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ORIGINAL RESEARCH

Analysis of Hematological Parameters in Relation to Genotypes in 497 Patients with Hemoglobin H Disease

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Background: Hemoglobin H (Hb H) disease is a common type of α-thalassemia, characterized by anemia caused by abnormal hemoglobin synthesis, and its hematological phenotype show significant heterogeneity. The purpose is to explore the relationship between genotypes and hematological parameters in Hb H disease, in order to provide scientific basis for the prevention and treatment of Hb H disease.

Methods: A total of 497 Hb H disease patients at Meizhou People's Hospital from December 2016 to December 2023, were retrospectively analyzed. Genotype testing was performed to determine the types of α-thalassemia and β-thalassemia. The hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hemoglobin electrophoresis results of the patients were collected to evaluate their hematological manifestations. The relationship between genotypes and hematological manifestations was analyzed.

Results: There were 449 (90.3%) cases with deletional Hb H disease and 48 (9.7%) with non-deletional Hb H disease. The detection rate of Hb H was higher in patients with non-deletional Hb H disease than in those with deletional Hb H disease (73.8% vs 66.8%). The proportion of severe anemia in patients with Hb H disease combined with β-thalassemia was lower than that of patients with isolated Hb H disease (11.1% vs 26.9%). Non-deletional Hb H disease exhibited more severe anemia compared to those with deletional Hb H disease (low Hb, p=0.002), accompanied by significantly higher MCV (p<0.001) and MCH (p=0.001). The degree of microcytosis and hypochromia in Hb H disease patients without β-thalassemia is less severe than that in patients with β-thalassemia.

Conclusion: Non-deletional Hb H disease exhibited higher detection rate of Hb H and proportion of severe anemia, and patients with $-^{SEA}/\alpha^{CS}\alpha$ have the highest proportion of severe anemia. There are differences in the genotypes distribution of Hb H disease among different populations.

Keywords: thalassemia, hemoglobin H disease, genotype, hematological parameters

Introduction

Thalassemia is a group of genetic hemolytic anemia diseases caused by the absence or defect of globin genes.[1] Thalassemia is a common condition among populations in Mediterranean countries, Africa, the Middle East, the Indian subcontinent, and Southeast Asia.[2,3] As a autosomal recessive genetic disorder, thalassemia is mainly classified into alpha (α)-thalassemia and beta (β)-thalassemia based on the different hemoglobin chains affected.[4] Alpha (α)-thalassemia is mainly caused by the absence or mutation of the alpha globin gene, and has a relatively high incidence in the Southeast Asia.[5] A total of four α-globin genes are present in humans (two located on each chromosome), and the number of deleted or defective α-globin genes dictates the subtype and severity of α-thalassemia: (1) silent type α thalassemia: defect in one α gene (-α/αα), where patients typically exhibit no overt clinical manifestations; (2) mild type α thalassemia: defect in two α genes (–/αα or -α/-α); (3) intermediate type α thalassemia: associated with defects in three α genes (–/-α); and (4) severe type α thalassemia: defect in four α genes (–/–), which is usually fatal in the fetal period or shortly after birth.[6]

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The common types of deletion α-thalassemia include $–^{SEA}$(Southeast Asian deletion, a type of α-globin deletion that is relatively common in Southeast Asia), $-\alpha^{3.7}$ (α-globin gene has a 3.7 kb fragment deletion), and $-\alpha^{4.2}$ (α-globin gene has a 3.7 kb fragment deletion).[7] And the common non-deletion α-thalassemia mutations including Hb Constant Spring (Hb CS) (CD142,TAA→CAA), Hb Quong Sze (Hb QS) (CD125,CTG→CCG), and Hb Westmead (CD122, CAC→CAG).[8] Hemoglobin H disease (Hb H disease), as a common type of α-thalassemia, is usually caused by the absence of three of the four α globin genes (–/-α, deletional Hb H disease, such as $–^{SEA}/-\alpha^{3.7}$, and $–^{SEA}/-\alpha^{4.2}$) or the combination of two absences and one functional mutation ($–/\alpha^{T}\alpha$, non-deletional Hb H disease, such as $–^{SEA}/\alpha^{CS}\alpha$, $–^{SEA}/\alpha^{WS}\alpha$, and $–^{SEA}/\alpha^{QS}\alpha$).[9,10] The core genetic mechanism of Hb H disease is that there is a severe defect in the α globin gene, resulting in a significant reduction in α chain synthesis, while the β chain is relatively excessive. The excessive β chain self-polymerizes to form Hb H (known as β-tetramers or β4).[11,12] The instability and precipitation of Hb H can damage the function of red blood cells, leading to hemolytic anemia.[13] The severity of clinical symptoms in Hb H disease lies between that of mild α-thalassemia and severe α-thalassemia.

The clinical phenotypes of patients with Hb H disease show significant heterogeneity, ranging from asymptomatic or mild anemia to severe anemia accompanied by hepatosplenomegaly, skeletal changes, and growth retardation.[6] Phenotypic heterogeneity is a significant feature of Hb H disease.[14] Patients with the same genotype may exhibit heterogeneous clinical symptoms, whereas those with different genotypes can present similar phenotypic characteristics. The clinical manifestations of Hb H disease are modulated by multiple contributing factors.[15] Some studies suggested that the phenotypic differences of Hb H disease are closely related to the genotypes of the patients.[15–17] Different types of α-globin gene deletions or mutations can impair the synthesis efficiency of α-globin chains, ultimately contributing to variations in hemoglobin levels and the severity of anemia. For example, patients with the $–^{SEA}$ deletion combined with $-\alpha^{3.7}$ deletion may have certain differences in clinical symptoms compared to those with $–^{SEA}$ deletion combined with $-\alpha^{4.2}$ deletion.[6] While patients with non-deletional Hb H disease often have more severe anemia.[18]

At present, studies on the association between the genotypes of Hb H disease and hemoglobin levels, as well as the severity of anemia, still have problems such as small sample sizes and incomplete coverage of genotypes. The consistency of some research results also needs to be further verified. Clarifying the characteristics of hemoglobin levels and the severity of anemia in patients with Hb H disease of different genotypes is of great clinical significance for precise assessment of the condition, formulation of individualized treatment plans, and prognosis judgment. Hb H disease is a common type of α-thalassemia, characterized by anemia caused by abnormal hemoglobin synthesis, and its clinical manifestations show significant heterogeneity. The purpose of this study is to explore the relationship between genotypes and hematological parameters in patients with Hb H disease, in order to provide scientific basis for the prevention and treatment of Hb H disease.

Materials and Methods

Subjects

This study is a retrospective observational study. Patients diagnosed with Hb H disease at Meizhou People's Hospital from December 2016 to December 2023 were collected in this study. Inclusion criteria were as follows: (1) genetic testing has confirmed the presence of α-globin gene deletion or mutation, which conforms to the genotype characteristics of Hb H disease; (2) complete clinical data, including blood routine, hemoglobin electrophoresis, and genetic test results; and (3) no blood transfusion treatment, iron depletion treatment or other intervention measures affecting hematological parameters within the past 4 months. Exclusion criteria were as follows: (1) complicated with other hemolytic diseases; (2) complicated with nutritional anemia (such as iron deficiency anemia, megaloblastic anemia), or severe liver and kidney dysfunction, malignant tumors, and autoimmune diseases; (3) incomplete clinical data or refusal to participate in this study; and (4) patients with treatment history within the past 4 months that affected the test results, such as blood transfusion, chemotherapy, and immunotherapy. Ultimately, a total of 497 Hb H disease patients were collected, including 219 (44.1%) males and 278 (55.9%) females, with an average age of 41.41 ± 21.38 years.

The clinical data of all the research subjects were collected from the electronic medical record system of our hospital, including their gender, age, and the results of hematological parameters (hemoglobin (Hb), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), and Hb electrophoresis (hemoglobin A_2 (Hb A_2), hemoglobin H (Hb H), and fetal hemoglobin (Hb F)). The results of the hematological indicators for patients requiring blood transfusion treatment are all the test data obtained before their blood transfusion treatment. Hematological parameters were determined by an automated cell counter (XE-2100, Sysmex Corporation, Japan), and Hb analysis was conducted using capillary electrophoresis (Capillarys 2 Flex piercing, Sebia, France). Present study was approved by the Ethics Committees of Meizhou People's Hospital according to the Declaration of Helsinki.

α-Thalassemia and β-Thalassemia Genotyping Analysis

Genotype testing was performed by gap-polymerase chain reaction (gap-PCR) and flow-through hybridization technology to determine the types of α-thalassemia and β-thalassemia mutations (Hybribio Limited, China). The mutations including: (1) three common deletion α-thalassemia mutations: $--^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$; (2) three common non-deletion α-thalassemia mutations: CD142 (TAA→CAA), CD125 (CTG→CCG), and CD122 (CAC→CAG); and (3) 16 common β-thalassemia mutations: 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). The data on thalassemia genotypes in this study were obtained from the electronic medical record system of Meizhou People's Hospital.

Severity Classification of Anemia

According to the diagnostic criteria and severity classification standards for anemia formulated by the World Health Organization (WHO), and based on the patient's hemoglobin level, the severity of anemia is classified as follows: (1) mild anemia: hemoglobin 110–119 g/L; (2) moderate anemia: hemoglobin 70–109 g/L; (3) severe anemia: hemoglobin <70 g/L.[19]

Statistical Analysis

Statistical analysis was performed with the SPSS statistical software version 26.0 (IBM, USA). Descriptive analysis was used to show the frequencies of genotype and allele in different populations. Continuous variables were compared either using Student's *t*-test or analysis of variance. The relationship between the genotypes and the severity of anemia in patients with deletional Hb H disease and non-deletional Hb H disease was analyzed using χ^2 test. $p<0.05$.

Results

Genotypes Distribution in Hb H Patients

A total of 497 patients with Hb H disease were enrolled in this study, including 449 (90.3%) cases of deletional Hb H disease and 48 (9.7%) cases of non-deletional Hb H disease. Among patients with deletional Hb H disease, the $--^{SEA}/-\alpha^{3.7}$ genotype was the most prevalent, accounting for 66.2% (329/497) of all enrolled patients, followed by the $--^{SEA}/-\alpha^{4.2}$ genotype, which accounted for 24.1% (120/497) of all patients. For non-deletional Hb H disease, the $--^{SEA}/\alpha^{CS}\alpha$ genotype was the most common, representing 7.8% (39/497) of all patients, followed by $--^{SEA}/\alpha^{WS}\alpha$ (1.0%, 5/497) and $--^{SEA}/\alpha^{QS}\alpha$ (0.8%, 4/497) (Table 1).

The percentage of allele $--^{SEA}$ in all patients was the highest (50.0%), followed by $-\alpha^{3.7}$ (33.1%), $-\alpha^{4.2}$ (12.1%), α^{CS} (CD142, TAA→CAA) (3.9%), α^{WS} (CD122, CAC→CAG) (0.5%), and α^{QS} (CD125, CTG→CCG) (0.4%) (Table 2).

Relationship of Genotypes and Hematological Phenotypes in Patients with Hb H Disease

The detection rate of Hb H was significantly higher in patients with non-deletional HbH disease (excluded patients with β-thalassemia) (eg, $--^{SEA}/\alpha^{CS}\alpha$, and $--^{SEA}/\alpha^{QS}\alpha$) than in those with deletional Hb H disease (excluded patients with β-thalassemia) (73.8% vs 66.8%). Notably, Hb H was not detected in any patients with Hb H disease complicated by β-thalassemia (Table 3).

Based on anemia severity classification by hemoglobin levels, 90.2% of patients with deletional Hb H disease (excluded patients with β-thalassemia) presented with moderate or severe anemia, compared with 92.7% of those with

Table 1 The Genotypes in Patients with Hb H Disease

Genotypes	Number of Patients (%)
Deletional Hb H disease	449(90.3%)
$--^{SEA}/-\alpha^{3.7}$	319(64.2%)
$--^{SEA}/-\alpha^{4.2}$	118(23.7%)
$--^{SEA}/-\alpha^{3.7}$, β^0/β^N or β^+/β^N	10(2.0%)
$--^{SEA}/-\alpha^{4.2}$, β^0/β^N or β^+/β^N	2(0.4%)
Non-deletional Hb H disease	48(9.7%)
$--^{SEA}/\alpha^{CS}\alpha$	33(6.6%)
$--^{SEA}/\alpha^{WS}\alpha$	5(1.0%)
$--^{SEA}/\alpha^{QS}\alpha$	4(0.8%)
$--^{SEA}/\alpha^{CS}\alpha$, β^0/β^N or β^+/β^N	6(1.2%)
Total	497(100.0%)

Abbreviation: Hb H, hemoglobin H.

Table 2 All Deferent Alpha Globin Genes Mutations Identified

Alpha Globin Genes Mutations	Number of Alleles in The Sample(n)	Percentage of Alleles in The Sample
$--^{SEA}$	497	50.0%
$-\alpha^{3.7}$	329	33.1%
$-\alpha^{4.2}$	120	12.1%
α^{CS} (CD142, TAA→CAA)	39	3.9%
α^{WS} (CD122, CAC→CAG)	5	0.5%
α^{QS} (CD125, CTG→CCG)	4	0.4%
Total	994	100.0%

Table 3 Genotypes and Hematological Phenotypes of Patients with Hb H Disease

Genotypes	Hb (g/L)	MCV(fL)	MCH (pg)	HbA_2 (%)	HbF (%)	HbH (n,%)
Deletional Hb H disease (included patients with β-thalassemia)	**83.99±20.62**	**62.58±8.39**	**18.59±2.60**	**1.26±0.68**	**0.97±8.15**	**292(65.0%)**
Deletional Hb H disease (excluded patients with β-thalassemia)	**83.89±20.71**	**62.75±8.40**	**18.61±2.62**	**1.17±0.39**	**0.98±8.25**	**292(66.8%)**
$--^{SEA}/-\alpha^{3.7}$	84.27±20.23	62.89±8.43	18.60±2.62	1.19±0.39	0.18±0.69	217(68.0%)
$--^{SEA}/-\alpha^{4.2}$	82.88±22.00	62.38±8.35	18.64±2.62	1.10±0.41	3.10±15.56	75(63.6%)
$--^{SEA}/-\alpha^{3.7}$, β^0/β^N or β^+/β^N	86.78±18.69	56.78±5.38	17.78±1.48	4.59±0.46	0.30±0.50	
$--^{SEA}/-\alpha^{4.2}$, β^0/β^N or β^+/β^N	92.00±8.49	53.50±0.71	17.50±2.12	4.70±0.57	0.30±0.42	
Non-deletional Hb H disease (included patients with β-thalassemia)	**73.41±23.02**	**72.68±6.00**	**20.05±2.09**	**1.11±0.69**	**0.20±0.51**	**31(64.6%)**
Non-deletional Hb H disease (excluded patients with β-thalassemia)	**76.21±23.37**	**70.09±8.88**	**19.49±2.48**	**1.46±1.10**	**0.49±1.09**	**31(73.8%)**
$--^{SEA}/\alpha^{CS}\alpha$	67.91±20.06	74.19±5.22	20.00±2.00	0.96±0.48	0.27±0.58	27(81.8%)
$--^{SEA}/\alpha^{WS}\alpha$	109.60±11.80	65.80±3.27	20.40±0.55	2.42±0.22	-	
$--^{SEA}/\alpha^{QS}\alpha$	72.25±14.64	69.25±8.06	20.00±4.00	0.48±0.50	-	4(100.0%)
$--^{SEA}/\alpha^{CS}\alpha$, β^0/β^N or β^+/β^N	95.33±16.81	52.33±2.07	15.67±1.21	3.53±0.66	2.23±1.91	
Total	83.24±21.00	63.31±8.72	18.68±2.60	1.28±0.73	0.92±7.77	323(67.4%)

Notes: Bolded values represent the results of the subgroups of the deletional Hb H disease and non-deletional Hb H disease (in order to distinguish the results from patients with different genotypes).

Abbreviations: Hb H, hemoglobin H; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Hb A_2, hemoglobin A_2; Hb F, fetal hemoglobin.

non-deletional Hb H disease (excluded patients with β-thalassemia). All patients carrying the $–^{SEA}/\alpha^{QS}\alpha$ genotype had moderate or severe anemia, while this proportion reached 96.9% among patients with the $–^{SEA}/\alpha^{CS}\alpha$ genotype. For patients with the $–^{SEA}/\text{-}\alpha^{4.2}$ and $–^{SEA}/\text{-}\alpha^{3.7}$ genotypes, the rates of moderate or severe anemia were 90.5% and 90.0%, respectively. Among patients with –/-α or $–/\alpha^{T}\alpha$ genotypes combined with β^{N}/β^{N}, 26.9% had severe anemia, and only 11.1% of patients with –/-α or $–/\alpha^{T}\alpha$ combined with β^{0}/β^{N} or β^{+}/β^{N} presented with severe anemia, which was lower than that in patients with isolated α-thalassemia-related Hb H disease. Additionally, the proportion of patients with severe anemia and non-deletional Hb H disease was significantly higher than in patients with deletional Hb H disease (43.9% vs 26.0%, p=0.018, χ^2=6.008) (Table 4).

Laboratory Parameter Comparisons: Deletional vs Non-Deletional Hb H Patients, and Hb H Patients with β-Thalassemia vs Those Without

Patients with non-deletional Hb H disease exhibited more severe anemia compared to those with deletional Hb H disease (Hb 73.41±23.02 vs 83.89±20.71 g/L, p=0.002), accompanied by significantly higher MCV (72.68±6.00 vs 62.75±8.40 fL, p<0.001) and MCH (20.05±2.09 vs 18.61±2.62 pg, p=0.001) values. Additionally, Hb H disease patients without β-thalassemia presented with more severe anemia symptoms and higher MCV (63.62±8.68 vs 54.82±4.53 fL, p<0.001) and MCH (18.74±2.61 vs 17.00±1.70 pg, p=0.007) levels than those carrying β-thalassemia. In other words, the manifestations of microcytic hypochromia were less pronounced in Hb H disease patients without β-thalassemia relative to those with β-thalassemia (Table 5).

Comparison of the Allele Constituent Ratios of Hb H Disease in Some Populations

We analyzed the proportion of various genotypes among Hb H disease patients reported in some populations (a populations from Guangdong Province of China,[20] some populations from Guangxi Province of China,[21–23] a group of people from Taiwan Province of China,[24] and some populations from Thailand[25–27]). We found that in most populations, the $–^{SEA}/\text{-}\alpha^{3.7}$ genotype accounted for the majority of Hb H patients. One notable feature was that in the populations of Guangxi Province in China, Taiwan Province, and Thailand, the proportion of $–^{SEA}/\alpha^{CS}\alpha$ was relatively higher (Table 6).

Table 4 Relationship of Genotypes and the Severity of Anemia Based on Hemoglobin Levels in Patients with Hb H Disease

Genotypes	Normal (Hb ≥120 g/L)	Mild (Hb 110–119 g/L)	Moderate (Hb 70–109 g/L)	Severe (Hb <70 g/L)
Deletional Hb H disease (included patients with β-thalassemia)	**18(4.1%)**	**24(5.5%)**	**283(64.6%)**	**113(25.8%)**
Deletional Hb H disease (excluded patients with β-thalassemia)	**18(4.2%)**	**24(5.6%)**	**274(64.2%)**	**111(26.0%)**
$–^{SEA}/\text{-}\alpha^{3.7}$	15(4.8%)	16(5.1%)	200(64.3%)	80(25.7%)
$–^{SEA}/\text{-}\alpha^{4.2}$	3(2.6%)	8(6.9%)	74(63.8%)	31(26.7%)
$–^{SEA}/\text{-}\alpha^{3.7}$, β^{0}/β^{N} or β^{+}/β^{N}	0(0)	0(0)	7(77.8%)	2(22.2%)
$–^{SEA}/\text{-}\alpha^{4.2}$, β^{0}/β^{N} or β^{+}/β^{N}	0(0)	0(0)	2(100.0%)	0(0)
Non-deletional Hb H disease (included patients with β-thalassemia)	**2(4.3%)**	**2(4.3%)**	**25(53.2%)**	**18(38.3%)**
Non-deletional Hb H disease (excluded patients with β-thalassemia)	**1(2.4%)**	**2(4.9%)**	**20(48.8%)**	**18(43.9%)**
$–^{SEA}/\alpha^{CS}\alpha$	0(0)	1(3.1%)	15(46.9%)	16(50.0%)
$–^{SEA}/\alpha^{WS}\alpha$	1(20.0%)	1(20.0%)	3(60.0%)	0(0)
$–^{SEA}/\alpha^{QS}\alpha$	0(0)	0(0)	2(50.0%)	2(50.0%)
$–^{SEA}/\alpha^{CS}\alpha$, β^{0}/β^{N} or β^{+}/β^{N}	1(16.7%)	0(0)	5(83.3%)	0(0)
Total	**20(4.1%)**	**26(5.4%)**	**308(63.5%)**	**131(27.0%)**
p values*	0.717 (χ^2=0.303)	1.000 (χ^2=0.039)	0.062 (χ^2=3.793)	0.018 (χ^2=6.008)

Notes: Bolded values represent the results of the subgroups of the deletional Hb H disease and non-deletional Hb H disease (in order to distinguish the results from patients with different genotypes).

Abbreviations: Hb H, hemoglobin H; Hb, hemoglobin; *, Deletional HbH disease (excluded patients with β-thalassemia) vs Non-deletional HbH disease (excluded patients with β-thalassemia).

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Table 5 Comparison of Laboratory Parameters Between Patients with Deletional Hb H and Non-Deletional Hb H, and Patients with and without β-Thalassemia, Respectively

Laboratory Parameters	Deletional (n=427)	Non-Deletional (n=41)	*p* values	Without β-Thalassemia (n=479)	With β-Thalassemia (n=18)	*p* values
Hb (g/L)	83.89±20.71	73.41±23.02	0.002	82.89±21.10	90.41±16.86	0.152
MCV(fL)	62.75±8.40	72.68±6.00	<0.001	63.62±8.68	54.82±4.53	<0.001
MCH (pg)	18.61±2.62	20.05±2.09	0.001	18.74±2.61	17.00±1.70	0.007
HbA_2 (%)	1.17±0.39	1.11±0.69	0.627	1.16±0.43	4.23±0.74	<0.001
HbF (%)	0.98±8.25	0.20±0.51	0.571	0.92±7.91	0.98±1.48	0.974

Abbreviations: Hb H, hemoglobin H; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Hb A_2, hemoglobin A_2; Hb F, fetal hemoglobin.

Discussion

This study analyzed the hematological data of 497 patients with Hb H disease and systematically explored the relationship between different genotypes and hemoglobin levels as well as the severity of anemia. It provided important evidence for understanding the clinical heterogeneity of Hb H disease. The results showed that the genetic differences of Hb H disease patients were significantly associated with their severity of anemia, which was consistent with the conclusions of most previous studies.[16,22,24,28]

The differences in the degree of α globin chain synthesis defect caused by different genotypes are the core factor determining the level of hemoglobin.[29,30] Deletional Hb H disease primarily results in a proportional decrease in α-globin chain synthesis due to the reduction in α-globin gene copy number.[14] The underlying mechanism is the gene dosage effect, where the transcriptional and translational products of the remaining functional α-globin genes cannot fully compensate for the expression loss of the deleted genes, thereby triggering an imbalance between α and β globin chains.[6] Since no structural mutations of the gene are involved, the defect in chain synthesis is relatively mild. Patients with the deletional Hb H disease (such as $--^{SEA}/-\alpha^{3.7}$ and $--^{SEA}/-\alpha^{4.2}$) retain one functional α globin gene (with the synthesis amount of the α chain being approximately 25% of the normal level), have a relatively mild excess of β chains.[31] The production of Hb H (β4) is less and its stability is relatively higher. Such patients mainly exhibit mild or moderate anemia, which is directly related to the relatively stable synthesis of the α chain.

In contrast, non-deletional Hb H is the combination of two absences and one functional mutation of α-globin genes.[32] Non-deletional Hb H disease is mostly caused by point mutations in the coding or regulatory regions of the α-globin gene.[14] These mutant genes may hinder the synthesis of abnormal α-globin chains or induce functional abnormalities through mechanisms such as interfering with mRNA stability, inhibiting ribosome binding, or disrupting peptide chain folding.[33] Even with the retention of some normal gene copies, more severe defects in α-globin chain synthesis still occur. Additionally, the mutant α-globin chains may form unstable complexes that exacerbate hemolysis.[34] The mRNA transcribed from the mutated α-globin gene is unstable, or the translated product exhibits impaired function—leading to a reduction in α-globin chain synthesis to 10%-15% of the normal level. This exacerbates the excess of β-globin chains, doubles the production of Hb H, and increases its tendency to precipitate. Clinically, most such patients present with moderate to severe anemia.[32,35]

The differences in the severity of anemia are essentially a quantitative reflection of the hemolytic pathological process caused by α-chain synthesis defects.[36] The genotype determines the hematological phenotype of anemia by regulating the intensity and duration of this process.[17] Patients with deletional Hb H disease have relatively sufficient α-chain synthesis, resulting in a slower Hb H precipitation rate.[37] Erythrocyte destruction primarily occurs within the mononuclear phagocyte system (MPS), particularly in the spleen, with moderate hemolysis that progresses gradually.[38] Therefore, anemia in these patients is predominantly characterized by persistent moderate anemia, with life-threatening acute hemolytic crises being rare. In clinical settings, the degree of spleen enlargement in such patients is relatively mild, and the incidence of iron overload is low, which conforms to the typical characteristics of intermediate anemia.[39]

The pathogenic mechanism underlying anemia in non-deletional Hb H disease is more intricate. Beyond the substantial production of Hb H resulting from severe impairment of α-globin chain synthesis, the inherent instability

Table 6 Comparison of the Allele Constituent Ratios of Hb H Disease in Some Populations

Populations	First	Second	Third	Fourth	Fifth	Ref
A group of people from Guangzhou city, Guangdong Province, China (n=435)	$--^{SEA}/-\alpha^{3.7}$(54.0%)	$--^{SEA}/-\alpha^{4.2}$(21.6%)	$--^{SEA}/\alpha^{CS}\alpha$(17.9%)	$--^{SEA}/\alpha^{QS}\alpha$(6.2%)		20
A group of people from Baise city, Guangxi Province, China (n=1246)	$--^{SEA}/\alpha^{CS}\alpha$(44.86%)	$--^{SEA}/-\alpha^{3.7}$(35.32%)	$--^{SEA}/-\alpha^{4.2}$(13.72%)	$--^{SEA}/\alpha^{WS}\alpha$(4.33%)	$--^{SEA}/\alpha^{QS}\alpha$(1.45%)	21
A group of people from Liuzhou city, Guangxi Province, China (n=615)	$--^{SEA}/-\alpha^{3.7}$(45.2%)	$--^{SEA}/-\alpha^{4.2}$(17.23%)	$--^{SEA}/\alpha^{CS}\alpha$(15.61%)	$--^{SEA}/\alpha^{WS}\alpha$(10.73%)	$--^{SEA}/\alpha^{QS}\alpha$(3.42%)	22
A group of people from Nanning city, Guangxi Province, China (n=357)	$--^{SEA}/\alpha^{CS}\alpha$(53.5%)	$--^{SEA}/-\alpha^{3.7}$(25.2%)	$--^{SEA}/-\alpha^{4.2}$(14.0%)	$--^{SEA}/\alpha^{WS}\alpha$(5.3%)	$--^{SEA}/\alpha^{QS}\alpha$(2.0%)	23
A group of people from Kaohsiung, Taiwan Province, China (n=38)	$--^{SEA}/-\alpha^{3.7}$(42.1%)	$--^{SEA}/\alpha^{CS}\alpha$(34.2%)	$--^{SEA}/\alpha^{QS}\alpha$(15.8%)	$--^{SEA}/-\alpha^{4.2}$(7.9%)		24
A group of people from Nakhon Nayok, Thailand (n=479)	$--^{SEA}/-\alpha^{3.7}$(65.14%)	$--^{SEA}/\alpha^{CS}\alpha$(27.35%)	$--^{SEA}/-\alpha^{4.2}$(5.43%)	$--^{SEA}/\alpha^{QS}\alpha$(0.63%)		25
A group of people from various provinces in southern Thailand (n=260)	$--^{SEA}/-\alpha^{3.7}$(66.15%)	$--^{SEA}/\alpha^{CS}\alpha$(28.46%)	$--^{SEA}/-\alpha^{4.2}$(1.92%)	$--^{SEA}/\alpha^{QS}\alpha$(1.54%)		26
A group of people from Chiang Mai, Thailand (n=102)	$--^{SEA}/\alpha^{CS}\alpha$(53.0%)	$--^{SEA}/-\alpha^{3.7}$(33.3%)	$--^{SEA}/-\alpha^{4.2}$(9.8%)			27
Present study	$--^{SEA}/-\alpha^{3.7}$(66.3%)	$--^{SEA}/-\alpha^{4.2}$(24.2%)	$--^{SEA}/\alpha^{CS}\alpha$(7.7%)	$--^{SEA}/\alpha^{WS}\alpha$(1.0%)	$--^{SEA}/\alpha^{QS}\alpha$(0.8%)	

Abbreviation: Hb H, hemoglobin H.

of the mutated α-globin chain itself can elicit additional oxidative stress responses,[40,41] thereby accelerating erythrocyte membrane damage and inclusion body formation.[42,43] This "double blow" significantly shortens erythrocyte survival, escalating hemolysis to a moderate-to-severe degree and frequently accompanied by an intravascular hemolysis components. Clinically, patients not only present with persistent severe anemia but are also more susceptible to hemolytic crises triggered by factors such as infection and pregnancy.[44] Moreover, the excessive compensatory hyperplasia of the bone marrow caused by long-term severe anemia may lead to skeletal deformities.[45] In patients with severe hemolytic anemia, frequent blood transfusions carry the risk of iron overload, which further aggravates damage to organs such as the heart and liver, forming a "anemia-compensation-damage" vicious cycle.[46]

Although the genotype is the core factor determining the level of hemoglobin and the severity of anemia, the existence of phenotypic heterogeneity makes this association not absolutely linear. The hematological phenotype of patients with the same genotype may vary due to the following mechanisms. Firstly, the polymorphism of modifier genes, such as variants in genes encoding red blood cell membrane proteins (alpha spectrin (SPTA1)),[47] antioxidant enzyme (glucose-6-phosphate dehydrogenase (G6PD)),[48] or iron metabolism-related proteins (HFE),[49] can regulate the severity of anemia by affecting the stability of red blood cells or the iron load status. Secondly, the interaction of environmental factors, such as infections (especially viral hepatitis),[50] exposure to oxidative drugs (such as sulfonamides),[51] or nutritional deficiencies (such as insufficient folic acid and vitamin B12),[52] can induce oxidative stress or inhibit hematopoietic function, exacerbating anemia symptoms. Studies have shown that malaria can exacerbate the symptoms of patients with anemia.[53,54] In addition to the variant types of core pathogenic genes, differences in gene interaction patterns within individual populations further amplify the heterogeneity of hematological phenotypes in Hb H disease.[13] Gene interaction mechanisms such as the compound inheritance of Hb H disease with β-thalassemia and polymorphisms in the α-globin gene regulatory regions within populations contribute to the enhanced phenotypic variability.[55] The population-specific nature of these variant-phenotype associations provides a critical basis for precise diagnosis, prognostic assessment, and genetic counseling. Future studies should focus on non-coding region variants and gene-environment interactions to refine the understanding of the underlying mechanisms.

In this study, the detection rate of Hb H in the hemoglobin electrophoresis of Hb H patients was 67.4% (323/479) (Table 3), which was relatively lower compared to other studies. It might be due to the lower stability of Hb H, as well as the fact that for a few Hb H patients, the Hb H content was extremely low and could result in false negatives due to the resolution limitations of the electrophoresis. In addition, we analyzed the proportion of various genotypes among Hb H disease patients reported in some populations. In most populations, the $--^{SEA}/-\alpha^{3.7}$ genotype accounted for the majority of Hb H patients. One notable feature was that in the populations of Guangxi Province in China, Taiwan Province, and Thailand, the proportion of $--^{SEA}/\alpha^{CS}\alpha$ was relatively higher (Table 6). The distribution of Hb H disease genotypes varies among different populations, which requires that the prevention and control of thalassemia in different regions should adopt strategies that are suitable for the actual conditions of each region.

Clarifying the association between the severity of anemia in patients with Hb H disease and their genotypes holds significant clinical practical value. Genotype testing can serve as a reliable indicator for predicting the prognosis of patients. For non-deletional Hb H patients, a long-term blood transfusion plan and iron chelation therapy should be initiated as early as possible, and close monitoring of spleen enlargement and bone lesions should be conducted.[14,18] For deletional Hb H patients, regular follow-up is the main approach, with a focus on preventing infections and avoiding oxidative drugs.[14,56] Moreover, in-depth research on phenotypic heterogeneity may provide new targets for individualized treatment, such as intervention targeting specific modified genes or the precise application of antioxidant therapy. However, this study still has certain limitations. Firstly, as a retrospective study, the information on some patients' blood transfusion history and iron overload treatment was not recorded in sufficient detail, which might interfere with the analysis of hemoglobin levels. Secondly, this study did not include other non-deletional and deletional α-thalassaemia types,[35,57,58] which limited the comprehensive clarification of the genotype-phenotype relationship. Finally, the lack of detection of iron metabolism indicators (such as serum ferritin) prevented further analysis of the cumulative effect of iron overload on the severity of anemia.

Future research could be advanced in the following directions. Firstly, conduct prospective cohort studies to dynamically monitor hemoglobin level fluctuations and treatment responses in Hb H disease patients with distinct

genotypes. Secondly, integrate sequencing technologies with multi-omics analytical approaches to investigate rare mutations and the underlying molecular mechanisms linking them to phenotypic variability.[13,59] Thirdly, pool data from multi-center studies to expand the sample size, thereby validating the generalizability of the conclusions drawn from this research. In summary, clarifying the genotype-phenotype correlations in Hb H disease patients facilitates individualized risk stratification and precision medicine, ultimately improving the long-term prognosis of patients.

Conclusions

Genotypes are closely related to the severity of anemia in patients with Hb H disease. Non-deletional Hb H disease exhibited higher detection rate of Hb H and proportion of severe anemia, and patients with $--^{SEA}/\alpha^{CS}\alpha$ have the highest proportion of severe anemia. There are differences in the genotypes distribution of Hb H disease among different populations. The results of this study provide an important basis for the precise clinical diagnosis and personalized treatment of Hb H disease in the local area. However, the study has limitations such as insufficient sample size, limited geographical and age coverage, and failure of genetic testing to cover all mutations. In the future, it is necessary to expand the sample size and broaden the geographical and age coverage through multi-center cooperation, and conduct in-depth research on molecular mechanisms, so as to improve the universality of the research results and provide a more solid theoretical basis for disease prevention and treatment.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Clearance No.: PY-C2024018). This retrospective study has legally obtained the access rights to the patients' medical records from Meizhou People's Hospital. The act of reviewing the medical records was in accordance with the regulations and was supervised by the Ethics Committee of Meizhou People's Hospital. All participants were informed on the study procedures and goals and the informed consent from all the participants was obtained.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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