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RESEARCH ARTICLE

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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 $-\alpha^{3.7}$ (19.93%), $-\alpha^{4.2}$ (7.73%), $\alpha^{CS}\alpha$ (3.97%), and $\alpha^{WS}\alpha$ (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 $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, $\alpha^{CS}\alpha/\alpha\alpha$, $\alpha^{WS}\alpha/\alpha\alpha$, and $\beta^{Cap+40-43}/\beta^{N}$.

Conclusions: The $--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$ 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

Heming Wu and Qingyan Huang Contributed equally to this work.

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.[1,2] It is prevalent in tropical and subtropical areas, such as Mediterranean countries, Africa, Middle East, Indian subcontinent, and Southeast Asia, including southern China.[3,4] 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.[5] 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.[6] 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.[7] 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 (Hybribio Limited) were used to detect the deletion α-thalassemia mutations (--^{SEA}, -$\alpha^{3.7}$, and -$\alpha^{4.2}$) and nondeletion 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), −28 (A>G), −29 (A>G), CD71-72 (+A), CD26 (G>A), IVS-I-1 (G>T), IVS-I-1 (G>A), CD27-28 (+C), IVS-I-5 (G>C), Cap+40–43 (-AAAC), 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 (8,173 with HbA_2 < 2.5%, 2,360 with HbA_2 > 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 $--^{SEA}/\alpha\alpha$ (62.78%), $-\alpha^{3.7}/\alpha\alpha$ (16.24%), and $-\alpha^{4.2}/\alpha\alpha$ (6.40%), accounted for 85.42%. Furthermore, several cases were identified to carrying the rare α-thalassemia mutations, such as 3 cases with carrying $--^{SEA}/HK\alpha\alpha$ 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 $--^{SEA}/\alpha\alpha$, 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 $-\alpha^{3.7}/\alpha\alpha$, 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 $-\alpha^{4.2}/\alpha\alpha$, $\alpha^{CS}\alpha/\alpha\alpha$, and $\alpha^{WS}\alpha/\alpha\alpha$ genotypes. MCV and MCH were sensitive markers for α-thalassemia except for $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, $\alpha^{CS}\alpha/\alpha\alpha$, and $\alpha^{WS}\alpha/\alpha\alpha$.

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 $\beta^{IVS-II-654}/\beta^{N}$ (40.14%), $\beta^{CD41-42}/\beta^{N}$ (33.21%), β^{CD17}/β^{N} (9.21%), and β^{-28}/β^{N} (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 $\beta^{IVS-II-654}/\beta^{N}$, 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 $\beta^{CD41-42}/\beta^{N}$, β^{CD17}/β^{N}, β^{-28}/β^{N}, $\beta^{CD27-28}/\beta^{N}$, $\beta^{CD71-72}/\beta^{N}$, and $\beta^{CD14-15}/\beta^{N}$ genotypes (all abnormal proportions >90%). Among the patients with $\beta^{Cap+40-43}/\beta^{N}$, the proportions of the patients with the normal MCV and MCH values were 52.38 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 $--^{SEA}/\alpha\alpha$ combined with $\beta^{IVS-II-654}/\beta^{N}$ (18.85%), $--^{SEA}/\alpha\alpha$ combined with $\beta^{CD41-42}/\beta^{N}$ (14.75%), $--^{SEA}/\alpha\alpha$ combined with β^{-28}/β^{N} (9.02%), $-\alpha^{3.7}/\alpha\alpha$ combined with $\beta^{IVS-II-654}/\beta^{N}$ (8.61%), and $-\alpha^{3.7}/\alpha\alpha$ combined with $\beta^{CD41-42}/\beta^{N}$ (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,

TABLE 1 Distribution genotypes and hematologic data of α-thalassemia patients in Meizhou area

			MCV			MCH		
Genotype	Cases	Constituent ratio (%)	MCV < 82 fl	MCV normal	Proportion of MCV <82 fl (%)	MCH < 27 pg	MCH normal	Proportion of MCH < 27 pg (%)
$--^{SEA}/\alpha\alpha$	3039	62.78	2910	129	95.76	2898	141	95.36
$-\alpha^{3.7}/\alpha\alpha$	786	16.24	387	399	49.24	390	396	49.62
$-\alpha^{4.2}/\alpha\alpha$	310	6.40	179	131	57.74	168	142	54.19
$--^{SEA}/-\alpha^{3.7}$	209	4.32	204	5	97.61	206	3	98.56
$\alpha^{CS}\alpha/\alpha\alpha$	132	2.73	76	56	57.58	72	60	54.55
$\alpha^{WS}\alpha/\alpha\alpha$	120	2.48	62	58	51.67	62	58	51.67
$--^{SEA}/-\alpha^{4.2}$	82	1.69	79	3	96.34	79	3	96.34
$--^{SEA}/\alpha^{CS}\alpha$	66	1.36	49	17	74.24	63	3	95.45
$\alpha^{QS}\alpha/\alpha\alpha$	37	0.76	33	4	89.19	35	2	94.59
$--^{SEA}/\alpha^{WS}\alpha$	17	0.35	15	2	88.24	14	3	82.35
$-\alpha^{3.7}/-\alpha^{3.7}$	12	0.25	12		100.00	12		100.00
$-\alpha^{3.7}/-\alpha^{4.2}$	11	0.23	11		100.00	11		100.00
$--^{SEA}/\alpha^{QS}\alpha$	5	0.10	5		100.00	5		100.00
$-\alpha^{3.7}/\alpha^{CS}\alpha$	5	0.10	3	2	60.00	4	1	80.00
$-\alpha^{3.7}/\alpha^{WS}\alpha$	4	0.08	4		100.00	2	2	50.00
$--^{SEA}/HK\alpha\alpha$	3	0.06	2	1	66.67	2	1	66.67
$\alpha^{WS}\alpha/\alpha^{WS}\alpha$	2	0.04	2		100.00	2		100.00
$-\alpha^{4.2}/\alpha^{WS}\alpha$	1	0.02	1		100.00	1		100.00
Total	4841	100	4034	807	83.33	4026	815	83.16

TABLE 2 Distribution genotypes and hematologic data of β-thalassemia patients in Meizhou area

			MCV			MCH		
Genotype	Cases	Constituent ratio (%)	MCV < 82 fl	MCV normal	Proportion of MCV < 82 fl (%)	MCH < 27 pg	MCH normal	Proportion of MCH < 27 pg (%)
$\beta^{IVS-II-654}/\beta^{N}$	898	40.14	865	33	96.33	859	39	95.66
$\beta^{CD41-42}/\beta^{N}$	743	33.21	714	29	96.10	704	39	94.75
β^{CD17}/β^{N}	206	9.21	195	11	94.66	194	12	94.17
β^{-28}/β^{N}	204	9.12	192	12	94.12	186	18	91.18
$\beta^{CD27-28}/\beta^{N}$	39	1.74	38	1	97.44	38	1	97.44
β^{CD26}/β^{N}	32	1.43	28	4	87.50	28	4	87.50
$\beta^{CD71-72}/\beta^{N}$	30	1.34	30		100.00	30		100.00
$\beta^{Cap+40-43}/\beta^{N}$	21	0.94	10	11	47.62	11	10	52.38
β^{-29}/β^{N}	12	0.54	11	1	91.67	10	2	83.33
$\beta^{CD14-15}/\beta^{N}$	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
β^{CD43}/β^{N}	6	0.27	6		100.00	6		100.00
$\beta^{IVS-II-654}/\beta^{IVS-II-654}$	6	0.27	3	3	50.00	3	3	50.00
$\beta^{CD41-42}/\beta^{CD26}$	4	0.18	4		100.00	4		100.00
$\beta^{IVS-II-654}/\beta^{CD26}$	3	0.13	2	1	66.67	3		100.00
$\beta^{-28}/\beta^{CD27-28}$	2	0.09	2		100.00	2		100.00
$\beta^{CD41-42}/\beta^{-28}$	2	0.09	2		100.00	1	1	50.00
$\beta^{CD41-42}/\beta^{CD41-42}$	2	0.09	2		100.00	1	1	50.00
$\beta^{IVS-I-1}/\beta^{N}$	1	0.04	1		100.00	1		100.00
$\beta^{-28}/\beta^{Cap+40-43}$	1	0.04	1		100.00	1		100.00
β^{CD17}/β^{-28}	1	0.04	1		100.00	1		100.00
$\beta^{CD17}/\beta^{CD26}$	1	0.04	1		100.00	1		100.00
$\beta^{CD41-42}/\beta^{Cap+40-43}$	1	0.04	1		100.00	1		100.00
$\beta^{CD41-42}/\beta^{CD27-28}$	1	0.04	1		100.00	1		100.00
$\beta^{CD41-42}/\beta^{IVS-II-654}$	1	0.04		1	-		1	-
$\beta^{IVS-I-5}/\beta^{N}$	1	0.04		1	-		1	-
$\beta^{IVS-II-654}/\beta^{-28}$	1	0.04	1		100.00		1	-
Total	2237	100	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

			MCV			MCH		
Genotype	Cases	Constituent ratio (%)	MCV < 82 fl	MCV normal	Proportion of MCV < 82 fl (%)	MCH < 27 pg	MCH normal	Proportion of MCH < 27 pg (%)
$--^{SEA}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	46	18.85	43	3	93.48	41	5	89.13
$--^{SEA}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	36	14.75	35	1	97.22	34	2	94.44
$--^{SEA}/\alpha\alpha$, β^{-28}/β^{N}	22	9.02	19	3	86.36	20	2	90.91
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	21	8.61	19	2	90.48	19	2	90.48
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	18	7.38	16	2	88.89	17	1	94.44
$--^{SEA}/\alpha\alpha$, β^{CD17}/β^{N}	11	4.51	11		100.00	11		100.00
$--^{SEA}/\alpha\alpha$, $\beta^{CD27-28}/\beta^{N}$	8	3.28	7	1	87.50	7	1	87.50
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	8	3.28	7	1	87.50	7	1	87.50
$--^{SEA}/\alpha\alpha$, β^{CD26}/β^{N}	6	2.46	6		100.00	6		100.00
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	6	2.46	6		100.00	6		100.00
$-\alpha^{3.7}/\alpha\alpha$, β^{-28}/β^{N}	5	2.05	5		100.00	5		100.00
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	5	2.05	5		100.00	5		100.00
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	5	2.05	5		100.00	5		100.00
$--^{SEA}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{-28}$	3	1.23	3		100.00	3		100.00
$-\alpha^{3.7}/\alpha\alpha$, β^{CD26}/β^{N}	3	1.23	1	2	33.33	1	2	33.33
$\alpha^{WS}\alpha/\alpha\alpha$, β^{CD17}/β^{N}	3	1.23	3		100.00	3		100.00
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	3	1.23	3		100.00	3		100.00
$--^{SEA}/-\alpha^{3.7}$, $\beta^{CD41-42}/\beta^{N}$	2	0.82	2		100.00	2		100.00
$--^{SEA}/-\alpha^{3.7}$, $\beta^{IVS-II-654}/\beta^{N}$	2	0.82	2		100.00	2		100.00
$-\alpha^{3.7}/\alpha\alpha$, β^{CD17}/β^{N}	2	0.82	2		100.00	2		100.00
$-\alpha^{4.2}/\alpha\alpha$, β^{-28}/β^{N}	2	0.82	2		100.00	1	1	50.00
$-\alpha^{4.2}/\alpha\alpha$, β^{CD17}/β^{N}	2	0.82	2		100.00	2		100.00
$--^{SEA}/HK\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/-\alpha^{3.7}$, $\beta^{Cap+40-43}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/-\alpha^{4.2}$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha^{CS}\alpha$, β^{CD17}/β^{N}	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha^{CS}\alpha$, $\beta^{CD41-42}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha^{CS}\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{CD27-28}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha\alpha$, $\beta^{Cap+40-43}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$--^{SEA}/\alpha\alpha$, β^{CD17}/β^{-28}	1	0.41	1		100.00		1	0.00
$--^{SEA}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	1	0.41		1	0.00		1	0.00
$-\alpha^{3.7}/\alpha\alpha$, β^{-28}/β^{-28}	1	0.41	1		100.00	1		100.00
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{Cap+40-43}/\beta^{N}$	1	0.41		1	0.00		1	0.00
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD17}/\beta^{CD27-28}$	1	0.41	1		100.00		1	0.00
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{IVS-II-654}$	1	0.41	1		100.00	1		100.00

(Continues)

TABLE 3 (Continued)

Genotype	Cases	Constituent ratio (%)	MCV			MCH		
			MCV < 82 fl	MCV normal	Proportion of MCV < 82 fl (%)	MCH < 27 pg	MCH normal	Proportion of MCH < 27 pg (%)
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$-\alpha^{4.2}/-\alpha^{4.2}$, $\beta^{CD41-42}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$-\alpha^{4.2}/\alpha\alpha$, β^{CD26}/β^{N}	1	0.41	1		100.00		1	0.00
$\alpha^{CS}\alpha/\alpha\alpha$, β^{-28}/β^{N}	1	0.41		1	0.00	1		100.00
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{CD27-28}/\beta^{N}$	1	0.41		1	0.00	1		100.00
$\alpha^{QS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{-28}$	1	0.41	1		100.00	1		100.00
$\alpha^{WS}\alpha/-\alpha^{3.7}$, $\beta^{CD41-42}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD27-28}/\beta^{N}$	1	0.41	1		100.00	1		100.00
$\alpha^{WS}\alpha/\alpha\alpha$, β^{CD26}/β^{N}	1	0.41	1		100.00	1		100.00
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.41	1		100.00		1	0.00
Total	244	100	225	19	92.21	221	23	90.57

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 $-\alpha^{3.7}$ (19.93%), $-\alpha^{4.2}$ (7.73%), $\alpha^{CS}\alpha$ (3.97%), and $\alpha^{WS}\alpha$ (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,[13] Chaozhou city,[13] Shaoguan city,[14] Heyuan city,[15] Zhuhai city,[16,17] Shenzhen city,[18] Shunde district in Foshan city,[19] and Nanhai district in Foshan city[20]) and some provinces of southern China (Fujian province,[21] Guangdong province,[7,22] Guangxi province,[6] Chongqing area,[23] Yunnan province,[24] and Jiangxi province[25]) was performed. For α-thalassemia, Chongqing people had different frequent mutations (descending order was $-\alpha^{3.7}$, $--^{SEA}$, $-\alpha^{4.2}$) from other Chinese people (including our data) (descending order was $--^{SEA}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$). People in Yunnan province had a higher constituent ratio of $\alpha^{CS}\alpha$ (15.5%) than $-\alpha^{4.2}$ (6.3%) and had different frequent mutations (descending order was $--^{SEA}$, $-\alpha^{3.7}$, $\alpha^{CS}\alpha$, $-\alpha^{4.2}$). 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 Chaozhou 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).

4 | DISCUSSION

Thalassemia is a significant health problem worldwide. In China, it is mainly prevalent in Guangdong, Guangxi, and Hainan province.[5] 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 α^{0}-thalassemia deletion ($--^{SEA}$), one carries α^{+}-thalassemia deletion (-α/), or α^{0}-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.[26]

In addition, nondeletional α-thalassemia is not rare. $\alpha^{CS}\alpha$ and $\alpha^{WS}\alpha$ 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 ($-/\alpha^{T}\alpha$) are usually more severe than the deletion types (--/-α). The patient may have greater anemia, jaundice, splenomegaly,

TABLE 4 Allele frequencies of α- and β-thalassemia mutations in the Meizhou area

	Allele	Constituent ratio (%)
α-thalassemia		
$--^{SEA}$	3567	64.69
$-\alpha^{3.7}$	1099	19.93
$-\alpha^{4.2}$	426	7.73
$\alpha^{CS}\alpha$	219	3.97
$\alpha^{WS}\alpha$	156	2.83
$\alpha^{QS}\alpha$	43	0.78
HKαα	4	0.07
Total	5514	100
β-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

and early anemic symptoms and a higher proportion of patients who require blood transfusion and splenectomy.[27,28] Therefore, the nondeletion α-thalassemia should be included in thalassemia prenatal diagnosis.

About β-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 (β^+/β^+) or compound heterozygous (β^+/β^0) for β (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[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*[30–33] and co-inherited *KLF1* variation.[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 $-\alpha^{4.2}/\alpha\alpha$ in Jiaoling county, $--^{SEA}/-\alpha^{3.7}$ and $\beta^{IVS-II-654}/\beta^N$ in Pingyuan county, $\alpha^{CS}\alpha/\alpha\alpha$ in Meixian county, $--^{SEA}/\alpha^{CS}\alpha$ in Dabu county than that in other counties, respectively. There are lower frequencies of $\alpha^{CS}\alpha/\alpha\alpha$ in Xingning county and β^{-28}/β^N in Dabu county than that in other counties.[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 prepregnancy 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.[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 nonprevalent areas bring challenges to the prevention and control of thalassemia in nonprevalent 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[39] and next-generation sequencing (NGS).[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.[41] have developed a new scoring system for nontransfusion-dependent thalassemia patients to assess disease severity and thus tailor therapy. While the scoring system is validated, it should be promising.

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

	First		Second		Third		Fourth		Fifth		Others
Area	Mutation	%	Mutation	%	Mutation	%	Mutation	%	Mutation	%	%
α-thalassemia											
Our data	$--^{SEA}$	64.7	$-\alpha^{3.7}$	19.9	$-\alpha^{4.2}$	7.7	$\alpha^{CS}\alpha$	4.0	$\alpha^{WS}\alpha$	2.8	0.9
Fujian province	$--^{SEA}$	66.1	$-\alpha^{3.7}$	20.7	$-\alpha^{4.2}$	7.2	$\alpha^{QS}\alpha$	3.3	$\alpha^{CS}\alpha$	1.4	1.3
Guangdong province	$--^{SEA}$	51.5	$-\alpha^{3.7}$	28.4	$-\alpha^{4.2}$	9.5	$\alpha^{WS}\alpha$	6.5	$\alpha^{CS}\alpha$	2.6	1.5
Guangxi province	$--^{SEA}$	45.5	$-\alpha^{3.7}$	25.4	$-\alpha^{4.2}$	11.3	$\alpha^{CS}\alpha$	7.9	$\alpha^{QS}\alpha$	5.6	4.3
Chongqing area	$-\alpha^{3.7}$	53.6	$--^{SEA}$	17.9	$-\alpha^{4.2}$	8.9	$\alpha^{QS}\alpha$	1.8	-	-	-
Yunnan province	$--^{SEA}$	59.2	$-\alpha^{3.7}$	19.0	$\alpha^{CS}\alpha$	15.5	$-\alpha^{4.2}$	6.3	-	-	-
Jiangxi province	$--^{SEA}$	61.8	$-\alpha^{3.7}$	41.9	$-\alpha^{4.2}$	9.7	$\alpha^{QS}\alpha$	1.4	$\alpha^{CS}\alpha$	1.1	-
Shantou city	$--^{SEA}$	52.7	$-\alpha^{3.7}$	30.9	$-\alpha^{4.2}$	9.1	$\alpha^{CS}\alpha$	3.6	$\alpha^{QS}\alpha$	3.6	-
Chaozhou city	$--^{SEA}$	58.7	$-\alpha^{3.7}$	22.0	$-\alpha^{4.2}$	14.8	$\alpha^{CS}\alpha$	3.1	$\alpha^{QS}\alpha$	1.4	-
Shaoguan city	$--^{SEA}$	51.4	$-\alpha^{3.7}$	41.0	$-\alpha^{4.2}$	4.8	$\alpha^{CS}\alpha$	2.9	-	-	-
Heyuan city	$--^{SEA}$	78.8	$-\alpha^{3.7}$	13.5	$-\alpha^{4.2}$	7.7	-	-	-	-	-
Zhuhai city	$--^{SEA}$	50.0	$-\alpha^{3.7}$	29.7	$-\alpha^{4.2}$	10.9	$\alpha^{CS}\alpha$	2.9	$\alpha^{QS}\alpha$	1.4	5.1
Shenzhen city	$--^{SEA}$	75.7	$-\alpha^{3.7}$	17.5	$-\alpha^{4.2}$	5.6	$\alpha^{CS}\alpha$	1.1	-	-	-
Shunde district, Foshan city	$--^{SEA}$	50.5	$-\alpha^{3.7}$	44.1	$-\alpha^{4.2}$	4.3	$\alpha^{CS}\alpha$	1.1	-	-	-
Nanhai district, Foshan city	$--^{SEA}$	77.6	$-\alpha^{3.7}$	15.0	$-\alpha^{4.2}$	7.4	-	-	-	-	-
Meta-analysis conducted by Lai et al.	$--^{SEA}$	51.0	$-\alpha^{3.7}$	27.5	$-\alpha^{4.2}$	9.5	$\alpha^{WS}\alpha$	6.1	$\alpha^{CS}\alpha$	4.3	-
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	-
Chaozhou 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)

TABLE 5 (Continued)

Area	First		Second		Third		Fourth		Fifth		Others
	Mutation	%	Mutation	%	Mutation	%	Mutation	%	Mutation	%	%
Heyuan city	CD41-42 (-TCTT)	47.9	IVS-II-654 (C>T)	33.9	CD14-15 (+G)	7.0	−28 (A>G)	5.6	CD17 (A>T)	2.8	2.8
Zhuhai city	CD41-42 (-TCTT)	41.0	−28 (A>G)	17.4	IVS-II-654 (C>T)	17.0	CD17 (A>T)	9.0	CD71-72 (+A)	4.7	10.9
Shenzhen city	CD41-42 (-TCTT)	41.3	IVS-II-654 (C>T)	27.5	−28 (A>G)	13.8	CD17 (A>T)	8.8	CD71-72 (+A)	2.5	6.3
Shunde district, Foshan city	CD41-42 (-TCTT)	39.9	IVS-II-654 (C>T)	21.0	CD17 (A>T)	19.4	−28 (A>G)	12.9	CD26 (G>A)	5.0	1.8
Nanhai district, Foshan city	CD41-42 (-TCTT)	38.2	IVS-II-654 (C>T)	22.8	−28 (A>G)	15.1	CD17 (A>T)	10.2	CD43 (G>T)	4.7	9.0
Meta-analysis conducted by Lai et al.	CD41-42 (-TCTT)	38.7	IVS-II-654 (C>T)	18.6	CD17 (A>T)	16.1	−28 (A>G)	10.9	CD71-72 (+A)	3.5	12.2

Epidemiological data regarding the occurrence and distribution of thalassemia are important for designing appropriate prevention strategies. In conclusion, $--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$ 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 were the principal mutations of β-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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