alpha^{QS}$, $-\alpha^{CS}/-\alpha^{WS}$, $-\alpha^{4.2}/-\alpha^{CS}$, $-\alpha^{CS}$/–SEA, $-\alpha^{3.7}/-\alpha^{CS}$) were observed at a frequency of less than 1% in eighteen regions. In general, the percentage of α-thalassemia mutation genes were in a similar tendency in different regions.

The geographical distribution of β-thalassemia mutations is shown in Table 2. Fourteen different point mutations of β-thalassemia were identified. CD41/42 (> 50%) was the most common mutation in eighteen regions, and four mutations (CD43, CD27/28, Cap, CD14/15) were observed at a frequency of less than 1%. Interestingly, the distribution trend of β-thalassemia mutation genes in eighteen regions was similar to that of α-thalassemia.

Fifty-six types of compound gene mutations were detected. The specific phenotype and frequency are shown in Table S3. 1319 cases carried both α- and β-globin gene mutations and, among them, the four most frequent types were $-\alpha^{4.2}$/αα, $-\alpha^{3.7}$/αα, $\alpha^{WS}$/αα, and − SEA/αα, all of which were accompanied by CD41/42 in eighteen regions of Hainan Province.

## Spectrum of α- and β-thalassemia mutations identified in Li and Han people of Hainan Province

Apart from geographical factors, ethnic difference also plays an important role in gene diversity. Since Hainan Province is a place, whose population is mainly composed of Han and other minority groups, which generates diverse genotypes. We then analyzed the frequency and spectrum of α- and β-thalassemia mutations of the Han people in Hainan Province and compared these findings with the minority. As is shown in Table 3, twenty-four α-globin genotypes were found in 4,597 cases of α-thalassemia, with –SEA/αα, $-\alpha^{4.2}$/αα and $-\alpha^{3.7}$/αα being the four most frequent α-thalassemia types among the Han and Li people, accounting for 57.50, 12.37, and 11.26% in Han people and 13.43, 16.43, and 16.76% in Li people, respectively. − SEA/αα and $-\alpha^{3.7}$/αα are the two most frequent in Miao and Zhuang people, accounting for 62.75 and 13.73% in Miao and 55.88 and 14.71% in Zhuang people.

Surprisingly, CD41/42 was the dominant mutation of β-thalassemia patients among the Han, Li, Miao, Zhuang people, accounting for 70.03, 94.84, 83.64, and 56.25%, respectively (Table 4).

Fifty-seven types of compound gene mutations were detected. The specific phenotypes and frequency are shown

**Table 2** Distribution of β-thalassemia mutations in Hainan Province

| Genotype | Haikou | Wenchang | Wuzhishan | Lingao | Ding'an | Sanya | Danzhou | Qionghai | Dongfang | Wanning | Tunchang | Chengmai | Changjiang | Qiongzhong | Baisha | Baoting | Lingshui | Ledong |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) | Ratio (%) |
| CD41/42 | 74.00 | 67.80 | 96.30 | 65.85 | 66.67 | 89.93 | 76.81 | 68.69 | 89.29 | 86.08 | 74.65 | 57.14 | 89.39 | 87.22 | 92.50 | 97.20 | 86.11 | 88.51 |
| − 28 | 13.60 | 6.78 | 2.78 | 24.39 | 23.81 | 2.24 | 10.14 | 15.15 | 6.55 | 5.06 | 16.90 | 21.43 | 1.52 | 4.51 | 0.83 | – | 2.22 | 1.35 |
| IVS-II-654 | 4.00 | 10.17 | – | 2.44 | 4.76 | 2.24 | 5.07 | 1.01 | 0.60 | 2.53 | – | 8.33 | 3.79 | – | 2.50 | 1.87 | 3.89 | 2.03 |
| CD17 | 4.00 | 1.69 | – | 4.88 | 4.76 | 1.49 | 1.45 | 8.08 | 1.19 | 1.27 | 2.82 | 3.57 | – | 3.76 | 0.83 | – | 1.11 | 4.05 |
| CD71/72 | 2.00 | 3.39 | 0.93 | 2.44 | – | 1.12 | 4.35 | 2.02 | 1.19 | 2.53 | 2.82 | 2.38 | 1.52 | 3.76 | 2.50 | – | 1.67 | 0.68 |
| CD26 | 2.00 | 8.47 | – | – | – | 0.75 | – | 2.02 | – | 1.27 | 1.41 | 2.38 | – | – | 0.83 | – | 3.33 | – |
| − 50 | – | – | – | – | – | 0.37 | 0.72 | – | 1.19 | – | 1.41 | 2.38 | 3.79 | – | – | 0.93 | 0.56 | 1.35 |
| − 29 | 0.40 | – | – | – | – | 0.75 | – | 1.01 | – | – | – | – | – | – | – | – | 0.56 | 2.03 |
| Int | – | 1.69 | – | – | – | – | – | 1.01 | – | – | – | 2.38 | – | – | – | – | – | – |
| IVS-I-1 | – | – | – | – | – | – | – | 1.01 | – | 1.27 | – | – | – | 0.75 | – | – | – | – |
| CD43 | – | – | – | – | – | 0.75 | 0.72 | – | – | – | – | – | – | – | – | – | – | – |
| CD27/28 | – | – | – | – | – | 0.37 | – | – | – | – | – | – | – | – | – | – | – | – |
| Cap | – | – | – | – | – | – | 0.72 | – | – | – | – | – | – | – | – | – | – | – |
| CD14/15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.56 | – |
| Total | 250 | 59 | 108 | 41 | 42 | 268 | 138 | 99 | 168 | 79 | 71 | 84 | 132 | 133 | 120 | 107 | 180 | 148 |

**Table 3** Spectrum of α-thalassemia mutations of Han people and minority in Hainan Province

| | Han | | Li | | Miao | | Zhuang | |
|---|---|---|---|---|---|---|---|---|
| | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) |
| – SEA/αα | 1557 | 57.50 | 278 | 13.43 | 64 | 62.75 | 199 | 55.88 |
| $-\alpha^{4.2}/\alpha\alpha$ | 335 | 12.37 | 340 | 16.43 | 4 | 3.92 | 1 | 2.94 |
| $-\alpha^{3.7}/\alpha\alpha$ | 305 | 11.26 | 347 | 16.76 | 14 | 13.73 | 5 | 14.71 |
| $-\alpha^{WS}/\alpha\alpha$ | 149 | 5.50 | 177 | 8.55 | 5 | 4.90 | 2 | 5.88 |
| $-\alpha^{3.7}/-\alpha^{4.2}$ | 42 | 1.55 | 184 | 8.89 | 3 | 2.94 | 1 | 2.94 |
| $-\alpha^{3.7}/-$ SEA | 62 | 2.29 | 73 | 3.53 | 5 | 4.90 | 1 | 2.94 |
| $-\alpha^{3.7}/-\alpha^{WS}$ | 22 | 0.81 | 104 | 5.02 | – | – | – | – |
| $-\alpha^{3.7}/-\alpha^{3.7}$ | 18 | 0.66 | 106 | 5.12 | – | – | 1 | 2.94 |
| $-\alpha^{4.2}/-\alpha^{WS}$ | 11 | 0.41 | 106 | 5.12 | – | – | – | – |
| $-\alpha^{4.2}/-$ SEA | 56 | 2.07 | 56 | 2.71 | 3 | 2.94 | 1 | 2.94 |
| $-\alpha^{QS}/\alpha\alpha$ | 65 | 2.40 | 46 | 2.22 | – | – | 1 | 2.94 |
| $-\alpha^{4.2}/-\alpha^{4.2}$ | 20 | 0.74 | 86 | 4.15 | – | – | – | – |
| $-\alpha^{WS}/-$ SEA | 18 | 0.66 | 39 | 1.88 | 3 | 2.94 | – | – |
| $-\alpha^{3.7}/-\alpha^{QS}$ | 4 | 0.15 | 42 | 2.03 | – | – | – | – |
| $-\alpha^{4.2}/-\alpha^{QS}$ | 10 | 0.37 | 31 | 1.50 | – | – | – | – |
| $-\alpha^{WS}/-\alpha^{WS}$ | 9 | 0.33 | 23 | 1.11 | – | – | – | – |
| $-\alpha^{CS}/\alpha\alpha$ | 19 | 0.70 | 4 | 0.19 | 1 | 0.98 | 2 | 5.88 |
| $-\alpha^{QS}/-\alpha^{WS}$ | 2 | 0.07 | 18 | 0.87 | – | – | – | – |
| $-\alpha^{QS}/-$ SEA | 3 | 0.11 | 5 | 0.24 | – | – | – | – |
| $-\alpha^{QS}/-\alpha^{QS}$ | – | – | 2 | 0.10 | – | – | – | – |
| $-\alpha^{CS}/-$ SEA | 1 | 0.04 | – | – | – | – | – | – |
| $-\alpha^{CS}/-\alpha^{WS}$ | – | – | 1 | 0.05 | – | – | – | – |
| $-\alpha^{4.2}/-\alpha^{CS}$ | – | – | 1 | 0.05 | – | – | – | – |
| $-\alpha^{3.7}/-\alpha^{CS}$ | – | – | 1 | 0.05 | – | – | – | – |
| Total | 270,888 | | 20,700 | | 1022 | | 34 | |

**Table 4** Spectrum of β-thalassemia mutations of Han people and minority in Hainan Province

| Genotype | Han | | Li | | Miao | | Zhuang | |
|---|---|---|---|---|---|---|---|---|
| | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) |
| CD41/42 | 694 | 70.03 | 1103 | 94.84 | 46 | 83.64 | 9 | 56.25 |
| – 28 | 135 | 13.62 | 20 | 1.72 | – | – | 1 | 6.25 |
| IVS-II-654 | 55 | 5.55 | 6 | 0.52 | 1 | 1.82 | – | – |
| CD17 | 33 | 3.15 | 7 | 0.60 | 5 | 9.09 | 5 | 31.25 |
| CD71/72 | 32 | 3.23 | 9 | 0.77 | 1 | 1.82 | – | – |
| CD26 | 17 | 1.72 | 7 | 0.60 | – | – | 1 | 6.25 |
| – 50 | 7 | 0.71 | 9 | 0.77 | – | – | – | – |
| – 29 | 7 | 0.71 | 1 | 0.09 | – | – | – | – |
| Int | 4 | 0.40 | – | – | – | – | – | – |
| IVS-I-1 | 1 | 0.10 | – | – | 2 | 3.64 | – | – |
| CD43 | 3 | 0.30 | – | – | – | – | – | – |
| CD14/15 | 1 | 0.10 | – | – | – | – | – | – |
| IVS-II-654// IVS-II-654 | 1 | 0.10 | – | – | – | – | – | – |
| Cap | – | – | 1 | 0.09 | – | – | – | – |
| CD27/28 | 1 | 0.10 | – | – | – | – | – | – |
| Total | 991 | | 1163 | | 55 | | 16 | |

in Table S4. The three most frequent types in Han people were $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, and $-\alpha^{WS}$, all of which were accompanied by CD41/42, accounting for 21.45, 21.29, and 11.28%, respectively. The three most frequent types in Li people were $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, and –SEA/αα, all of which were accompanied by CD41/42, accounting for 19.48, 16.29, and 16.86%, respectively. On the contrary, the patients who carried compound gene mutations in Miao and Zhuang were less than 1% of the total subjects.

## Prenatal diagnosis

The frequencies of thalassemia genotypes obtained from 3049 prenatal diagnosis in Hainan Province are shown in Table 5. Among these 3049 cases of α-thalassemia, 3.80% of the fetus were α-thalassemia hydrops, 70.35% were α-thalassemia carriers, 4.39% were β-thalassemia major, 27.75% were β-thalassemia carriers, and 18.24% were normal. The spectrum of α-thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province are shown in Table 6. The most frequent genotype was –SEA/αα, accounting for 21.42% of total fetuses, and is followed, in order of frequency, by the mutations $-\alpha^{4.2}/\alpha\alpha$ and $-\alpha^{3.7}/\alpha\alpha$, accounting for 15.45 and 14.89%, respectively. The top three α-globin gene mutations in the fetuses were the same as those observed in the pregnant women and husbands.

For the β-thalassemia mutations, the most frequent genotype was CD41-42 (67.36%), followed by CD41/42/CD41-42, − 28, CD71/72 and − 28/CD41/42, accounting for 15.28, 5.01, 3.42, and 2.93%, respectively. In the pregnant women and husbands, the most common mutation was CD41/42 followed by IVS-II-65 and CD17 in parents (Table 7).

A total of 485 genotypes were determined in both α and β-thalassemia carriers. The top three frequent types were $-\alpha^{3.7}/\alpha\alpha$ (17.53%), $-\alpha^{4.2}/\alpha\alpha$ (16.91%), and $-\alpha^{WS}/\alpha\alpha$ (8.87%), all of which were accompanied by CD41/42. The top 3 α- and β-combination globin gene mutations in the fetuses were the same to those of the pregnant women and husbands (Table S5).

For couples undergoing prenatal diagnosis, we conducted telephone follow-ups 6 months after the expected date of delivery, and a total of 2842 follow-ups were completed. 241 cases of termination of pregnancy, of which 208 cases were thalassemia severe, 33 cases were due to abnormalities including chromosomal and embryonic development, and 2601 successfully delivered newborns.

## Discussion

Previous studies have shown the prevalence and molecular spectrum of α- and β-thalassemia mutations in Hainan Province, but those studies had limited sample size and information [9, 10]. This study was the first to provide a large scale, sampling of the eighteen regions of Hainan Province. Also, we analyzed the family-based sampling, as we knew that the couples who carried the same type of thalassemia mutations had high risk to have a moderate or severe thalassemia fetus. These pregnant women and their husbands are critical subjects of thalassemia intervention, which will help to prevent birth of moderate to severe thalassemia fetus. Our center is the earliest established Prenatal Diagnosis Center in Hainan Province, and nearly all carriers of thalassemia in the whole province come here for prenatal diagnosis. Hence, our study presented the prevalence and molecular variations of α- and β-globin gene mutations in Hainan Province.

Our data indicated a high prevalence of thalassemia in Hainan Province. The frequencies of α- and β-thalassemia were 80.73 and 34.69% in the total subjects of our study, respectively. The frequency of α-thalassemia shown in our study were higher than that reported in other provinces of China, such as Guangdong[3], Fujian[5], Guangxi[4], Sichuan[11] and the frequency of β-thalassemia reported were lower than that reported in Fujian[5], but higher than that in Guangdong[3], Guangxi[4] and Sichuan[11]. In addition, the frequency of α-thalassemia demonstrated in our report were higher than that reported in other countries of Southeast Asia, such as Cambodia, Laos, Thailand, Vietnam,

**Table 5** Genotypes of thalassemia identified by prenatal diagnosis in Hainan Province

| Variable | One of parents was carrier | | Both parents were carriers | | Both parents were normal | | Total | Ratio (%) |
|---|---|---|---|---|---|---|---|---|
| | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) | | |
| α-thalassemia hydrops | – | – | 116 | 4.16 | – | – | 116 | 3.80 |
| α-thalassemia carrier | 119 | 48.18 | 1926 | 69.06 | – | – | 2145 | 70.35 |
| β-thalassemia major | – | – | 134 | 4.80 | – | – | 134 | 4.39 |
| β-thalassemia carrier | 39 | 15.79 | 807 | 28.94 | – | – | 820 | 27.75 |
| α + β-thalassemia carrier | 9 | 3.64 | 476 | 17.07 | – | – | 485 | 15.91 |
| Normal | 98 | 39.68 | 445 | 15.96 | 13 | 100 | 556 | 18.24 |
| Total | 247 | | 2789 | | 13 | | 3049 | |

**Table 6** Spectrum of α-thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province

| Genotype | Pregnant women | | Husbands | | Fetus | |
|---|---|---|---|---|---|---|
| | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) |
| –SEA/αα | 946 | 37.27 | 977 | 32.15 | 463 | 21.42 |
| $-\alpha^{4.2}/\alpha\alpha$ | 367 | 14.46 | 313 | 10.30 | 334 | 15.45 |
| $-\alpha^{3.7}/\alpha\alpha$ | 360 | 14.18 | 312 | 10.27 | 322 | 14.89 |
| $-\alpha^{WS}/\alpha\alpha$ | 166 | 6.54 | 168 | 5.53 | 160 | 7.40 |
| $-\alpha^{3.7}/-\alpha^{4.2}$ | 130 | 5.12 | 101 | 3.32 | 76 | 3.52 |
| $-\alpha^{3.7}/-\alpha^{WS}$ | 75 | 2.96 | 51 | 1.68 | 42 | 1.94 |
| $-\alpha^{3.7}$/—SEA | 72 | 2.84 | 69 | 2.27 | 139 | 6.43 |
| $-\alpha^{3.7}/-\alpha^{3.7}$ | 68 | 2.68 | 57 | 1.88 | 52 | 2.41 |
| $-\alpha^{4.2}$/–SEA | 66 | 2.60 | 50 | 1.65 | 162 | 7.49 |
| $-\alpha^{4.2}/-\alpha^{WS}$ | 66 | 2.60 | 51 | 1.68 | 39 | 1.80 |
| $-\alpha^{QS}/\alpha\alpha$ | 53 | 2.09 | 59 | 1.94 | 39 | 1.80 |
| $-\alpha^{4.2}/-\alpha^{4.2}$ | 46 | 1.81 | 60 | 1.97 | 45 | 2.08 |
| $-\alpha^{3.7}/-\alpha^{QS}$ | 28 | 1.10 | 18 | 0.59 | 23 | 1.06 |
| $-\alpha^{WS}$/–SEA | 25 | 0.99 | 35 | 1.15 | 63 | 2.91 |
| $-\alpha^{4.2}/-\alpha^{QS}$ | 21 | 0.83 | 20 | 0.66 | 17 | 0.79 |
| $-\alpha^{CS}/\alpha\alpha$ | 19 | 0.75 | 8 | 0.26 | 8 | 0.37 |
| $-\alpha^{WS}/-\alpha^{WS}$ | 11 | 0.43 | 21 | 0.69 | 18 | 0.83 |
| $-\alpha^{QS}/-\alpha^{WS}$ | 11 | 0.43 | 9 | 0.30 | 17 | 0.79 |
| $-\alpha^{QS}$/–SEA | 3 | 0.12 | 5 | 0.16 | 15 | 0.69 |
| $-\alpha^{CS}$/–SEA | 1 | 0.04 | – | – | 8 | 0.37 |
| $-\alpha^{QS}/-\alpha^{QS}$ | 1 | 0.04 | 1 | 0.03 | 1 | 0.05 |
| $-\alpha^{3.7}/-\alpha^{CS}$ | 1 | 0.04 | – | – | 1 | 0.05 |
| $-\alpha^{4.2}/-\alpha^{CS}$ | 1 | 0.04 | – | – | 1 | 0.05 |
| $-\alpha^{CS}/-\alpha^{WS}$ | 1 | 0.04 | – | – | 1 | 0.05 |
| –SEA/–SEA | – | – | – | – | 116 | 5.37 |
| Total | 2538 | | 3039 | | 2162 | |

and Malaysia [12] and in other countries/regions of the world, such as the north of Southwest Iran [13] and lower than that reported in Pakistan [8].

In this study, the regions with the highest prevalence of α-thalassemia were Haikou, followed by the Sanya, Lingshui, Danzhou, and Dongfang. The three regions with the highest prevalence of β-thalassemia mutations were Sanya, Haikou, and Lingshui. The lowest prevalence of α- and β-thalassemia was Lingao, Wenchang, and Ding'an. In our study, the prevalence of thalassemia carrier status in Sanya City was lower than that reported by Li [14]. Among α-thalassemia genotypes, –SEA/αα, which is consistent with the previous research by Sanya City and Ding'an County of Hainan Province, Sichuan, Fujian, Guangdong, and Guangxi [3–5, 11, 14, 15], but different from that of Lingshui Autonomous County of Hainan Province [16], accounts for the highest frequency in the total couples of eighteen regions. Comparing with other countries, the most frequent α-thalassemia mutations are consistent with that Laos [17], but different from those reported Thailand [18], Cambodia [19], and Iran [20]. Among the β-thalassemia genotypes, CD41/42 accounts for the greatest proportion among couples of eighteen regions and it is consistent with the previous study that reported to be the most frequent genotype in Sanya City, Lingshui, and Ding'an County of Hainan Province, Yunnan [21], Guangdong [3], Sichuan [11], Guangxi Province [4], while IVS-II-654 ranked first in Hubei Province [22]. These findings may be explained with the special location of Hainan Province which is located on a small island in southeast China. Comparing with other countries, the most frequent genotype of β-thalassemia is similar to that in Thailand [23], while that were different from Pakistan [24], Syria [25], Iran [20], Vietnam [26] Malaysia [27], Philippines, and India [28].

Race difference may also play a significant role in gene difference, so we do not exclude racial diversity. As population of Hainan Province is composed of Han and the minority ethnic group such as Li, Miao, Zhuang, so we also analyzed thalassemia genotype of couples based on their nations. Interestingly, –SEA/αα and CD41/42 are the most frequent thalassemia genotype in Han, Li, Miao, and Zhuang people, different from that in an early study [10], whose dominant genotype is $-\alpha^{4.2}/\alpha\alpha$. This result may be

**Table 7** Spectrum of β-thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province

| Genotypes | Pregnant women | | Husbands | | Fetus | |
|---|---|---|---|---|---|---|
| | *N* | Ratio (%) | *N* | Ratio (%) | *N* | Ratio (%) |
| CD41/42 | 976 | 85.54 | 876 | 80.81 | 551 | 67.36 |
| IVS-II-654 | 29 | 2.54 | 34 | 3.14 | 1 | 0.12 |
| CD17 | 23 | 2.02 | 28 | 2.58 | 6 | 0.73 |
| CD26 | 13 | 1.14 | 12 | 1.11 | 6 | 0.73 |
| − 50 | 6 | 0.53 | 10 | 0.92 | – | – |
| − 29 | 5 | 0.44 | 3 | 0.28 | 1 | 0.12 |
| IVS-I-1 | 2 | 0.18 | 1 | 0.09 | 3 | 0.37 |
| CD27/28 | 1 | 0.09 | – | – | 1 | 0.12 |
| CD14/15 | 1 | 0.09 | – | – | 1 | 0.12 |
| CD41/42/CD41/42 | – | – | – | – | 125 | 15.28 |
| − 28 | – | – | 71 | 6.55 | 41 | 5.01 |
| CD71/72 | – | – | 41 | 3.78 | 28 | 3.42 |
| − 28/CD41/42 | – | – | – | – | 24 | 2.93 |
| CD41/42/IVS-II-654 | – | – | – | – | 5 | 0.61 |
| CD41/42/-50 | – | – | – | – | 4 | 0.49 |
| − 28/IVS-II-654 | – | – | – | – | 4 | 0.49 |
| − 28/-28 | – | – | – | – | 4 | 0.49 |
| CD17CD41/42 | – | – | – | – | 4 | 0.49 |
| IVS-II-654/IVS-II-654 | – | – | 1 | 0.09 | 1 | 0.12 |
| CD26/CD26 | – | – | – | – | 1 | 0.12 |
| CD41/42/CD26 | – | – | – | – | 1 | 0.12 |
| CD43 | – | – | 3 | 0.28 | 2 | 0.24 |
| Int | – | – | 4 | 0.37 | 1 | 0.12 |
| Cap | – | – | 1 | 0.09 | 1 | 0.12 |
| Total | 1141 | | 1084 | | 818 | |

explained by continued migration or the screened population we selected.

Due to geography, culture, customs, and other reasons, the proportion of inter-marriages between some regions of Hainan Province and other regions is relatively small, which consequently led to an overlapping distribution of thalassemia genes and resulting in a high number of thalassemia carriers. Prenatal diagnosis is one of the most effective and direct method to prevent thalassemia major. In this study, we showed the results of prenatal diagnosis in our regions from 2004 to 2020, all participants were lived in Hainan Province. This study is the first systematic family-based prenatal diagnosis of thalassemia in the Hainan Province.

The findings of fetal diagnosis presented 9.17% at risk for thalassemia major, 18.26% for the normal type. Compared with one parent being a carrier, both parents that were carriers were more likely to have a child with thalassemia major or carrier. Also, the couples who carried the same thalassemia genotype had a high risk to have a fetus with thalassemia major. Interestingly, –SEA/αα deletion and CD41/42 were found to be the most common types of thalassemia of prenatal diagnosis, which were also the most predominant mutations of the full set of couples in our study, such couples had a 1/4 chance of producing a fetus with Hb Bart's and β-thalassemia major. We identified 118 fetuses with Bart's hydrops syndrome, accounting for 3.88%. We also identified 161 fetuses with β-thalassemia major, accounting for 5.29%. This study had a limitation that we did not test the α12 allele which also have the impact with α-thalassemia on clinical phenotype [29].

At present, Hainan Province has established several Prenatal Diagnosis Centers and Health Departments which have initiated free screening program since 2011. Other organizations such Li Ka-shing Health Poverty Alleviation fund projects and Prevention and Control Committee and Volunteers Association of Hainan Province, will help the prevention and diagnosis of thalassemia. We also recommend further studies on the factors that can affect the accessibility of thalassemia interventions to provide a scientific basis for government decision-making.

## Conclusion

The gene types of α- and β-thalassemia in Hainan Province in China are characterized by a wide range of distribution, high carrier rate, genetic diversity, genetic heterogeneity,

geographical, and ethnic differences. Therefore, it is necessary to detect the thalassemia genes in the suspected population in this area so as to provide clinical genetic counseling and prenatal diagnosis of for thalassemia major in Hainan Province.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12185-021-03173-z.

**Acknowledgements** We are thankful to Jun Wang from Texas Heart Institute for writing instruction.

**Author contributions** Zhen Wang, Wenye Sun, Qi Li, and Yanlin Ma conducted the laboratory work, data analysis, and manuscript draft writing. Huaye Chen, Yongfang Zhang, Fei Wang, Yanhua Huang, Hongjian Chen, Yao Zhou, and Xixi Zhou assisted in clinical information collection, interpretation and to improve the manuscript. Qi Li and Yanlin Ma designed the study plan and supervised the overall project. All authors read, critically reviewed the draft and approved the final version.

**Funding** This work was supported by Hainan Provincial Natural Science Foundation of China (No.2019CXTD408), Major Science and Technology Program of Hainan Province (No. ZDKJ2017007), National Natural Science Foundation of China (81460034, 81660433, 81960283, 82072880), Key Research and Development projects of Hainan Province (ZDYF2020117), Tropical Translational Medicine Education Key Laboratory Open Project (2020TTM006, 2020TTM008).

## Declarations

**Conflict of interest** The authors have no conflict of interests.

## References

1. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet (London, England). 2018;391(10116):155–67. https://doi.org/10.1016/s0140-6736(17)31822-6.
2. Lai K, Huang G, Su L, He Y. The prevalence of thalassemia in mainland China: evidence from epidemiological surveys. Sci Rep. 2017;7(1):920. https://doi.org/10.1038/s41598-017-00967-2.
3. Yin A, Li B, Luo M, Xu L, Wu L, Zhang L, et al. The prevalence and molecular spectrum of α- and β-globin gene mutations in 14,332 families of Guangdong Province, China. PLoS ONE. 2014;9(2):e89855. https://doi.org/10.1371/journal.pone.0089855.
4. He S, Li J, Li DM, Yi S, Lu X, Luo Y, et al. Molecular characterization of α- and β-thalassemia in the Yulin region of Southern China. Gene. 2018;655:61–4. https://doi.org/10.1016/j.gene.2018.02.058.
5. Xu C, Liao B, Qi Y, Huangfu Z, Chen J, Chen Y. Analysis of gene mutation Types of α- and β-Thalassemia in Fuzhou Fujian Province in China. Hemoglobin. 2018;42(3):143–7. https://doi.org/10.1080/03630269.2018.1496096.
6. Sargolzaie N, Montazer Zohour M, Ayubi E, Shahraki F. Relationship between social determinants of health and the Thalassemia prenatal diagnosis test in Zahedan South Eastern Iran. Hemoglobin. 2018;42(4):231–5. https://doi.org/10.1080/03630269.2018.1520718.
7. Nopparatana C, Nopparatana C, Saechan V, Karnchanaopas S, Srewaradachpisal K. Prenatal diagnosis of α- and β-thalassemias in southern Thailand. Int J Hematol. 2020;111(2):284–92. https://doi.org/10.1007/s12185-019-02761-4.
8. Kanwal S, Bukhari S, Perveen S. Molecular genetics and prenatal diagnosis of beta thalassemia to control transfusion dependent births in carrier Pakistani couples. J Pak Med Assoc. 2017;67(7):1030–4.
9. Liang C, Chen X-Y, Gao X, Chen H-J, Jin Y-X, Zhou Y, et al. Spectrum of thalassemia mutations in fetuses of Han and Li ethinicities in Hainan Province, China. Asian Pac J Trop Med. 2019;12(12):537–44. https://doi.org/10.4103/1995-7645.272483.
10. Yao H, Chen X, Lin L, Wu C, Fu X, Wang H, et al. The spectrum of α- and β-thalassemia mutations of the Li people in Hainan Province of China. Blood Cells Mol Dis. 2014;53(1–2):16–20. https://doi.org/10.1016/j.bcmd.2014.01.003.
11. Yu X, Yang LY, Yang HT, Liu CG, Cao DC, Shen W, et al. Molecular epidemiological investigation of Thalassemia in the Chengdu Region, Sichuan Province Southwest China. Hemoglobin. 2015;39(6):393–7. https://doi.org/10.3109/03630269.2015.1070733.
12. Goh LPW, Chong ETJ, Lee PC. Prevalence of Alpha(α)-Thalassemia in Southeast Asia (2010–2020): a meta-analysis involving 83,674 subjects. Int J Environ Res Public Health. 2020;17(20):7354. https://doi.org/10.3390/ijerph17207354.
13. Nezhad FH, Nezhad KH, Choghakabodi PM, Keikhaei B. Prevalence and genetic analysis of α- and β-Thalassemia and sickle cell anemia in Southwest Iran. J Epidemiol Global Health. 2018;8(3–4):189–95. https://doi.org/10.2991/j.jegh.2018.04.103.
14. Li M, Xiang SH, Ding Y, Liu WW, Xu YY, Bo J. Genotype analysis of patients with Thalassemia in Sanya Area of Hainan Province in China. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2018;26(4):1146–50. https://doi.org/10.7534/j.issn.1009-2137.2018.04.033.
15. Tu ZH, Wang J, Hu JJ, Zhao LQ, Ran HL, Wang AG, et al. Genetic screening of Thalassemia among the couples of childbearing age in Ding'an County of Hainan Province and its analysis. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2019;27(5):1592–5. https://doi.org/10.19746/j.cnki.issn.1009-2137.2019.05.035.
16. Tu ZH, Zhou Z, Wu WX, Wang XP, Zhou YZ, Huang CD, et al. Analysis of genetic screening in couples of reproductive age for Thalassemia in Lingshui Li Autonomous County of Hainan Province. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2019;27(4):1227–31. https://doi.org/10.19746/j.cnki.issn.1009-2137.2019.04.038.
17. Phanmany S, Chanprasert S, Munkongdee T, Svasti S, Leecharoenkiat K. Molecular prevalence of thalassemia and hemoglobinopathies among the Lao Loum Group in the Lao People's Democratic Republic. Int J Lab Hematol. 2019;41(5):650–6. https://doi.org/10.1111/ijlh.13080.
18. Pharephan S, Sirivatanapa P, Makonkawkeyoon S, Tuntiwechapikul W, Makonkawkeyoon L. Prevalence of α-thalassaemia genotypes in pregnant women in northern Thailand. Indian J Med Res. 2016;143(3):315–22. https://doi.org/10.4103/0971-5916.182622.
19. Munkongdee T, Tanakulmas J, Butthep P, Winichagoon P, Main B, Yiannakis M, et al. Molecular epidemiology of hemoglobinopathies in Cambodia. Hemoglobin. 2016;40(3):163–7. https://doi.org/10.3109/03630269.2016.1158723.
20. Jaripour ME, Hayatigolkhatmi K, Iranmanesh V, Zand FK, Badiei Z, Farhangi H, et al. Prevalence of β-Thalassemia mutations among Northeastern Iranian Population and their impacts on Hematological indices and application of prenatal diagnosis, a seven-years study. Mediterr J Hematol Infect Dis. 2018;10(1):e2018042. https://doi.org/10.4084/mjhid.2018.042.
21. Zhang J, He J, Mao X, Zeng X, Chen H, Su J, et al. Haematological and electrophoretic characterisation of β-thalassaemia in Yunnan province of Southwestern China. BMJ Open. 2017;7(1):e013367. https://doi.org/10.1136/bmjopen-2016-013367.

22. Zhu Y, Shen N, Wang X, Xiao J, Lu Y. Alpha and beta-Thalassemia mutations in Hubei area of China. BMC Med Genet. 2020;21(1):6. https://doi.org/10.1186/s12881-019-0925-5.
23. Singha K, Taweenan W, Fucharoen G, Fucharoen S. Erythrocyte indices in a large cohort of β-thalassemia carrier: implication for population screening in an area with high prevalence and heterogeneity of thalassemia. Int J Lab Hematol. 2019;41(4):513–8. https://doi.org/10.1111/ijlh.13035.
24. Muhammad R, Shakeel M, Rehman SU, Lodhi MA. Population-based genetic study of β-Thalassemia mutations in Mardan Division, Khyber Pakhtunkhwa Province Pakistan. Hemoglobin. 2017;41(2):104–9. https://doi.org/10.1080/03630269.2017.1330210.
25. Murad H, Moasses F, Dabboul A, Mukhalalaty Y, Bakoor AO, Al-Achkar W, et al. Geographical distribution of β-globin gene mutations in Syria. Hematology (Amsterdam, Netherlands). 2018;23(9):697–704. https://doi.org/10.1080/10245332.2018.1461291.
26. Vo LTT, Nguyen TT, Le HX, Le HTT. Analysis of common β-Thalassemia mutations in North Vietnam. Hemoglobin. 2018;42(1):16–22. https://doi.org/10.1080/03630269.2018.1428621.
27. Abdullah UYH, Ibrahim HM, Mahmud NB, Salleh MZ, Teh LK, Noorizhab M, et al. Genotype-phenotype correlation of β-Thalassemia in Malaysian population: toward effective genetic counseling. Hemoglobin. 2020;44(3):184–9. https://doi.org/10.1080/03630269.2020.1781652.
28. Shah PS, Shah ND, Ray HSP, Khatri NB, Vaghasia KK, Raval RJ, et al. Mutation analysis of β-thalassemia in East-Western Indian population: a recent molecular approach. Appl Clin Genet. 2017;10:27–35. https://doi.org/10.2147/tacg.s127531.
29. Luo SQ, Chen XY, Tang N, Huang J, Zhong QY, Cai R, et al. Pedigree analysis of nonhomologous sequence recombination of HBA1 and HBA2 genes. Hemoglobin. 2020;44(5):329–33. https://doi.org/10.1080/03630269.2020.1807355.