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Original Article

Genotype analysis of 55,281 cases of thalassemia in northern Guangxi

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Abstract: Objective: To understand the genotype and distribution of thalassemia in northern Guangxi. Methods: The study subjects were 55,281 individuals who came to the Affiliated Hospital of Guilin Medical University for genetic diagnosis of thalassemia from January 2012 to August 2023. All of their household registration was in the precincts of Guibei District and its affiliated counties. Red blood cell parameters and hemoglobin analysis were used for thalassemia screening. Gap-PCR, PCR-reverse dot blot hybridization (PCR-RDB), and multicolor melting curve analysis (MMCA) were used to identify common thalassemia genes. Multiplex ligation-dependent probe amplification (MLPA), Sanger sequencing, and third-generation single-molecule real-time (SMRT) sequencing were employed to identify rare thalassemia genes. Results: Among the 55,281 samples, 16,442 (29.74%) were diagnosed with thalassemia. The detection rates of α, β, and α combined β-thalassemia were 18.57%, 9.99% and 1.18%, respectively. Among ethnical groups, allele mutation frequency of thalassemia was the highest in Zhuang (44.97%), followed by Yao (40.11%), Dong (31.33%), Han (29.85%), Miao (24.31%), and Hui (20.6%). A total of 11,659 alleles (21.09%) of 8 types of α-thalassemia were identified in 55,281 samples, primarily $--^{SEA}$ (53.9%), followed by $-\alpha^{3.7}$ (21.3%), including rare alleles: $--^{THAI}$ (0.45%) and HKαα (0.38%). A total of 6367 (11.52%) and 14 types of β-thalassemia alleles were identified, mainly CD41-42 (50.12%), followed by CD17 (22.22%), including rare alleles: β^{CD37} (0.16%) and $G\gamma^{+}(A\gamma\delta\beta)^{0}/\beta^{N}$ (0.05%). A total of 31 genotypes were detected in 10,264 cases of α-thalassemia, and the main types were $--^{SEA}/\alpha\alpha$ (53.23%), $-\alpha^{3.7}/\alpha\alpha$ (19.15%), and $-\alpha^{4.2}/\alpha\alpha$ (7.21%). A total of 34 genotypes were detected in 5525 cases of β-thalassemia, and the main types were $\beta^{CD41-42}/\beta^{N}$ (50.53%), β^{CD17}/β^{N} (21.77%), and $\beta^{IVS-II-654}/\beta^{N}$ (12.16%). A total of 78 gene types were detected in 653 cases of α- and β-thalassemia, and the main types were $--^{SEA}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$ (18.68%) and $-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$ (13.02%). There were 580 cases (5.65%) of HbH disease (α^{0}/α^{+}), and 4 cases of Hemoglobin Bart's Hydrops Foetus syndrome ($--^{SEA}/--^{SEA}$). In addition, there were 92 cases (1.67%) of intermedia or severe types of β-thalassemia (β^{0}/β^{0}, β^{0}/β^{+}, β^{+}/β^{+}), including 23 cases of combined α-thalassemia. Among the samples screened negative for thalassemia, 3.7% of them were found to carry thalassemia genes, and 91.35% of the genotypes were $\alpha^{WS}\alpha/\alpha\alpha$, $-\alpha^{3.7}/\alpha\alpha$, and $-\alpha^{4.2}/\alpha\alpha$. In addition, 40.26% of $\alpha^{WS}\alpha/\alpha\alpha$, 22.89% of $-\alpha^{3.7}/\alpha\alpha$, and 18.51% of $-\alpha^{4.2}/\alpha\alpha$ had no hematological phenotype. Conclusion: The population in northern Guangxi exhibited rich ethnic diversity, with high allelic carrying rates among the Zhuang, Yao and Dong ethnic groups. Thalassemia gene mutations are diverse, encompassing a variety of gene types, with α thalassemia predominating, notably the $--^{SEA}/\alpha\alpha$ gene type. The prevalence of intermedia or severe types of thalassemia is not low, but there are still some carriers of thalassemia in people who are initially tested negative.

Keywords: Thalassemia, genotype, minority, phenotype

Introduction

Southern China has a high incidence of thalassemia [1]. In different regions, the carrier rate of thalassemia in the Chinese population varies from 1% to 24% [2]. In southern provinces such as Guangdong, Guangxi, and Hainan, the incidence of thalassemia is high [3-7], and there are also differences in the frequency and distribution of thalassemia gene mutations

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Table 1. Distribution of α-thalassemia alleles

Allele distribution	Phenotype	n	Constituent ratio	Frequency
$--^{SEA}$	α^0	6285	53.91%	11.37%
$-\alpha^{3.7}$	α^+	2484	21.31%	4.49%
$-\alpha^{4.2}$	α^+	993	8.52%	1.80%
$\alpha^{CS}\alpha$	α^+	942	8.08%	1.70%
$\alpha^{WS}\alpha$	α^+	583	5.00%	1.05%
$\alpha^{QS}\alpha$	α^+	275	2.36%	0.50%
$--^{THAI}$	α^0	53	0.45%	0.10%
Hkaa	α^+	44	0.38%	0.08%
Total		11659	100.00%	21.09%

among different ethnic groups [8-10]. In the northern part of Guibei, renowned for its tourist cities in China, frequent population movements and intermarriages among various ethnic groups may have induced significant changes in the molecular epidemiological profile of thalassemia in the region, leading to a heightened prevalence rate [11]. Also, due to the limitations of medical conditions and the level of health education, many patients are unable to receive timely diagnosis and treatment, exacerbating the spread of the disease [12]. Since thalassemia is a genetic disorder, it is critical to provide accurate genetic counseling and prenatal diagnosis. However, the medical resources in northern Guangxi are relatively limited, so many patients and families do not have access to this information and services [13]. Thus, it is particularly important to explore the genotypes and distribution characteristics of thalassemia. Through the investigation and analysis of the genotype of local residents, we can better understand the type and distribution characteristics of thalassemia and provide a scientific basis for the development of targeted prevention and control measures. Therefore, this study conducted a retrospective analysis of 55,281 individuals who came to the Affiliated Hospital of Guilin Medical University for genetic diagnosis of thalassemia from January 1, 2012, to August 31, 2023, to fully understand the genotype and distribution of thalassemia in the region, so as to understand the major and rare genotypes and to provide information for the continuous improvement of thalassemia prevention and treatment in this region.

Material and methods

Research subjects

A total of 55,281 individuals who came to the Affiliated Hospital of Guilin Medical University for genetic diagnosis of thalassemia from January 2012 to August 2023 were selected, including those receiving physical examination for marriage and childbearing, as well as anemic patient. Among them, 40,692 were female and 14,589 were male, aged from birth to 80 years, from 32 ethnic groups. Inclusion criteria: (1) individuals who underwent physical examination, premarital examination, prenatal diagnosis or genetic counseling, requiring genetic diagnosis for thalassemia; (2) residents of northern Guangxi area and its counties. Exclusion criteria: those with sample contamination. All patients included in this study signed an informed consent form. This study has been ethical approved by the Affiliated Hospital of Guilin Medical University.

Thalassemia screening

Blood cell analysis, red blood cell hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) combined with hemoglobin analysis were employed for thalassemia screening. (1) Blood count: MCV<82 fl and/or MCH<27 pg. (2) Hemoglobin analysis showed Hb $A_2 \geq 3.5\%$ or Hb $A_2 < 2.4\%$, increased Hb F and or abnormal hemoglobin. Any of the above abnormalities was considered as positive for thalassemia.

Common thalassemia gene detection

Three types of α deletion ($--^{SEA}$, $-\alpha^{4.2}$, $-\alpha^{3.7}$) were identified by α-thalassemia gene detection kit (Gap-PCR method, Shenzhen Yaneng Biotechnology Co., LTD.: 20193401915), and four types of α deletion ($--^{THAI}$, $--^{SEA}$, $-\alpha^{4.2}$, $-\alpha^{3.7}$) were identified by Yishentang α-thalassemia gene detection kit (Gap-PCR method, Shenzhen Yishentang Biological Enterprise Co., LTD.: 20153400626). The non-deletion α-thalassemia kit (PCR reverse dot hybridization, Shenzhen Yaneng Biotechnology Co., LTD.: 20173401107) identified three α mutation types ($\alpha^{WS}\alpha$, α^{CS}-α, α^{QS}-α). β-thalassemia gene test kit (PCR reverse dot hybridization,

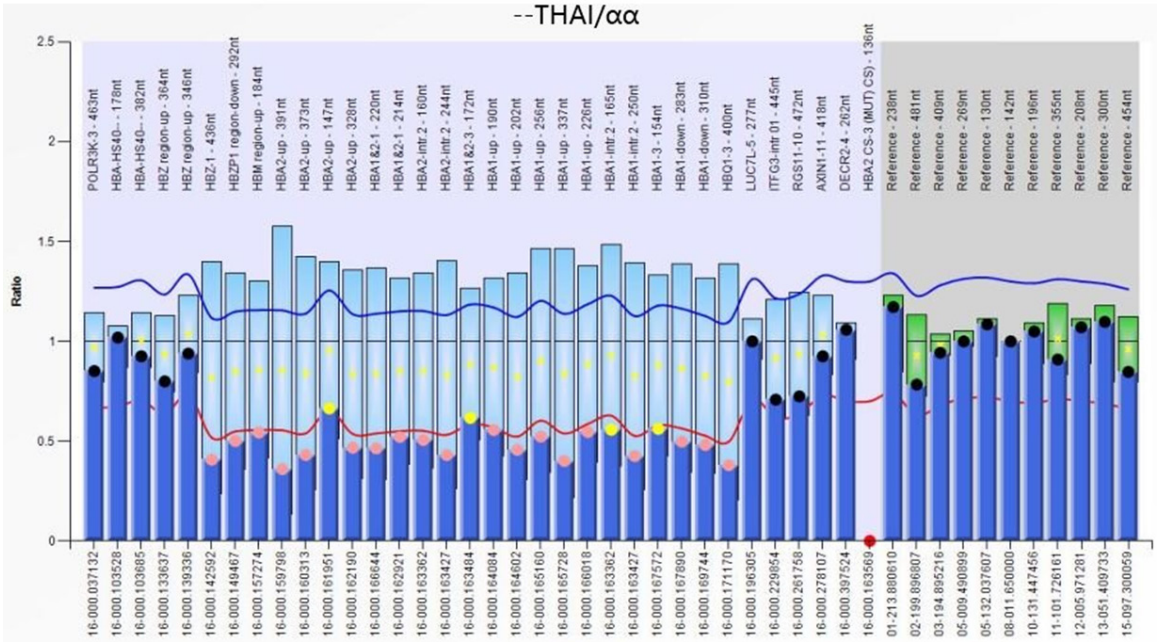

Figure 1. Rare α-thalassemia gene: $--^{THAI}/\alpha\alpha$.

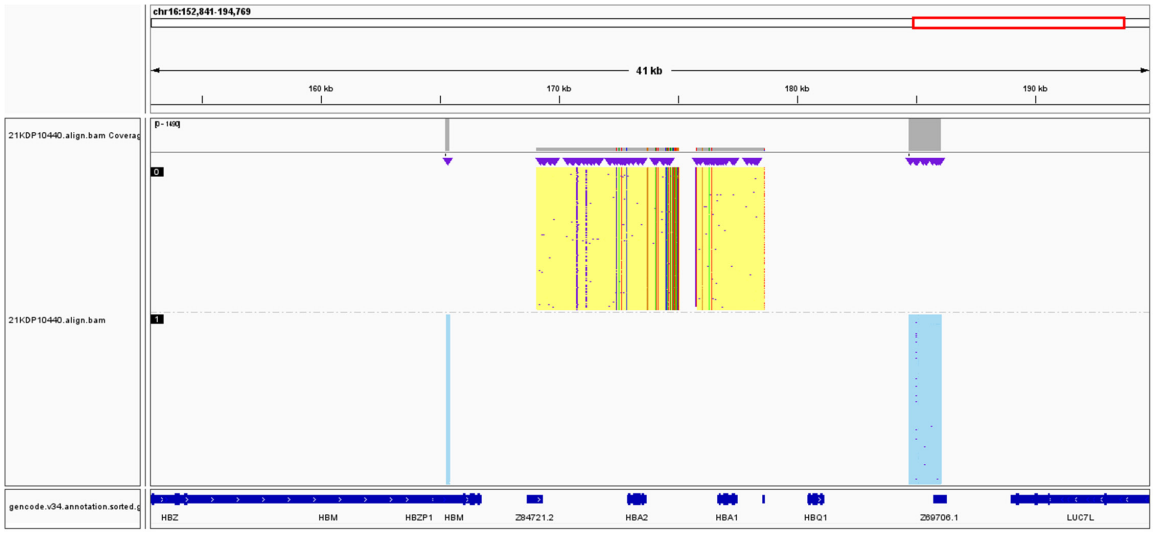

Figure 2. Rare α-thalassemia gene: HKαα.

Shenzhen Yaneng Biotechnology Co., LTD.: 20163400463) identified 17 common β-point mutations.

Rare thalassemia gene detection

When thalassemia screening shows positive, but common thalassemia gene tests are normal, a rare thalassemia gene test should be further performed. Multiplex ligation-dependent probe amplification (MLPA) was applied to identify rare thalassemia with deletion. Sanger sequencing was used to identify rare or unknown thalassemia with point mutation. Single-molecule real-time (SMRT) sequencing was conducted to identify complex thalassemia.

Statistical analysis

SPSS27.0 software was used for statistical analysis. The measurement data were repre-

Table 2. Distribution of β-thalassemia alleles

Allele distribution	Phenotype	n	Constituent ratio	Frequency
CD41-42	β^0	3191	50.38%	5.77%
CD17	β^0	1415	22.34%	2.56%
IVS-II-654	β^0	754	11.90%	1.36%
-28	β^+	364	5.75%	0.66%
CD71-72	β^0	214	3.38%	0.39%
CD26	β^+	167	2.64%	0.30%
-29	β^+	125	1.97%	0.23%
IVS-I-1	β^0	42	0.66%	0.08%
CD43	β^+	23	0.36%	0.04%
CD27-28	β^0	13	0.20%	0.02%
Cap	β^+	9	0.14%	0.02%
CD14-15	β^0	4	0.06%	0.01%
CD37	β^0	10	0.16%	0.02%
$G\gamma^+ (A\gamma\delta\beta)^0$	β^0	3	0.05%	0.01%
Total		6334	100.00%	11.46%

sented by mean ± standard deviation, and t-test was used for comparison between groups. Count data were expressed as cases (%), and chi-square test was carried out for comparison. $P<0.05$ was considered statistically significant.

Results

Genetic diagnosis of thalassemia

Among the 55,281 individuals, 16,442 were found to be positive for the thalassemia gene, with a positive rate of 29.74%. There were 10,264 cases (18.57%) of α-thalassemia, 5,525 cases (9.99%) of β-thalassemia, and 653 cases (1.18%) of α-complex β-thalassemia. α-thalassemia was predominantly attributed to deletions. A total of 11659 alleles of 8 types of α-thalassemia were identified, with a population frequency of 21.09% (**Table 1**). Among them, $--^{SEA}$ gene was the most common one (53.91%), followed by $-\alpha^{3.7}$ (21.31%), $-\alpha^{4.2}$ (8.52%), $\alpha^{CS}\alpha$ (8.08%), $\alpha^{WS}\alpha$ (1.05%), $\alpha^{QS}\alpha$ (0.5%), and rare genes $--^{THAI}$ (0.1%) (**Figure 1**) and HKαα (0.08%) (**Figure 2**). β-thalassemia primarily resulted from point mutations. A total of 14 types of β-thalassemia were identified, encompassing a total of 6367 alleles (**Table 2**), with a population frequency of 11.52%. The main type was CD41-42 (50.12%), followed by CD17 (22.22%), IVS-II-654M (11.84%), etc. There were two rare genotypes: CD37 (0.02%) (**Figure 3**), $G\gamma^+ (A\gamma\delta\beta)^0$ (0.01%) (**Figure 4**). The common deficient α-thalassemia genes, non-deficient α-thalassemia genes, and β-thalassemia genes are shown in **Figures 5**-**7**.

Genetic diagnosis of thalassemia in different ethnical groups

The mutation frequency of α thalassemia allele in Zhuang, Yao, Hui, Miao, Dong, and Han was higher than that of β thalassemia. The highest allelic mutation frequency was found in Zhuang (44.97%), Yao (40.11%), Dong (31.33%), Han (29.85%), Miao (24.31%) and Hui (20.6%) ethnic groups. The total allelic mutation frequency of the other 26 ethnic groups was 30.13%. These results indicate that most ethnic minorities carry the thalassemia gene.

The most common α-thalassemia gene in different ethnical groups was $--^{SEA}$, followed by $-\alpha^{3.7}$. The occurrence frequency of $\alpha^{CS}\alpha$ was higher than that of $-\alpha^{4.2}$ in Zhuang and Yao ethnic groups, while lower than that of $-\alpha^{4.2}$ in Han, Miao, Dong, and Hui ethnic groups. In non-deletion α-thalassemia, $\alpha^{WS}\alpha$ mutation was the most common in Miao ethnic group, while $\alpha^{CS}\alpha$ mutation was the most common in other ethnic groups. $--^{THAI}$ was found to be carried by mainly Han (0.1%) and Zhuang (0.23%) people (**Table 3**).

CD41-42 was the most common β-thalassemia gene in different ethnic groups, and the other common ones were CD17, IVS-II-654, -28, and CD71-72M. The carrying rate of IVS-II-654 was higher than that of CD17 in Hui ethnic group, while in other ethnic groups, the carrying rate of IVS-II-654 was lower than that of CD17. No IVS-II-654 carriers were found in Dong ethnic group. Although the Han population did not have the highest β-carrying rate, they carried the most β-alleles (**Table 4**).

Distribution of thalassemia gene types

A total of 31 genotypes were identified in 10,264 cases of α-thalassemia (**Table 5**). $--^{SEA}$/

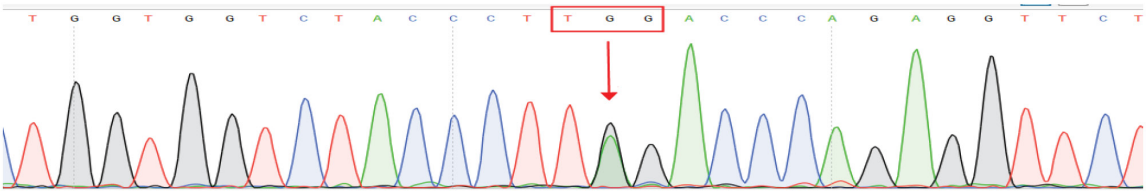

Figure 3. Rare β-thalassemia gene: β^{CD37}/β^{N}.

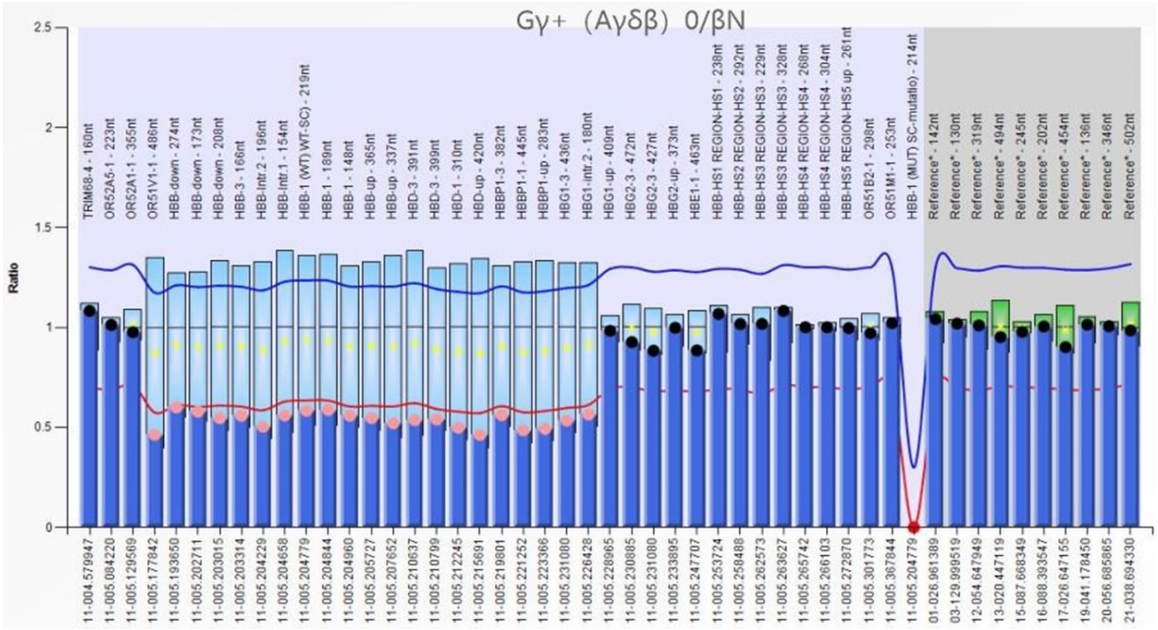

Figure 4. Rare β-thalassemia gene: $G\gamma^{+}(A\gamma\delta\beta)^{0}/\beta^{N}$.

αα was also the most common genotype of thalassemia in northern Guangxi. A total of 34 genotypes were identified in 5,525 cases of β-thalassemia (**Table 6**), with the most common genotype being $\beta^{CD41\text{-}42}/\beta^{N}$. The CD31, IVS-I-5, Int M, -30, and -32 gene types in the β-thalassemia gene detection kit (PCR-reverse dot blot) were not found in the population in northern Guangxi. $\beta^{CD41\text{-}42}/\beta^{CD41\text{-}42}$ is the most common homozygote/double heterozygote of β-thalassemia. A total of 78 genotypes were identified in 653 cases of α-complex β-thalassemia (**Table 7**). Alpha-complex β-thalassemia gene types are extremely abundant in the population of northern Guangxi, and the most common combination is $--^{SEA}/αα$ and $\beta^{CD41\text{-}42}/\beta^{N}$. There were 580 cases (5.65%) of HbH disease (α^{0}/α^{+}), including 27 cases of β-thalassemia. There were 4 cases of edema fetus Pasteurelli ($--^{SEA}/--^{SEA}$). There were 92 cases (1.67%) of moderate and severe β-thalassemia (β^{0}/β^{0}, β^{0}/β^{+}, β^{+}/β^{+}), including 23 cases of combined α-thalassemia.

Thalassemia genotypes with negative thalassemia screening

In this study, 3.7% of the samples initially tested negative for thalassemia were found to carry thalassemia genes, and the most common genotype among them was $-\alpha^{3.7}/αα$, followed by $\alpha^{WS}α/αα$, and $-\alpha^{4.2}/αα$. These three types of non-deletional α-thalassemia accounted for 94.83% (771/813) of the total α-thalassemia cases identified in the initially negative screenings. By analyzing the number of confirmed cases and phenotypes of each genotype, it was found that 40.26% (184/457) of $\alpha^{WS}α/αα$, 22.89% (450/1966) of $-\alpha^{3.7}/αα$, 18.51% (137/740) of $-\alpha^{4.2}/αα$, and 80% (4/5) of β^{CAP}/β^{N} thalassemia were negative. A small percentage of $\alpha^{CS}α/αα$, $\alpha^{QS}α/αα$, and $--^{SEA}/αα$ phenotypes were normal. See **Table 8**.

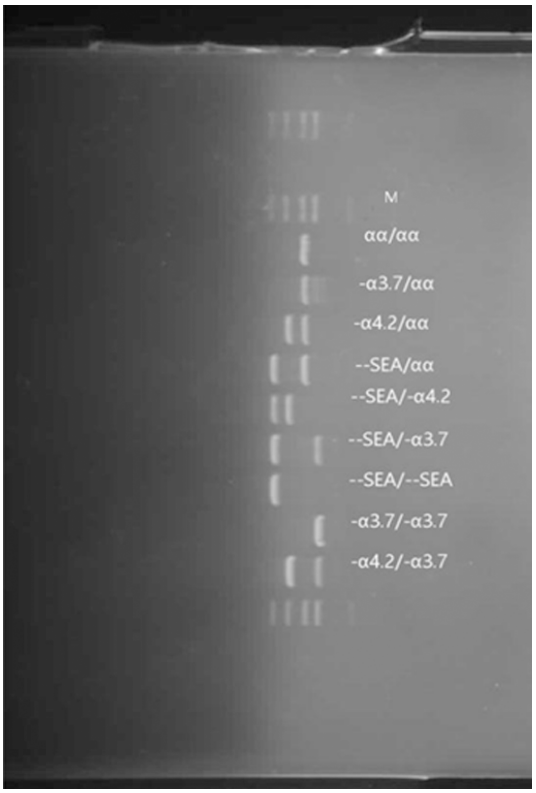

Figure 5. Common deletion α-thalassemia genes.

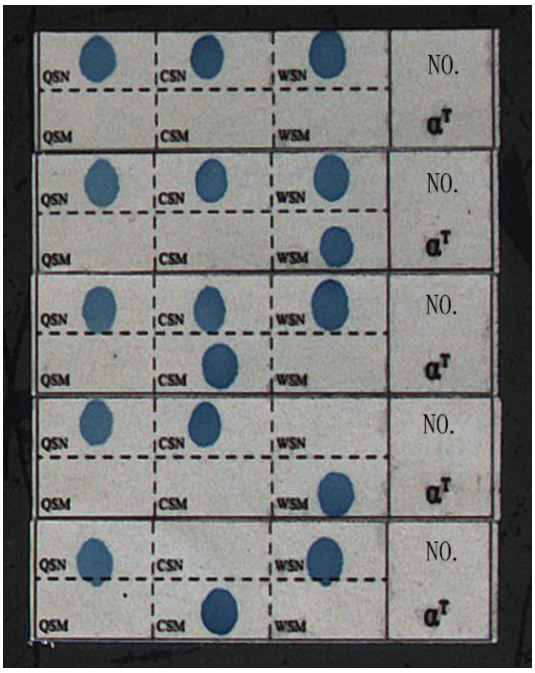

Figure 6. Common non-deletion α-thalassemia genes. Genotypes: αα/αα, α^{WS}-α/αα, α^{Cs}-α/αα, α^{WS}-α/α^{WS}-α, α^{Cs}-α/α^{Cs}-α.

Discussion

Thalassemia is an autosomal recessive genetic disease and one of the most common hemoglobinopathies. The disease is widely distributed worldwide, and Southeast Asia is one of the high-incidence areas. In China, it is more common in Guangdong, Guangxi, and Sichuan, while sporadic cases have been found in provinces south of the Yangtze River, and rare in the north [14-19]. A related study [20] screened 47,500 individuals in the Baise area, and reported that 11,432 (24.07%) subjects were diagnosed as carriers or patients with thalassemia, including 7,290 (15.35%) with α-thalassemia, 3152 (6.64%) with β-thalassemia, and 990 (2.08%) with α-complex β-thalassemia. A total of 16,442 cases (29.74%) of thalassemia were reported in this study. Compared with other regions of Guangxi, the positive rate of the thalassemia gene was higher in northern Guangxi [21]. The positive rates of α, β, and α complex β thalassemia were 18.57%, 9.99%, and 1.18%, respectively, which are similar to the conclusions reported in Baise and southeast Guizhou province [7, 20].

The results of this study showed that the distribution of alleles in the population in northern Guangxi is consistent with the type of thalassemia gene mutation in Chinese people studied by Chen [1]. However, the thalassemia carrying rate (29.74%) of the population in northern Guangxi is higher than that of the population in Guangxi studied by Chen et al. (24.51%). The reason may be that some people from the department of hematology, pediatrics, etc., who had anemia symptoms did genetic testing, which increased the thalassemia carrier rate. In addition, ethnic minorities are abundant, accounting for 18% of the total population, and the high proportion and diversity of ethnic minorities may have increased the thalassemia-carrying rate of people in this study.

The study also found that Zhuang and Yao populations had a higher incidence of thalassemia gene than Han people, which is consistent with the research results of Xu et al. [1], but the positive rate of all ethnic groups in this study is higher. The number of ethnic group in this study is large, which theoretically can better reflect the situation of thalassemia gene in all ethnic groups.

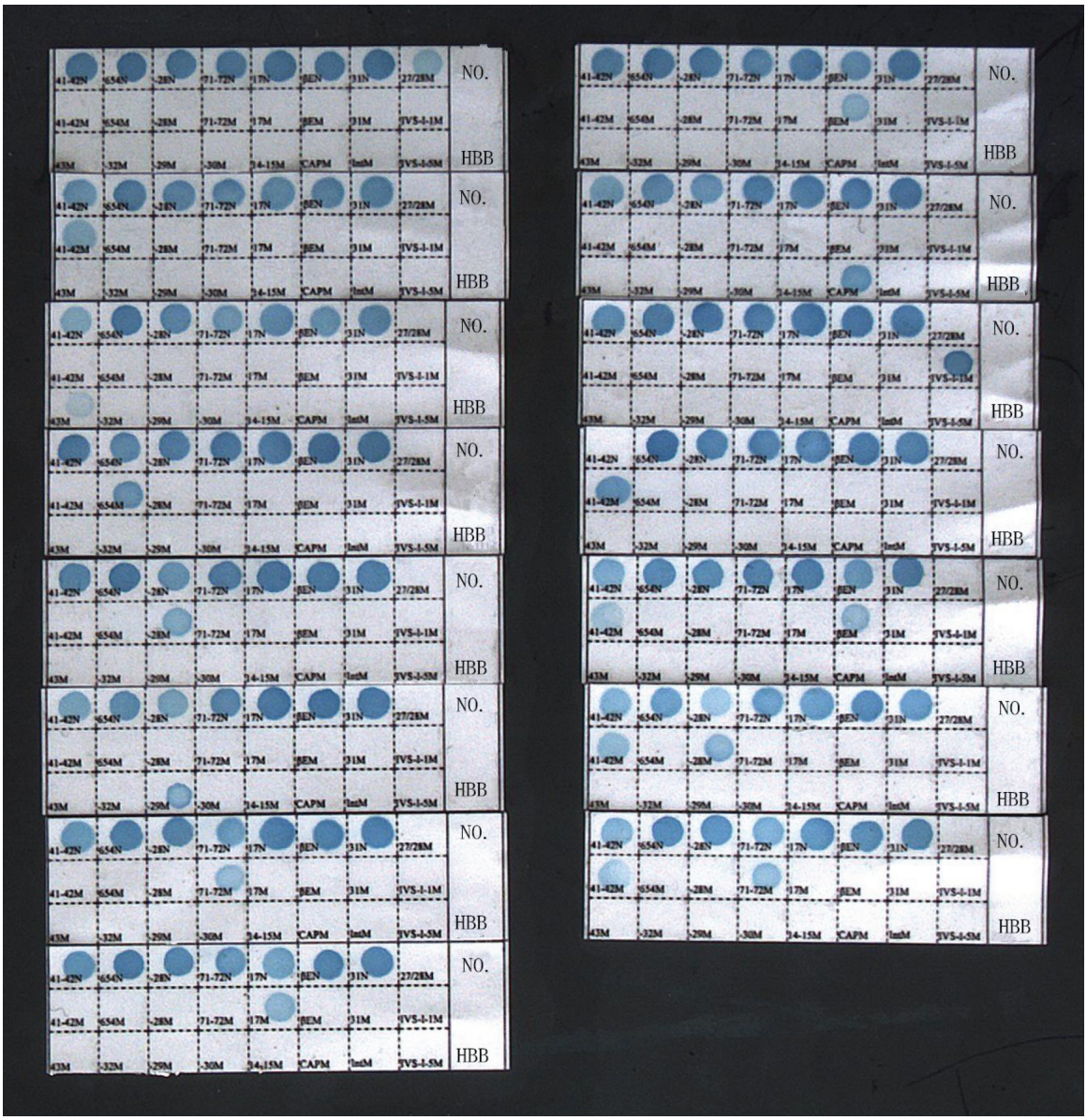

Figure 7. Common β-thalassemia genes. Genotypes: β^N/β^N, $\beta^{CD41-42}/\beta^N$, β^{CD43}/β^N, $\beta^{IVS-II-654}/\beta^N$, β^{-28}/β^N, β^{-29}/β^N, $\beta^{CD71-72}/\beta^N$, β^{CD17}/β^N, β^{CD26}/β^N, β^{CAP}/β^N, $\beta^{IVS-I-1}/\beta^N$, $\beta^{CD41-42}/\beta^{CD41-42}$, $\beta^{CD41-42}/\beta^{CD26}$, $\beta^{CD41-42}/\beta^{-28}$, $\beta^{CD41-42}/\beta^{CD71-72}$.

Table 3. Frequency distribution of α-thalassemia in different ethnic groups

Mutant type	Han (n=36,802)		Zhuang (n=3551)		Yao (n=2920)		Miao (n=502)		Dong (n=482)		Hui (n=301)		Other (n=302)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
$--^{SEA}$	3908	10.62%	550	15.49%	440	15.07%	48	9.56%	46	9.54%	19	6.31%	25	8.28%
$-\alpha^{3.7}$	1332	3.62%	212	5.97%	135	4.62%	6	1.20%	28	5.81%	13	4.32%	22	7.28%
$-\alpha^{4.2}$	569	1.55%	94	2.65%	48	1.64%	4	0.80%	9	1.87%	5	1.66%	4	1.32%
$\alpha^{CS}\alpha$	525	1.43%	140	3.94%	59	2.02%	2	0.40%	3	0.62%	3	1.00%	13	4.30%
$\alpha^{WS}\alpha$	348	0.95%	67	1.89%	43	1.47%	5	1.00%	3	0.62%	0	0.00%	1	0.33%
$\alpha^{QS}\alpha$	188	0.51%	22	0.62%	15	0.51%	2	0.40%	1	0.21%	0	0.00%	1	0.33%
$--^{THAI}$	38	0.10%	8	0.23%	1	0.03%	0	0.00%	0	0.00%	1	0.33%	0	0.00%
Total	6908	18.77%	1093	30.78%	741	25.38%	67	13.35%	90	18.67%	41	13.62%	66	21.85%

Table 4. Frequency distribution of β-thalassemia in different ethnic groups

Mutant type	Han (n=36,802)		Zhuang (n=3551)		Yao (n=2920)		Miao (n=502)		Dong (n=482)		Hui (n=301)		Other (n=302)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
CD41-42	1916	5.21%	237	6.67%	221	7.57%	37	7.37%	40	8.30%	13	4.32%	11	3.64%
CD17	772	2.10%	168	4.73%	72	2.47%	10	1.99%	13	2.70%	1	0.33%	8	2.65%
IVS-II-654	482	1.31%	31	0.87%	63	2.16%	4	0.80%	0	0.00%	3	1.00%	1	0.33%
-28	203	0.55%	26	0.73%	34	1.16%	1	0.20%	5	1.04%	2	0.66%	2	0.66%
CD71-72	137	0.37%	14	0.39%	14	0.48%	3	0.60%	2	0.41%	1	0.33%	0	0.00%
CD26	97	0.26%	14	0.39%	9	0.31%	0	0.00%	0	0.00%	1	0.33%	2	0.66%
-29	87	0.24%	6	0.17%	12	0.41%	0	0.00%	1	0.21%	0	0.00%	0	0.00%
IVS-I-1	25	0.07%	5	0.14%	1	0.03%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
CD43	16	0.04%	2	0.06%	1	0.03%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
CD27-28	5	0.01%	0	0.00%	2	0.07%	0	0.00%	0	0.00%	0	0.00%	1	0.33%
CD14-15	4	0.01%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Cap	4	0.01%	1	0.03%	1	0.03%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
Total	3748	10.18%	504	14.19%	430	14.73%	55	10.96%	61	12.66%	21	6.98%	25	8.28%

Table 5. Genotype distribution of α-thalassemia in 10,264 cases

α-thalassemia	Gene type-α	n	Constituent ratio
α-thalassemia minima	$-\alpha^{3.7}/\alpha\alpha$	1966	19.15%
	$-\alpha^{4.2}/\alpha\alpha$	740	7.21%
	$\alpha^{CS}\alpha/\alpha\alpha$	666	6.49%
	$\alpha^{WS}\alpha/\alpha\alpha$	457	4.45%
	$\alpha^{QS}\alpha/\alpha\alpha$	224	2.18%
α-thalassemia minor	$--^{SEA}/\alpha\alpha$	5464	53.23%
	$--^{THAI}/\alpha\alpha$	47	0.46%
	$-\alpha^{3.7}/-\alpha^{3.7}$	33	0.32%
	$-\alpha^{4.2}/-\alpha^{4.2}$	10	0.10%
	$-\alpha^{4.2}/-\alpha^{3.7}$	16	0.16%
	$-\alpha^{3.7}/\alpha^{CS}\alpha$	26	0.25%
	$-\alpha^{3.7}/\alpha^{WS}\alpha$	9	0.09%
	$-\alpha^{4.2}/\alpha^{WS}\alpha$	10	0.10%
	$-\alpha^{4.2}/\alpha^{CS}\alpha$	3	0.03%
	$\alpha^{CS}\alpha/\alpha^{CS}\alpha$	11	0.11%
	$\alpha^{WS}\alpha/\alpha^{WS}\alpha$	4	0.04%
	$\alpha^{QS}\alpha/\alpha^{CS}\alpha$	7	0.07%
	$\alpha^{QS}\alpha/\alpha^{QS}\alpha$	3	0.03%
	$\alpha^{CS}\alpha/\alpha^{WS}\alpha$	6	0.06%
	$\alpha^{WS}\alpha/\alpha^{QS}\alpha$	2	0.02%
Hemoglobin H	$--^{SEA}/-\alpha^{3.7}$	243	2.37%
	$--^{SEA}/-\alpha^{4.2}$	119	1.16%
	$--^{SEA}/\alpha^{CS}\alpha$	132	1.29%
	$--^{SEA}/\alpha^{WS}\alpha$	29	0.28%
	$--^{SEA}/\alpha^{QS}\alpha$	22	0.21%
	$HK\alpha\alpha/-\alpha^{4.2}$	3	0.03%
	$--^{SEA}/HK\alpha\alpha$	4	0.04%
	$--^{THAI}/-\alpha^{3.7}$	2	0.02%
	$--^{THAI}/-\alpha^{4.2}$	1	0.01%
	$--^{THAI}/\alpha^{QS}\alpha$	1	0.01%
hydrops fetails	$--^{SEA}/--^{SEA}$	4	0.04%
Total		10264	100.00%

This study revealed that 3.7% of the samples, initially screened as negative for thalassemia, still carried thalassemia genes. The primary reasons for this occurrence are as follows: Static thalassemia presents no alterations in hematological phenotype and hemoglobin electrophoresis, making identification possible only through genetic testing [22]. This topic found that 40.26% (184/457) of $\alpha^{WS}\alpha/\alpha\alpha$, 22.89% (450/1966) of $-\alpha^{3.7}/\alpha\alpha$, 18.51% (137/740) of $-\alpha^{4.2}/\alpha\alpha$, and 44.44% (4/9) of β^{CAP}/β^{N} screening were negative.

Thalassemia screening includes blood cell parameter analysis and hemoglobin content analysis. The increase of Hb A_2 content suggests β-thalassemia. However, when hemoglobin band CS and hemoglobin band E are found in the hemoglobin content analysis, the sample is highly likely to carry the ($\alpha^{CS}\alpha/$), ($\beta^{CD26}/$) and other thalassemia genes [23]. However, in clinical work, the examination of hemoglobin electrophoresis is often ignored. This topic found that study uncovered that a subset of individuals with mild anemia may have entirely normal cell parameters, so the omission of hemoglobin content analysis could lead to overlooking this group of patients. Combining hematological parameters with hemoglobin content analysis can reduce missed diagnosis [24, 25].

Table 6. Distribution of genotypes in 5,525 cases of β-thalassemia

β-thalassemia	Gene type	n	Constituent ratio
Thalassemia minor	$\beta^{CD41-42}/\beta^{N}$	2792	50.53%
	β^{CD17}/β^{N}	1203	21.77%
	$\beta^{IVS-II-654}/\beta^{N}$	672	12.16%
	β^{-28}/β^{N}	297	5.38%
	$\beta^{CD71-72}/\beta^{N}$	181	3.28%
	β^{CD26}/β^{N}	127	2.30%
	β^{-29}/β^{N}	109	1.97%
	$\beta^{IVS-I-1}/\beta^{N}$	33	0.60%
	β^{CD43}/β^{N}	21	0.38%
	$\beta^{CD27/28}/\beta^{N}$	12	0.22%
	β^{CAP}/β^{N}	5	0.09%
	$\beta^{CD14-15}/\beta^{N}$	4	0.07%
Thalassemia intermedia/major	$\beta^{CD41-42}/\beta^{CD41-42}$	12	0.22%
	$\beta^{CD41-42}/\beta^{CD26}$	10	0.18%
	$\beta^{CD41-42}/\beta^{CD17}$	7	0.13%
	$\beta^{CD41-42}/\beta^{IVS-II-654}$	6	0.11%
	$\beta^{CD41-42}/\beta^{-28}$	5	0.09%
	$\beta^{CD41-42}/\beta^{-29}$	2	0.04%
	$\beta^{CD41-42}/\beta^{CD43}$	1	0.02%
	$\beta^{CD41-42}/\beta^{CD71-72}$	1	0.02%
	$\beta^{CD41-42}/\beta^{CD27/28}$	1	0.02%
	$\beta^{CD41-42}/\beta^{IVS-I-1}$	1	0.02%
	$\beta^{CD17}/\beta^{CD17}$	4	0.07%
	$\beta^{CD17}/\beta^{CD26}$	3	0.05%
	$\beta^{CD17}/\beta^{IVS-II-654}$	3	0.05%
	$\beta^{CD17}/\beta^{CD71-72}$	2	0.04%
	β^{CD17}/β^{-29}	1	0.02%
	β^{CD17}/β^{CAP}	1	0.02%
	β^{CD17}/β^{-29}	1	0.02%
	$\beta^{CD17}/\beta^{IVS-I-1}$	1	0.02%
	$\beta^{IVS-II-654}/\beta^{-29}$	2	0.04%
	$\beta^{IVS-II-654}/\beta^{CD71-72}$	1	0.02%
	$\beta^{CD71-72}/\beta^{CD26}$	3	0.05%
	β^{-28}/β^{-28}	1	0.02%
Total		5525	100.00%

In Zhuang's study [26], newborn erythrocyte parameters were significantly higher than those of adults. When newborns carried thalassemia, MCV and MCH would decrease, but they were still higher than the positive index of thalassemia screening (MCV<82 fl, MCH<27 pg). Therefore, the hematological parameters of neonatal thalassemia carriers may appear normal.

The detection strategy of thalassemia in most studies is to first screen the population for thalassemia, and then test the thalassemia gene if the screening is positive. Such a strategy may miss the diagnosis of some thalassemia carriers [27]. In our hospital, with the informed consent of the pregnant women, blood routine, hemoglobin electrophoresis and thalassemia gene detection were performed. If the pregant woman carried the thalassemia gene, the spouse were recommended to take further tests for thalassemia. The advantage of this thalassemia screening strategy is the timely identification of thalassemia carriers who do not have a hematological phenotype.

The missed diagnosis of thalassemia minima or minor and the lack of timely diagnosis of rare thalassemia may lead to newborn baby suffered from intermedia or severe types of thalassemia. Therefore, it is recommended to conduct comprehensive screening of thalassemia in the clinic, including blood cell parameter analysis, hemoglobin content analysis, ferritin, and genetic diagnosis of thalassemia to avoid missed diagnosis of static thalassemia. If thalassemia screening does not match thalassemia genes, appropriate molecular diagnostic technology should be adopted to further identify rare thalassemia genes or reduce the birth rate of children with thalassemia intermedia and major.

Disclosure of conflict of interest

None.

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Table 7. Distribution of genotypes in 653 cases of α-combined β-thalassemia

Gene type	n	Constituent ratio
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	85	13.02%
$-\alpha^{3.7}/\alpha\alpha$, β^{CD17}/β^{N}	36	5.51%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	19	2.91%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	6	0.92%
$-\alpha^{3.7}/\alpha\alpha$, β^{-28}/β^{N}	8	1.23%
$-\alpha^{3.7}/\alpha\alpha$, β^{-29}/β^{N}	2	0.31%
$-\alpha^{3.7}/\alpha\alpha$, β^{CAP}/β^{N}	2	0.31%
$-\alpha^{3.7}/\alpha\alpha$, β^{CD26}/β^{N}	5	0.77%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{IVS-I-1}/\beta^{N}$	2	0.31%
$-\alpha^{3.7}/\alpha\alpha$, β^{CD43}/β^{N}	1	0.15%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	28	4.29%
$-\alpha^{4.2}/\alpha\alpha$, β^{CD17}/β^{N}	11	1.68%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	4	0.61%
$-\alpha^{4.2}/\alpha\alpha$, β^{-28}/β^{N}	4	0.61%
$-\alpha^{4.2}/\alpha\alpha$, β^{-29}/β^{N}	2	0.31%
$-\alpha^{4.2}/\alpha\alpha$, β^{CD26}/β^{N}	2	0.31%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{IVS-I-1}/\beta^{N}$	1	0.15%
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	30	4.59%
$\alpha^{CS}\alpha/\alpha\alpha$, β^{CD17}/β^{N}	19	2.91%
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	12	1.84%
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	3	0.46%
$\alpha^{CS}\alpha/\alpha\alpha$, β^{CD26}/β^{N}	2	0.31%
$\alpha^{CS}\alpha/\alpha\alpha$, β^{-28}/β^{N}	1	0.15%
$\alpha^{CS}\alpha/\alpha\alpha$, $\beta^{IVS-I-1}/\beta^{N}$	1	0.15%
$\alpha^{CS}\alpha/\alpha\alpha$, β^{CAP}/β^{N}	1	0.15%
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	32	4.90%
$\alpha^{WS}\alpha/\alpha\alpha$, β^{CD17}/β^{N}	13	1.99%
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	5	0.77%
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	4	0.61%
$\alpha^{WS}\alpha/\alpha\alpha$, β^{CD26}/β^{N}	3	0.46%
$\alpha^{WS}\alpha/\alpha\alpha$, β^{-28}/β^{N}	1	0.15%
$\alpha^{QS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	9	1.38%
$\alpha^{QS}\alpha/\alpha\alpha$, β^{CD26}/β^{N}	2	0.31%
$\alpha^{QS}\alpha/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.15%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{-28}$	2	0.31%
$-\alpha^{3.7}/\alpha\alpha$, β^{-28}/β^{-28}	1	0.15%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD17}$	1	0.15%
$-\alpha^{3.7}/\alpha\alpha$, $\beta^{CD26}/\beta^{CD17}$	1	0.15%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{CD26}$	4	0.61%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD41-42}$	3	0.46%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{-28}/\beta^{IVS-II-654}$	1	0.15%
$-\alpha^{4.2}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD17}$	1	0.15%
$\alpha^{CS}\alpha/\alpha\alpha$, β^{-28}/β^{CD17}	1	0.15%
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD17}$	1	0.15%
$\alpha^{WS}\alpha/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD41-42}$	1	0.15%
$--^{SEA}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{N}$	122	18.68%
$--^{SEA}/\alpha\alpha$, β^{CD17}/β^{N}	42	6.43%
$--^{SEA}/\alpha\alpha$, β^{-28}/β^{N}	23	3.52%
$--^{SEA}/\alpha\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	22	3.37%
$--^{SEA}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{N}$	7	1.07%
$--^{SEA}/\alpha\alpha$, β^{-29}/β^{N}	6	0.92%
$--^{SEA}/\alpha\alpha$, β^{CD26}/β^{N}	3	0.46%
$--^{SEA}/\alpha\alpha$, $\beta^{IVS-I-1}/\beta^{N}$	3	0.46%
$--^{THAI}/\alpha\alpha$, β^{CD17}/β^{N}	2	0.31%
$-\alpha^{3.7}/-\alpha^{4.2}$, β^{-28}/β^{N}	12	1.84%
$-\alpha^{3.7}/-\alpha^{3.7}$, β^{CD17}/β^{N}	1	0.15%
$-\alpha^{3.7}/\alpha^{WS}\alpha$, β^{CD17}/β^{N}	1	0.15%
$-\alpha^{4.2}/\alpha^{CS}\alpha$, β^{-28}/β^{N}	1	0.15%
$\alpha^{CS}\alpha/\alpha^{CS}\alpha$, $\beta^{CD41-42}/\beta^{N}$	1	0.15%
HKαα/$-\alpha^{4.2}$, $\beta^{CD41-42}/\beta^{CD41-42}$	1	0.15%
$--^{SEA}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{CD41-42}$	2	0.31%
$--^{SEA}/\alpha\alpha$, $\beta^{CD41-42}/\beta^{-28}$	2	0.31%
$--^{SEA}/\alpha\alpha$, $\beta^{CD71-72}/\beta^{-28}$	1	0.15%
$--^{SEA}/\alpha\alpha$, $\beta^{CD17}/\beta^{CD26}$	1	0.15%
$--^{SEA}/-\alpha^{3.7}$, $\beta^{CD41-42}/\beta^{N}$	6	0.92%
$--^{SEA}/-\alpha^{3.7}$, $\beta^{IVS-II-654}/\beta^{N}$	3	0.46%
$--^{SEA}/-\alpha^{3.7}$, $\beta^{CD71-72}/\beta^{N}$	1	0.15%
$--^{SEA}/-\alpha^{3.7}$, β^{CD17}/β^{N}	1	0.15%
$--^{SEA}/-\alpha^{4.2}$, β^{CD17}/β^{N}	3	0.46%
$--^{SEA}/-\alpha^{4.2}$, β^{-28}/β^{N}	1	0.15%
$--^{SEA}/-\alpha^{4.2}$, $\beta^{CD41-42}/\beta^{N}$	1	0.15%
$--^{SEA}/-\alpha^{4.2}$, $\beta^{IVS-II-654}/\beta^{N}$	1	0.15%
$--^{SEA}/\alpha^{CS}\alpha$, $\beta^{CD41-42}/\beta^{N}$	4	0.61%
$--^{SEA}/\alpha^{CS}\alpha$, $\beta^{IVS-II-654}/\beta^{N}$	2	0.31%
$--^{SEA}/\alpha^{CS}\alpha$, β^{CD26}/β^{N}	1	0.15%
$--^{SEA}/\alpha^{QS}\alpha$, $\beta^{CD41-42}/\beta^{N}$	1	0.15%
$--^{SEA}/\alpha^{WS}\alpha$, $\beta^{CD41-42}/\beta^{N}$	1	0.15%
Total	653	100%

Table 8. Thalassemia gene types of carriers with initial negative screening for thalassemia

Gene type	Confirmed cases (n)	Negative cases (n)	Percentage
$-\alpha^{3.7}/\alpha\alpha$	1966	450	22.89%
$\alpha^{WS}\alpha/\alpha\alpha$	457	184	40.26%
$-\alpha^{4.2}/\alpha\alpha$	740	137	18.51%
$\alpha^{CS}\alpha/\alpha\alpha$	666	22	3.30%
$--^{SEA}/\alpha\alpha$	5464	14	0.26%
$\alpha^{QS}\alpha/\alpha\alpha$	224	3	1.34%
$\alpha^{WS}\alpha/\alpha^{WS}\alpha$	4	2	50.00%
$-\alpha^{3.7}/-\alpha^{3.7}$	33	1	3.03%
β^{CAP}/β^{N}	5	4	80.00%

References

[1] Chen P, Lin WX and Li SQ. THALASSEMIA in ASIA 2021: thalassemia in Guangxi Province, People's Republic of China. Hemoglobin 2022; 46: 33-35.

[2] Lai K, Huang G, Su L and He Y. The prevalence of thalassemia in mainland China: evidence from epidemiological surveys. Sci Rep 2017; 7: 920.

[3] Xian J, Wang Y, He J, Li S, He W, Ma X and Li Q. Molecular epidemiology and hematologic characterization of thalassemia in Guangdong Province, Southern China. Clin Appl Thromb Hemost 2022; 28: 10760296221119807.

[4] Wang L, Zuo YJ, Lin L, Chen QL, Chen BY, Chen FQ and He S. Genotyping of thalassemia in Guangxi population. Chongqing Med 2022; 51: 491-494.

[5] Wang M, Zhang X, Zhao Y, Lu Z and Xiao M. Prevalence and genetic analysis of thalassemia in childbearing age population of Hainan, The Free Trade Island in Southern China. J Clin Lab Anal 2022; 36: e24260.

[6] Wang Z, Sun W, Chen H, Zhang Y, Wang F, Chen H, Zhou Y, Huang Y, Zhou X, Li Q and Ma Y. Prevalence and molecular spectrum of alpha- and beta-globin gene mutations in Hainan, China. Int J Hematol 2021; 114: 307-318.

[7] Xu G, Wang C, Wang J, Lin M, Chang Z, Liang J, Chen X, Zhong S, Nong X, Wei W and Deng Y. Prevalence and molecular characterization of common thalassemia among people of reproductive age in the border area of Guangxi-Yunnan-Guizhou province in Southwestern China. Hematology 2022; 27: 672-683.

[8] Zhao P, Weng R and Wu H. Molecular spectrum of alpha- and beta-thalassemia mutations in a large ethnic Hakka population in Southern China. Hemoglobin 2018; 42: 117-121.

[9] He S, Wei Y, Lin L, Chen Q, Yi S, Zuo Y, Wei H, Zheng C, Chen B and Qiu X. The prevalence and molecular characterization of (deltabeta)(0)-thalassemia and hereditary persistence of fetal hemoglobin in the Chinese Zhuang population. J Clin Lab Anal 2018; 32: e22304.

[10] Yu Y, Lu C, Gao Y, Li C, Li D, Wang J, Wei H, Lu Z and You G. Molecular spectrum, ethnic and geographical distribution of thalassemia in the Southern Area of Hainan, China. Front Pediatr 2022; 10: 894444.

[11] Tang W, Zhang C, Lu F, Tang J, Lu Y, Cui X, Qin X and Li S. Spectrum of α-thalassemia and β-thalassemia mutations in the Guilin Region of southern China. Clin Biochem 2015; 48: 1068-72.

[12] Wang WD, Hu F, Zhou DH, Gale RP, Lai YR, Yao HX, Li C, Wu BY, Chen Z, Fang JP, Chen SJ and Liang Y. Thalassaemia in China. Blood Rev 2023; 60: 101074.

[13] Li DM, Li JH, Chen DM and He S. Analysis of gene mutation types of thalassemia in Yulin childbearing-age population of Guangxi China. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2020; 28: 2011-2016.

[14] Liang HF, Liang WM, Xie WG, Lin F, Liu LL, Li LJ, Ge YY, Lu M, Liao YW, Zeng GK, Yao JX, Situ JW and Yang LY. The gene spectrum of thalassemia in Yangjiang of western Guangdong Province. Front Genet 2023; 14: 1126099.

[15] Huang TL, Zhang TY, Song CY, Lin YB, Sang BH, Lei QL, Lv Y, Yang CH, Li N, Tian X, Yang YH and Zhang XW. Gene mutation spectrum of thalassemia among children in Yunnan Province. Front Pediatr 2020; 8: 159.

[16] Chen MF, Huang MZ, Lin Q, Huang J, Chen F, Zhang JY and Xue F. Analysis of the types of thalassemia gene mutations in Nanping Area of Fujian, China. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2020; 28: 918-926.

[17] Zhong K, Shi H, Wu W, Xu H, Wang H and Zhao Z. Genotypic spectrum of α-thalassemia and β-thalassemia in newborns of the Li minority in Hainan province, China. Front Pediatr 2023; 11: 1139387.

[18] Wang F, Zhang RY, Deng DY, Xu D, Zou Y and Zhou YY. Gene mutation types and ethnic distribution characteristic of thalassemia in Guiyang. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2021; 29: 1887-1891.

[19] Wang M, Zhang X, Zhang Y and Xiao M. Prevalence and genetic analysis of thalassemia and hemoglobinopathy in different ethnic groups and regions in Hainan Island, Southeast China. Front Genet 2022; 13: 874624.

[20] He S, Qin Q, Yi S, Wei Y, Lin L, Chen S, Deng J, Xu X, Zheng C and Chen B. Prevalence and genetic analysis of alpha- and beta-thalassemia in Baise region, a multi-ethnic region in southern China. Gene 2017; 619: 71-75.

[21] Zheng HQ, Yu XY, Zeng D, Feng Q and Zhu CJ. Analysis of thalassemia gene carriers in 19,482 pregnant women from 2015 to 2019 in Guilin, Guangxi. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2021; 29: 1892-1895.

[22] Yu X, Lin M, Liu C, Liao Z, Wei Y, Liu R and Zhu J. Genetic investigation of haemoglobinopathies in a large cohort of asymptomatic individuals reveals a higher carrier rate for beta-thalassaemia in Sichuan Province (Southwestern China). Genes Dis 2019; 8: 224-231.

[23] Lin HZ, Mai YM, Huang CY and Huang ZD. Value of hemoglobin electrophoresis combined with thalassemia gene detection in early diagnosis of thalassemia. Clin Med 2022; 42: 59-62.

[24] Xie CL and Chen MF. Evaluation of the diagnostic value of screening and combination tests for thalassemia. Heilong Med J 2022; 46: 809-813.
[25] Ming SS, Zhang DL, Chen L and Shi Y. Effects of anemia and red blood cell transfusion in preterm infants on the development of bronchopulmonary dysplasia: a propensity score analysis. All Life 2021; 14: 830-839.
[26] Zhuang CJ and Wan ZD. Application value of cord blood erythrocyte parameter test in the screening of α-thalassemia. Chin J Clin Pathologist 2021; 13: 34-37.
[27] Li YQ, He S, Qiu XX, Dong BQ, Chen BY, Wei H, Huang XN, Zhao L, Liang L, Qin T, Tian M and Li JJ. Current status and prevention strategy of thalassemia in Guangxi from 2010 to 2019. Chin J New Clin Med 2020; 13: 955-959.