Molecular analysis of alpha- and beta-thalassemia in Meizhou region and comparison of gene mutation spectrum with different regions of southern China

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Abstract

Background: Thalassemia is a group of inherited autosomal recessive hemolytic anemia disease caused by reduced or absent synthesis of globin chain/chains of hemoglobin. Only few studies showed the molecular characterization of α- and β-thalassemia in Meizhou city of China.

Methods: A total of 22,401 individuals were collected; hematological and hemoglobin electrophoresis analysis and thalassemia genetic testing were performed.

Results: Eleven thousand and thirty (49.24%) cases with microcytosis (mean corpuscular volume (MCV) < 82 fl), 11,074 (49.44%) cases with hypochromia (mean corpuscular Hb (MCH) < 27 pg) in 22,401 subjects, 11,085 cases with abnormal hemoglobin results were identified in subjects aged ≥ 6 months. 7,322 (32.69%) subjects harbored thalassemia mutations, including 4,841 (21.61%) subjects with α-thalassemia, 2,237 (9.99%) with β-thalassemia, and 244 (1.09%) with α-thalassemia combined β-thalassemia. 18 genotypes of α-thalassemia mutations and 27 genotypes of β-thalassemia mutations were characterized. The most frequent α gene mutation was --SEA (64.69%), followed by -α3.7 (19.93%), -α4.2 (7.73%), αCSα (3.97%), and αWSα (2.83%).

The six most common β-thalassemia mutations were IVS-II-654 (C>T) (39.79%), CD41-42 (-TCTT) (33.02%), –28 (A>G) (10.38%), CD17 (A>T) (9.08%), CD27-28 (+C) (2.14%), and CD26 (G>A) (2.02%). In addition, MCV and MCH were sensitive markers for α- and β-thalassemia except for -α3.7/αα, -α4.2/αα, αCSα/αα, αWSα/αα, and βCap+40-42/βN.

Conclusions: The --SEA, -α3.7, and -α4.2 deletions were the main mutations of α-thalassemia, while IVS-II-654 (C>T), CD41-42 (-TCTT), –28 (A>G), and CD17 (A>T) mutations of β-thalassemia in Meizhou. There were some differences in thalassemia mutation frequencies in Meizhou city from other populations in China.

KEYWORDS
genotype distribution, Meizhou city, thalassemia
1 | INTRODUCTION

Thalassemia is a group of inherited autosomal recessive hemolytic anemia disease caused by reduced or absent synthesis of globin chain/chains of hemoglobin, and it is one of the most common monogenic disorders in the world.\(^1\) It is prevalent in tropical and subtropical areas, such as Mediterranean countries, Africa, Middle East, Indian subcontinent, and Southeast Asia, including southern China.\(^2\,\(^3\) Thalassemia can be divided into some types: the most common forms are α-thalassemia (OMIM: #604131) and β-thalassemia (OMIM: #613985), which affect the synthesis of α- and β-globin subunits, respectively. Almost asymptomatic or only slight changes in hematology showed in thalassemia silent and thalassemia trait patients, and lethal hemolytic anemia in the thalassemia major patients. Thalassemia can be divided into three groups according to the clinical severity: thalassemia trait, thalassemia intermedia, and thalassemia major. Patients with thalassemia major or intermediate usually present with life-long anemia and require blood transfusions and iron removal, placing a huge burden on the family and society.

In China, thalassemia was mainly prevalent in the population of southern areas of the Yangtze River, especially in the provinces of Guangxi, Guangdong, and Hainan, according to the reports of previous researches.\(^4\) The prevalence of α-thalassemia and β-thalassemia was 17.55% and 6.43%, respectively, in a cohort of 5,789 consecutive samples from Guangxi province.\(^5\) The prevalence of α-thalassemia, β-thalassemia, and both α- and β-thalassemia was 8.53%, 2.54%, and 0.26%, respectively, in Guangdong province.\(^2\) The prevalence of α-thalassemia, β-thalassemia, and both α- and β-thalassemia was 53.45%, 3.83%, and 7.99%, respectively, in 8,600 subjects of the Li people from Hainan province; however, those were 12.16%, 6.11%, and 4.85%, respectively, in 9,800 subjects of the Han people.\(^8\) There are differences in globin gene mutations among different ethnic groups in different geographical regions. Ethnic background may partially explain these differences.

Meizhou is an underdeveloped city, located in the northeast of Guangdong province. Thalassemia has brought significant challenges to improving the quality of the population in Meizhou region. The prevalence of α-thalassemia and β-thalassemia in population in Meizhou area has been investigated by Lin et al.\(^9\) and Zhao et al.\(^10\) respectively. Herein, a more large-scale survey of thalassemia to analyze the feature of genotypes distribution and frequencies in Meizhou city was performed in the present study. A larger sample size and more detailed study of the mutation frequencies of α- and β-globin genes will help to provide better reference data for the prevention and control of thalassemia in this region.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 22,401 unrelated subjects who visited Meizhou people's hospital from January 2015 to June 2020 were collected. These subjects visited our hospital for routine examination. These cases were mainly collected from outpatients and inpatients who underwent molecular detection for thalassemia in the departments of Pediatrics, Hematology, Obstetrics & Gynecology, and Reproductive Medicine Center of our hospital. The present study was approved by the Ethics Committees of Meizhou People’s Hospital (Huangtang Hospital), Guangdong province, China, and was conducted according to the Declaration of Helsinki.

2.2 | Hematological studies and hemoglobin electrophoresis analysis

Two millilitre of blood sample was taken via venipuncture of an antecubital vein from each subject and collected in tube with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Erythrocyte correlative indices were detected by Sysmex XE-2100 blood analyzer (Sysmex Corporation) according to the standard operating procedures (SOP). The composition and content of hemoglobin was analyzed by Sebia capillary electrophoresis system (Sebia, Inc.) according to the SOP. Mean corpuscular volume (MCV) <82 fl and (or) corpuscular hemoglobin (MCH) values <27 pg were thought as suspicious thalassemia carriers.\(^11\) Subjects with hemoglobin A\(_2\) (HbA\(_2\)) < 2.5% and HbA\(_2\) > 3.5% were considered probable α-thalassemia carriers and β-thalassemia carriers, respectively.\(^12\)

2.3 | Genetic analysis

Two millilitre of peripheral blood sample was collected in tube with EDTA as anticoagulant, and subjects’ genomic DNA was extracted. Gap-polymerase chain reaction (gap-PCR) and flow-through hybridization technology (Hybríbio Limited) were used to detect the deletion α-thalassemia mutations (−66A, −α\(_{3.7}\), and −α\(_{4.2}\)) and non-deletion α-thalassemia mutations (Hb Constant Spring (Hb CS) (CD142, TAA→CAA), Hb Quong Sze (Hb QS) (CD125, CTG→CCG), and Hb Westmead (CD122, CAC→CAG)), and 16 common nondeletion mutations in β-globin gene, including CD41-42 (→TCTT), CD43 (G>T), IVS-II-654 (C>T), CD17 (A>T), CD14-15 (G→C), −28 (A>G), −29 (A>G), CD71-72 (+A), CD26 (G>A), IVS-1 (G>T), IVS-1 (G>A), CD27-28 (+C), IVS-1 (G>C), Cap+40–43 (→AAC), initiation codon (ATG→AGG), and CD31 (C).

Multiplex ligation-dependent probe amplification (MLPA) assay was performed to detect the unknown deletions by using the SALSA MLPA probemix P140-C1HBA (MRC-Holland).

2.4 | Statistical analysis

Statistical analysis was performed with the SPSS statistical software version 20.0 (International Business Machines Corporation). Descriptive analysis was used to show the frequencies of genotype and allele in different populations. The ratio of α- and β-thalassemia alleles was calculated.
3 | RESULTS

Among the 22,401 subjects, 11,030 (49.24%) cases with microcytosis (MCV < 82 fl), 11,074 (49.44%) cases with hypochromia (MCH < 27 pg), 10,438 (46.60%) cases both with microcytosis and hypochromia, and 11,085 cases with abnormal hemoglobin results (8.137 with HbA2 < 2.5%, 2.360 with Hba2 > 3.5%, 552 with abnormal hemoglobin zone) were found in subjects aged ≥6 months, respectively.

As shown in Table 1, among the 22,401 subjects, 18 genotypes and 4,841 (21.61%) subjects with α-thalassemia were identified. The common α-thalassemia genotypes were -SEa/αα (62.78%), -α^3.7/αα (16.24%), and -α^4.2/αα (6.40%), accounted for 85.42%. Furthermore, several cases were identified to carry the rare α-thalassemia mutations, such as 3 cases with carrying -SEa/HKαe genotype were identified. In these α-thalassemia carriers subjects, 4,034 cases (83.33%, 4,034/4,841) were with MCV < 82 fl and 4,026 cases (83.16%, 4,026/4,841) with MCH < 27 pg. Of the patients with -SEa/αα, the levels of MCV and MCH in most cases (>95.0%) were lower than the normal reference; only 129 (4.24%) and 141 cases (4.64%) had the normal MCV and MCH values, respectively. Among the patients with -α^3.7/αα, the proportions of the patients with the normal MCV and MCH values were 50.76 and 50.38%, respectively, and the similar results were seen in the patients with -α^4.2/αα, α^CΔα/αα, and α^WSα/αα genotypes. MCV and MCH were sensitive markers for α-thalassemia except for -α^3.7/αα, -α^4.2/αα, α^CΔα/αα, and α^WSα/αα.

Among the 22,401 subjects, 27 types of β-globin gene mutation and 2,237 (9.99%) subjects with β-thalassemia were identified. The common four genotypes of β-thalassemia being βIVS−II−654/βN (40.14%), βCD41-42/βN (33.21%), βCD71/βN (9.21%), and ββ−28/βN (9.12%), accounted for 91.68%. In these β-thalassemia carriers subjects, 2,127 cases (95.08%, 2,127/2,237) were with MCV < 82 fl and 2,103 cases (94.01%, 2,103/2,237) with MCH < 27 pg (Table 2). Of the patients with βIVS−II−654/βN, the level of MCV and MCH in most cases (>95.0%) were lower than the normal reference; only 33 (3.67%) and 39 cases (4.34%) had the normal MCV and MCH value, respectively, and the similar results were seen in the patients with βCD41-42/βN, βCD71/βN, ββ−28/βN, βCD71−72/βN, and βCD14-15/βN genotypes (all abnormal proportions >90%). Among the patients with βCD41-42/βN, the proportions of the patients with the normal MCV and MCH values were 53.92 and 47.62% respectively.

As shown in Table 3, among the 22,401 subjects, 244 (1.09%) subjects had been found to carry compound α/β-thalassemia mutations, and the top five genotypes were -SEa/αα combined with βIVS−II−654/βN (18.85%), -SEa/αα combined with βCD41-42/βN (14.75%), -SEa/αα combined with ββ−28/βN (9.02%), -α^3.7/αα combined with βIVS−II−654/βN (8.56%), and -α^3.7/αα combined with βCD41-42/βN (7.38%), accounted for 58.61%. In these subjects with composite α-thalassemia and β-thalassemia, 225 cases (92.21%, 225/244) were with MCV < 82 fl,

| Genotype   | Cases | Constituent ratio (%) | MCV | MCH | Proportion of MCV < 27 pg (%) | Proportion of MCH < 27 pg (%) |
|------------|-------|-----------------------|-----|-----|-----------------------------|-------------------------------|
| -SEa/αα    | 3039  | 62.78                 | 2910| 129 | 95.76                       | 2898                         |
| -α^3.7/αα  | 786   | 16.24                 | 387 | 399 | 49.24                       | 390                          |
| -α^4.2/αα  | 310   | 6.40                  | 179 | 131 | 57.74                       | 168                          |
| -SEa/α^3.7 | 209   | 4.32                  | 204 | 5   | 97.61                       | 206                          |
| α^CΔα/αα   | 132   | 2.73                  | 76  | 56  | 57.58                       | 72                           |
| α^WSα/αα   | 120   | 2.48                  | 62  | 58  | 51.67                       | 62                           |
| -SEa/α^4.2 | 82    | 1.69                  | 79  | 3  | 96.34                       | 79                           |
| -SEa/α^CΔα | 66    | 1.36                  | 49  | 17  | 74.24                       | 63                           |
| α^WSα/αα   | 37    | 0.76                  | 33  | 4  | 89.19                       | 35                           |
| -α^3.7/α^3.7 | 12  | 0.25                  | 12  | 2  | 100.00                      | 12                           |
| -α^3.7/α^4.2 | 11  | 0.23                  | 11  | 0  | 100.00                      | 11                           |
| -SEa/α^WSα | 5     | 0.10                  | 5   | 0  | 100.00                      | 5                            |
| -α^3.7/α^CΔα | 5   | 0.10                  | 3   | 2  | 60.00                       | 4                            |
| -α^3.7/α^WSα | 4   | 0.08                  | 4   | 0  | 100.00                      | 2                            |
| -SEa/αHKαα | 3     | 0.06                  | 2   | 1  | 66.67                       | 2                            |
| α^WSα/α^WSα | 2   | 0.04                  | 2   | 0  | 100.00                      | 2                            |
| -α^4.2/α^WSα | 1   | 0.02                  | 1   | 0  | 100.00                      | 1                            |
| Total      | 4841  | 100                   | 4034| 807 | 83.33                       | 4026                         |

TABLE 1 Distribution genotypes and hematologic data of α-thalassemia patients in Meizhou area
| Genotype                  | Cases | Constituent ratio (%) | MCV     | Proportion of MCV < 82 fl (%) | MCH     | Proportion of MCH < 27 pg (%) |
|--------------------------|-------|-----------------------|---------|--------------------------------|---------|-------------------------------|
|                          |       |                       | MCV < 82 fl |                             | MCV normal |                             | MCH < 27 pg | MCH normal |
| βIVS−II−654/βN           | 898   | 40.14                 | 865     | 33                            | 96.33   | 859                           | 39          | 95.66      |
| βCD41−42/βN              | 743   | 33.21                 | 714     | 29                            | 96.10   | 704                           | 39          | 94.75      |
| βCD17/βN                 | 206   | 9.21                  | 195     | 11                            | 94.66   | 194                           | 12          | 94.17      |
| β−28/βN                  | 204   | 9.12                  | 192     | 12                            | 94.12   | 186                           | 18          | 91.18      |
| βCD27−28/βN              | 39    | 1.74                  | 38      | 1                             | 97.44   | 38                            | 1           | 97.44      |
| βCD26/βN                 | 32    | 1.43                  | 28      | 4                             | 87.50   | 28                            | 4           | 87.50      |
| βCD17−22/βN              | 30    | 1.34                  | 30      |                               | 100.00 | 30                            | 100.00      |            |
| βCap+40−43/βN            | 21    | 0.94                  | 10      | 11                            | 47.62   | 11                            | 10          | 52.38      |
| β−29/βN                  | 12    | 0.54                  | 11      | 1                             | 91.67   | 10                            | 2           | 83.33      |
| βCD14−15/βN              | 11    | 0.49                  | 10      | 1                             | 90.91   | 11                            | 100.00      |            |
| β−28/β−28                | 7     | 0.31                  | 6       | 1                             | 85.71   | 6                             | 1           | 85.71      |
| βCD43/βN                 | 6     | 0.27                  | 6       |                               | 100.00 | 6                             | 100.00      |            |
| βIVS−II−654/βIVS−II−654  | 6     | 0.27                  | 3       | 3                             | 50.00   | 3                             | 3           | 50.00      |
| βCD41−42/βCD26           | 4     | 0.18                  | 4       |                               | 100.00 | 4                             | 100.00      |            |
| βIVS−II−654/βCD26        | 3     | 0.13                  | 2       | 1                             | 66.67   | 3                             | 100.00      |            |
| β−28/βCD27−28            | 2     | 0.09                  | 2       |                               | 100.00 | 2                             | 100.00      |            |
| βCD41−42/β−28            | 2     | 0.09                  | 2       |                               | 100.00 | 1                             | 1           | 50.00      |
| βCD41−42/βCD41−42        | 2     | 0.09                  | 2       |                               | 100.00 | 1                             | 1           | 50.00      |
| βIVS−II−1−1/βN           | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD17/β−28               | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD17/βCD26              | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD41−42/βCap+40−43      | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD17/β−28               | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| β−28/βCap+40−43          | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD41−42/βCD27−28        | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βCD41−42/βIVS−II−654     | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βIVS−II−654/β−28         | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βIVS−II−5βN              | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| βIVS−II−654/β28          | 1     | 0.04                  | 1       |                               | 100.00 | 1                             | 100.00      |            |
| **Total**                | 2237  |                       | 2127    | 110                           | 95.08   | 2103                          | 134         | 94.01      |
### TABLE 3 Distribution genotypes and hematologic data of composite α-thalassemia and β-thalassemia patients in Meizhou area

| Genotype                              | Cases | Constituent ratio (%) | MCV < 82 fl | MCV normal | Proportion of MCV < 82 fl (%) | MCH < 27 pg | MCH normal | Proportion of MCH < 27 pg (%) |
|---------------------------------------|-------|-----------------------|-------------|------------|-------------------------------|-------------|------------|-------------------------------|
| -SEA/αα, β^IVS-II-654/β^N            | 46    | 18.85                 | 43          | 3          | 93.48                         | 41          | 5          | 89.13                         |
| -SEA/αα, β^CD41-42/β^N               | 36    | 14.75                 | 35          | 1          | 97.22                         | 34          | 2          | 94.44                         |
| -SEA/αα, β^-28/β^N                   | 22    | 9.02                  | 19          | 3          | 86.36                         | 20          | 2          | 90.91                         |
| -α^3.7/αα, β^IVS-II-654/β^N          | 21    | 8.61                  | 19          | 2          | 90.48                         | 19          | 2          | 90.48                         |
| -α^3.7/αα, β^CD41-42/β^N             | 18    | 7.38                  | 16          | 2          | 88.89                         | 17          | 1          | 94.44                         |
| -SEA/αα, β^CD17/β^N                  | 11    | 4.51                  | 11          |            | 100.00                        | 11          |            | 100.00                        |
| -SEA/αα, β^CD27-28/β^N               | 8     | 3.28                  | 7           | 1          | 87.50                         | 7           | 1          | 87.50                         |
| -α^2.2/αα, β^CD41-42/β^N             | 8     | 3.28                  | 7           | 1          | 87.50                         | 7           | 1          | 87.50                         |
| α^CS/αα, β^IVS-II-654/β^N            | 6     | 2.46                  | 6           |            | 100.00                        | 6           |            | 100.00                        |
| -α^3.7/αα, β^-28/β^N                 | 5     | 2.05                  | 5           |            | 100.00                        | 5           |            | 100.00                        |
| -α^3.7/αα, β^CD41-42/β^N             | 5     | 2.05                  | 5           |            | 100.00                        | 5           |            | 100.00                        |
| α^CS/αα, β^CD17/β^N                  | 5     | 2.05                  | 5           |            | 100.00                        | 5           |            | 100.00                        |
| -SEA/αα, β^IVS-II-654/β^-28          | 3     | 1.23                  | 3           |            | 100.00                        | 3           |            | 100.00                        |
| -α^3/αα, β^CD26/β^N                  | 3     | 1.23                  | 1           | 2          | 33.33                         | 1           | 2          | 33.33                         |
| α^WS/αα, β^CD17/β^N                  | 3     | 1.23                  | 3           |            | 100.00                        | 3           |            | 100.00                        |
| α^WS/αα, β^CD41-42/β^N               | 3     | 1.23                  | 3           |            | 100.00                        | 3           |            | 100.00                        |
| -SEA/-α^3.7, β^CD41-42/β^N           | 2     | 0.82                  | 2           |            | 100.00                        | 2           |            | 100.00                        |
| -SEA/-α^3.7, β^IVS-II-654/β^-28      | 2     | 0.82                  | 2           |            | 100.00                        | 2           |            | 100.00                        |
| -α^3.7/αα, β^CD17/β^N                | 2     | 0.82                  | 2           |            | 100.00                        | 2           |            | 100.00                        |
| -α^4.2/αα, β^-28/β^N                 | 2     | 0.82                  | 2           |            | 100.00                        | 2           |            | 100.00                        |
| -α^4.2/αα, β^CD17/β^N                | 2     | 0.82                  | 2           |            | 100.00                        | 2           |            | 100.00                        |
| -SEA/β^HKαα, β^IVS-II-654/β^-28      | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/-α^3.7, β^CD41-40-43/β^-28      | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/-α^4.2, β^IVS-II-654/β^-28      | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| α^CS/αα, β^CD17/β^N                  | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -α^CS/αα, β^CD41-42/β^-28            | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/αα, β^IVS-II-654/β^CD27-28      | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/αα, β^CD17/β^-28                | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/αα, β^CD17/β^-28                | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -SEA/αα, β^CD71-72/β^-28             | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -α^3/αα, β^-28/β^-28                 | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -α^3/αα, β^CD17/β^-28                | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -α^3/αα, β^CD17/β^-28                | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |
| -α^3/αα, β^CD41-42/β^IVS-II-654      | 1     | 0.41                  | 1           |            | 100.00                        | 1           |            | 100.00                        |

(Continues)
221 cases (90.57%), 221/244) with MCH < 27 pg, and 215 cases (88.11%, 215/244) with both MCV < 82 fl and MCH < 27 pg.

The results of allele frequencies of α- and β-globin gene mutations were shown in Table 4. There were 5,514 chromosomes carrying α-globin gene mutations, and 7 types of α-globin gene mutations were identified. The most frequent mutation was -SEA, accounting for 64.69%, followed by -αβ-(19.93%), -αβ+(7.73%), αCSα (3.97%), and αWSα (2.83%). There were 2,523 chromosomes carrying β-globin gene mutations, and 13 types of β-globin gene mutations were identified. Of these cases, the six most common β-globin gene mutations were IVS-II-654 (C>T) (39.79%), CD41-42 (-TCTT) (33.02%), -28 (A>G) (10.38%), CD17 (A>T) (9.08%), CD27-28 (+C) (2.14%), and CD26 (G>A) (2.02%), accounting for 96.43%.

Comparison of the allele frequencies of α- and β-thalassemia common mutations in the populations of Meizhou and some regions of Guangdong province (such as Shantou city, Chaohu city, Heyuan city, Zhuhai city, Shunde district in Foshan city, and Nanhai district in Foshan city) and some provinces of southern China (Fujian province, Guangdong province, Guangxi province, Chunqing area, Yunnan province, and Jiangxi province) was performed. For α-thalassemia, Chunqing people had different frequent mutations (descending order was -αβ− (3.7%)) from other Chinese people (including our data) (descending order was -SEα, -αβ−). People in Yunnan province had a higher constituent ratio of αCSα (15.5%) than -αβ+. 6.3% and had different frequent mutations (descending order was -SEα, -αβ−). For β-thalassemia, people in Meizhou area had similar frequent mutations (descending order was IVS-II-654 (C>T), CD41-42 (-TCTT), and -28 (A>G)) with Chinese people in Jiangxi province, Shantou city, and Chaohu city in Guangdong province. Guangxi province (descending order was CD41-42 (-TCTT), CD17 (A>T), CD71-72 (+A)) and Yunnan province (descending order was CD26 (G>A), CD17 (A>T), and CD41-42 (-TCTT)) had different dominant mutation types, in which CD17 (A>T) mutation has more frequency (Table 5).

### Table 3 (Continued)

| Genotype | Cases | Constituent ratio (%) | MCV < 82 fl | MCH normal | Proportion of MCV < 82 fl (%) | MCH < 27 pg | MCH normal | Proportion of MCH < 27 pg (%) |
|----------|-------|-----------------------|-------------|------------|-----------------------------|-------------|------------|-------------------------------|
| -αβ3.7/αa, βCD71-72/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| -α4.2/α4.2, βCD41-42/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| -α2/αa, βCD71-72/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| -α4.2/αa, βCD26/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 0.00 |
| αCSa/αa, β-28/βN | 1 | 0.41 | 1 | | 0.00 | 1 | | 100.00 |
| αCSa/αa, βCD27-28/βN | 1 | 0.41 | 1 | | 0.00 | 1 | | 100.00 |
| αOSa/αa, βCD41-42/β28 | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| αWSα/αa, β3.7, βCD41-42/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| αWSα/αa, βCD27-28/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| αWSα/αa, βIVS-II-654/βN | 1 | 0.41 | 1 | | 100.00 | 1 | | 100.00 |
| Total | 244 | 100 | 225 | 19 | 92.21 | 221 | 23 | 90.57 |

4 | DISCUSSION

Thalassemia is a significant health problem worldwide. In China, it is mainly prevalent in Guangdong, Guangxi, and Hainan province. Meizhou, located in the northeast of Guangdong province, is an underdeveloped city, although certain effects have been achieved through prevention and control; the prevention and treatment of thalassemia in Meizhou is still a difficult task. In broad terms, due to the higher prevalence of thalassemia in southern China, it is necessary to study whether there are differences in Meizhou population and other populations.

Based on the present study, similar to most parts of mainland China, the most common α-thalassemia mutation in Meizhou is -SEA. The high gene frequency of -SEA shows that the health burden resulting from Hb H diseases and Hb Bart’s hydrops fetalis may be severe in these areas. Because, when both parents are carriers (one carries α0-thalassemia deletion (-SEA), one carries α−-thalassemia deletion (-α−), or α0-thalassemia deletion (-SEA)), there is a 25% risk that the fetus will be a Hb H and Hb Bart’s hydrops fetalis patient in every pregnancy, respectively.

In addition, nondeletional α-thalassemia is not rare. αCSα and αWSα are the most prevalent nondeletion type of α-thalassemia in the Meizhou area, with a constituent ratio of 6.8% in α-thalassemia common mutations. Several studies on different populations have suggested that the clinical signs and symptoms of nondeletion Hb H disease (-/α−) are usually more severe than the deletion types (-/-α−). The patient may have greater anemia, jaundice, splenomegaly,
and early anemic symptoms and a higher proportion of patients who require blood transfusion and splenectomy.\(^\text{27,28}\) Therefore, the non-deletion \(\alpha\)-thalassemia should be included in thalassemia prenatal diagnosis.

About \(\beta\)-thalassemia, the present study showed that IVS-II-654 (C>T) was the most common mutation in Meizhou population, followed by CD41-42 (-TCTT), -28 (A>G), and CD17 (A>T). There were 4 patients with homozygous (\(\beta^+/-\beta^+\)) or compound heterozygous (\(\beta^+/-\beta^\theta\)) for \(\beta\) (3 with \(\beta^{IVS-II-654}/\beta^{IVS-II-654}\) and 1 with \(\beta^{CD41-42}/\beta^{IVS-II-654}\) have normal MCV and MCH. It showed that thalassemia intermedia cases encompass a wide phenotypic spectrum from mild anemia to more severe anemia.\(^\text{29}\) The genotype-phenotypic relationship of thalassemia intermedia is so complex that the pathogenesis of some patients remains uncertain. It may due to some genetic modifications linked to the globin gene locus, associated with disease severity, for example, SNPs rs11886868, rs766432, rs4671393, rs7557939, rs6732518, and rs1427407 in BCL11A\(^\text{30-33}\) and co-inherited KLF1 variation.\(^\text{34,35}\)

The genotypes distribution of thalassemia had regional characteristics, and there are some differences in Meizhou population from other populations. The previous results show that there were some differences of the distribution of thalassemia mutations among eight counties in Meizhou city. There were higher genotype frequencies of \(\alpha^{+s}/\alpha\) in Jiaoling county, \(\alpha^{SEA}/\alpha\) in Pingyuan county, \(\alpha^{CS}/\alpha\) in Meixian county, \(\alpha^{SEA}/\alpha^{CS}\) in Dabus county than that in other counties, respectively. There are lower frequencies of \(\alpha^{CS}/\alpha\) in Xingning county and \(\beta^{28}/\beta^N\) in Dabus county than that in other counties.\(^\text{36}\) It also indicates that the frequency distribution of thalassemia gene mutations is population and geographically diverse.

At present, the intervention mode to prevent and control thalassemia in China is a three-level prevention strategy. Primary prevention is a measure with the highest prevention efficiency to reduce the occurrence of congenital thalassemia disabilities, through comprehensive interventions such as health education, genetic screening, and genetic counseling before pregnancy and in the early stage of pregnancy. Secondary prevention of thalassemia birth defects is to identify the fetus’s congenital disabilities through pre-pregnancy screening and prenatal diagnosis, and try to achieve early detection and early intervention, to reduce the birth of fetuses with thalassemia major. Tertiary prevention of birth defects caused by thalassemia disabilities is the treatment of children diagnosed as thalassemia intermedia or major at an early stage.\(^\text{37,38}\) Thalassemia is a kind of genetic disease, and there is no good cure at present, mainly rely on prevention. At present, the way to prevent thalassemia major is prenatal diagnosis and birth defects intervention.

The number of children born with thalassemia major has been significantly reduced through prevention and control measures, but some challenges remain. First, with the rapid development of economy, the population migration occurs more and more frequently. Migrants from the thalassemia prevalent areas to non-prevalent areas bring challenges to the prevention and control of thalassemia in non-prevalent areas where there is no perfect prenatal diagnosis system. Second, common thalassemia mutations do not fully explain the phenotype. The genotype-phenotypic relationships of some thalassemia types are complex and may be related to some genetic modifications linked to globin gene loci. These mechanisms are not fully understood.

There are some future prospects in the prevention and control of thalassemia. First, the establishment of a rapid, high-throughput, low-cost, and covering more mutations DNA-based assay is essential for clinical diagnosis and mass screening in thalassemia-prevention programs. Up to now, it is not possible to use a single technique to completely meet the needs of detection of thalassemia mutations. In recent years, some scholars have also carried out research on this aspect, such as asymmetric PCR melting curve analysis\(^\text{39}\) and next-generation sequencing (NGS).\(^\text{40}\) Second, in addition to genetic testing, for some thalassemia types with complex genotype-phenotypic relationships (such as thalassemia intermedia), clinical diagnosis and treatment require a comprehensive scoring system to assess disease severity. Cappellini et al.\(^\text{41}\) have developed a new scoring system for non-transfusion-dependent thalassemia patients to assess disease severity and thus tailor therapy. While the scoring system is validated, it should be promising.

### TABLE 4 Allele frequencies of \(\alpha\)- and \(\beta\)-thalassemia mutations in the Meizhou area

| Allele                  | Constituent ratio (%) |
|-------------------------|-----------------------|
| \(\alpha\)-thalassemia  |                       |
| \(\alpha^{SEA}\)        | 3567                  | 64.69                  |
| \(\alpha^{2.7}\)        | 1099                  | 19.93                  |
| \(\alpha^{4.2}\)        | 426                   | 7.73                   |
| \(\alpha^{CS}\)         | 219                   | 3.97                   |
| \(\alpha^{W5}\)         | 156                   | 2.83                   |
| \(\alpha^{OS}\)         | 43                    | 0.78                   |
| HK\(\alpha\)            | 4                     | 0.07                   |
| Total                   | 5514                  | 100                    |
| \(\beta\)-thalassemia   |                       |
| IVS-II-654 (C>T)        | 1004                  | 39.79                  |
| VCD41-42 (-TCTT)        | 833                   | 33.02                  |
| -28 (A>G)               | 262                   | 10.38                  |
| CD17 (A>T)              | 229                   | 9.08                   |
| CD27-28 (+C)            | 54                    | 2.14                   |
| CD26 (G>A)              | 51                    | 2.02                   |
| CD71-72 (+A)            | 33                    | 1.31                   |
| CAP +40-43 (AAAC)       | 26                    | 1.03                   |
| -29 (A>G)               | 12                    | 0.48                   |
| CD14-15 (+G)            | 11                    | 0.44                   |
| CD43 (G>T)              | 6                     | 0.24                   |
| IVS-I-1                 | 1                     | 0.04                   |
| IVS-I-5                 | 1                     | 0.04                   |
| Total                   | 2523                  | 100                    |
### Table 5
Comparison of the allele constituent ratios of α- and β-thalassemia common mutations in the populations of Meizhou, some regions of Guangdong province and some provinces of southern China

| Area                  | First Mutation | Second Mutation | Third Mutation | Fourth Mutation | Fifth Mutation | Others |
|-----------------------|----------------|-----------------|----------------|----------------|----------------|--------|
|                       | Mutation %     | Mutation %      | Mutation %     | Mutation %     | Mutation %     |        |
| α-thalassemia         |                |                 |                |                |                |        |
| Our data              | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Fujian province       | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Guangdong province    | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Guangxi province      | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Chongqing area        | -α<sup>3.7</sup> | -SEA           | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Yunnan province       | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Jiangxi province      | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Shantou city          | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Chaohua city          | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Shaoguan city         | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Heyuan city           | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Zhubai city           | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Shenzhen city         | -SEA           | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Shunde district, Foshan city | -SEA     | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Nanhai district, Foshan city | -SEA     | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| Meta-analysis conducted by Lai et al. | -SEA         | α<sup>3.7</sup> | α<sup>4.2</sup> | α<sup>CS</sup> | α<sup>WS</sup> |        |
| β-thalassemia         |                |                 |                |                |                |        |
| Our data              | IVS-II-654 (C>T) | 39.8             | CD41-42 (-TCTT) | 33.0           | -28 (A>G) | 10.4        | CD17 (A>T) | 9.1  | CD27-28 (+C) | 2.1  | 5.6  |
| Fujian province       | IVS-II-654 (C>T) | 43.9             | CD41-42 (-TCTT) | 27.0           | CD17 (A>T) | 8.1         | CD71-72 (+A) | 6.8  | CD26 (G>A) | 1.4  | 12.8 |
| Guangdong province    | CD41-42 (-TCTT) | 39.2             | IVS-II-654 (C>T) | 26.0           | CD71-72 (+A) | 14.2        | CD17 (A>T) | 8.2  | CD26 (G>A) | 2.6  | 9.8  |
| Guangxi province      | CD41-42 (-TCTT) | 42.3             | CD17 (A>T) | 28.1           | CD71-72 (+A) | 7.7         | IVS-II-654 (C>T) | 6.6  | -28 (A>G) | 6.4  | 8.9  |
| Chongqing area        | CD41-42 (-TCTT) | 46.7             | IVS-II-654 (C>T) | 20.0           | CD17 (A>T) | 11.1        | CD26 (G>A) | 11.1 | -29 (A>G) | 8.9  | 2.2  |
| Yunnan province       | CD26 (G>A) | 30.5             | CD17 (A>T) | 20.8           | CD41-42 (-TCTT) | 17.5        | IVS-II-654 (C>T) | 17.2 | -28 (A>G) | 6.9  | 7.1  |
| Jiangxi province      | IVS-II-654 (C>T) | 39.1             | CD41-42 (-TCTT) | 30.4           | -28 (A>G) | 18.3        | CD17 (A>T) | 4.3  | CD27-28 (+C) | 4.3  | 3.6  |
| Shantou city          | IVS-II-654 (C>T) | 46.7             | CD41-42 (-TCTT) | 20.0           | -28 (A>G) | 13.3        | CD17 (A>T) | 13.3 | Cap +1 (A>C) | 6.7  | -    |
| Chaohua city          | IVS-II-654 (C>T) | 36.8             | CD41-42 (-TCTT) | 34.2           | -28 (A>G) | 13.2        | CD26 (G>A) | 7.9  | CD17 (A>T) | 5.3  | 2.6  |
| Shaoguan city         | CD41-42 (-TCTT) | 47.3             | IVS-II-654 (C>T) | 16.4           | -28 (A>G) | 12.7        | CD43 (G>T) | 9.1  | CD71-72 (+A) | 3.6  | 10.9 |

(Continues)
Epidemiological data regarding the occurrence and distribution of thalassemia are important for designing appropriate prevention strategies. In conclusion, $\alpha^{3.7}$, $\alpha^{4.2}$ deletions were the main mutations of $\alpha$-thalassemia, while IVS-II-654 (C>T), CD41-42 (-TCTT), $\sim$28 (A>G), and CD17 (A>T) mutations were the principal mutations of $\beta$-thalassemia in Meizhou area. There were some differences in thalassemia mutation frequencies in Meizhou city from some populations in China. Local governments can formulate corresponding measures and detection projects to prevent and control thalassemia major according to the genotype distributions, effectively saving costs and enhancing social benefits.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
Zhixiong Zhong and Heming Wu designed the study. Heming Wu and Qingyan Huang collected clinical data. Heming Wu and Zhikang Yu analyzed the data. Heming Wu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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