Genotypic and hematological profiling of thalassemia in reproductive-age and pediatric populations

YANHONG LIU[1*], WEI YE[2*], YAN ZHANG[1], TAILANG YIN[3] and JINGWEI WANG[1]

[1]Department of Clinical Laboratory, Institute of Translational Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China; [2]School of Laboratory Medicine, Hubei University of Chinese Medicine, Wuhan, Hubei 430065, P.R. China; [3]Reproductive Medicine Center, Department of Obstetrics and Gynecology, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China

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Abstract. Thalassemia is mainly prevalent in southern China, but consistent regional epidemiological data remain scarce in Hubei Province (central China). The present study characterized thalassemia genotypes and hematological parameters in 2,604 non-selectively screened individuals of reproductive-age (mean age, 31.39±5.72 years) and 407 pediatric subjects (mean age, 3.19±4.31 years) with clinical indications enrolled at Renmin Hospital of Wuhan University (Wuhan, Hubei), from January 2019 to September 2024. Peripheral blood and serum samples were analyzed for red blood cell count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum iron, total iron binding capacity, serum ferritin (SF), folate, transferrin saturation and vitamin B12 (VITB12) levels. Genetic testing was performed using gap-polymerase chain reaction (gap-PCR), reverse dot blot, Sanger sequencing and research-grade gap-PCR. The overall thalassemia carrier rates were 14.94% (reproductive-age group), 37.35% (pediatric group), and 17.97% (combined). Common α-thalassemia genotypes included--SEA/αα and -α3.7/αα, and dominant β-thalassemia mutations were β654, β41-42 and β17, aligning with neighboring provinces, differing from southern high-prevalence regions. Most phenotypes exhibited reduced RBC, Hb, MCV, MCH (all P<0.01) and elevated SF, VITB12. The high pediatric carrier rate may stem from selection bias due to clinical enrollment criteria. Notably, Hb and MCH were effective screening markers. In conclusion, Hubei has a substantial thalassemia burden (especially in children), and enhanced prenatal screening/counseling is urgently needed.

Correspondence to: Dr Jingwei Wang, Department of Clinical Laboratory, Institute of Translational Medicine, Renmin Hospital of Wuhan University, 99 Ziyang Road, Wuchang, Wuhan, Hubei 430060, P.R. China
E-mail: sophia_wang03@whu.edu.cn

*Contributed equally

Key words: thalassemia, genotype characteristics, hematological indicators, reproductive-age population, pediatric population

Introduction

Thalassemia is one of the most common hematological disorders globally, characterized by impaired synthesis of hemoglobin (Hb) chains. The primary form of adult hemoglobin is hemoglobin A (HbA), which accounts for >95% of total Hb and exists as a tetramer composed of two α-globin chains and two β-like globin chains ($\alpha_2\beta_2$). Disruption of the balanced synthesis of these α- and β-globin chains, triggered by mutations or deletions in the α-globin-encoding genes *HBA1* (OMIM **141800*) and *HBA2* (OMIM **141850*), or the β-globin-encoding gene *HBB* (OMIM * *141900*), leads to red blood cell damage and hemolysis, ultimately resulting in α-thalassemia or β-thalassemia, respectively (1,2). Genetically, the human genome normally contains four functional copies of *HBA* gene (genotype: αα/αα) and two functional copies of the *HBB* gene (genotype: β^N/β^N). The clinical severity of thalassemia is directly tied to the number of functionally defective *HBA* or *HBB* alleles. Based on this allele dosage effect, α-thalassemia is classified into four subtypes: α-thalassemia minima (α^+ trait, mildest, often asymptomatic), α-thalassemia trait (αTT, mild anemia), α-thalassemia intermedia (αTI, moderate anemia requiring intermittent transfusion), and α-thalassemia major (αTM, severe, life-threatening anemia); β-thalassemia is classified into three subtypes: β-thalassemia trait (βTT, mild asymptomatic anemia), β-thalassemia intermedia (βTI, moderate anemia with infrequent transfusion needs), and β-thalassemia major (βTM, severe anemia requiring lifelong regular transfusions) (3-4).

Among the regions heavily affected by thalassemia worldwide, China, especially the southern provinces, stands out due to high disease prevalence and significant associated health burdens, making it a key focus for thalassemia epidemiological and clinical research (5). In recent years, due to population mobility and the growing number of inter-regional marriages, the thalassemia carrier rate has been exhibiting a trend towards northward expansion (6).

In accordance with national health policies, Hubei Province incorporated thalassemia into the newborn screening program in 2016, and subsequently the screening was extended to the prenatal stage in 2022. However, the epidemiological data on thalassemia in Hubei exhibit regional variability. In light of these disparities, the present study focused on individuals of reproductive age and the pediatric population in Hubei in an aim to analyze thalassemia-associated genetic mutations and hematological changes. It is hoped that the results of the present study can provide comprehensive and solid data for genetic counseling, disease prevention and early intervention strategies.

Materials and methods

Study subjects. Between January, 2019 and September, 2024, a total of 2,604 individuals of reproductive age and 407 pediatric subjects, who underwent thalassemia gene testing at Renmin Hospital of Wuhan University (Wuhan, China), were enrolled in the present study. The individuals of reproductive age, which were non-selectively screened, had a mean age of 31.39±5.72 years (age range, 17-60 years) and included 526 males (20.20%; mean age, 33.25±6.36 years) and 2,078 females (79.80%; mean age, 30.93±5.45 years). The pediatric subjects had a mean age of 3.19±4.31 years (age range, 1 day to 16 years), consisting of 230 males (mean age, 2.72±3.88 years) and 177 females (mean age, 3.78±4.76 years). These pediatric subjects were enrolled based on clinical indications, including jaundice, anemia, hemolytic anemia, or a family history of thalassemia. Hematological and anemia-related biochemical data collected during their hospital visits were analyzed. Ethical approval for the present study was obtained from the Medical Ethics Committee of Renmin Hospital of Wuhan University (Wuhan, China), with the approval no. WDRY2023-K175. Individual informed consent was waived by the Ethics Committee of Renmin Hospital of Wuhan University, as the study involved no additional interventions, and all data were analyzed in a de-identified manner.

Instruments and reagents. Hematological analysis was performed using the Sysmex XN-9000 automated hematology analyzer (Sysmex Corporation). The following reagents were used: the CELLPACK DCL diluent (Sysmex Corporation) for cell counting and hemoglobin measurement, the SULFOLYSER lysing reagent (Sysmex Corporation) for hemoglobin release, and the STROMATOLYSER-FB reagent (Sysmex Corporation) for fluorescent staining of nucleic acids to perform leukocyte differential counting. Biochemical assays for serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF), folate (FOL), and vitamin B12 (VITB12) were performed on an ADVIA 1800 analyzer (Siemens Healthineers) using the manufacturer's corresponding reagent kits, namely the ADVIA Chemistry Iron Assay (cat. no. 10377510), ADVIA Chemistry TIBC Assay (cat. no. 03940010), ADVIA Centaur Ferritin Assay (cat. no. 10309969), ADVIA Centaur Folate Assay (cat. no. 124838), and ADVIA Centaur Vitamin B12 Assay (cat. no. 10330218), respectively. Transferrin saturation (TS) was calculated from the SI and TIBC results. The GeneRotex 96 nucleic acid extraction system (Xi'an Tianlong Science and Technology Co., Ltd.) and whole blood genomic DNA extraction kits (cat. no. T146H; Xi'an Tianlong Science and Technology Co., Ltd.) were utilized for genomic DNA extraction. A NanoDrop spectrophotometer (Thermo Fisher Scientific) was used to assess DNA quality. Thalassemia mutations were analyzed using commercial detection kits from Shenzhen Yaneng Bioscience, including the deletion α-thalassemia gene assay kit (cat. no. 20243401205), the non-deletion α-thalassemia gene assay kit (cat. no. 20193401915), and the β-thalassemia gene assay kit (cat. no. 20163400463). An Automatic Hybridization System (Shenzhen Yaneng Bioscience) was used to identify thalassemia gene mutations. A 3500 Genetic Analyzer (Thermo Fisher Scientific) and a Veriti 96-well Fast Thermal Cycler (Thermo Fisher Scientific) were used for further genetic analysis.

Hematology and biochemical analyses. Peripheral venous blood (2 ml) was collected in EDTA anticoagulant tubes for the analysis of red blood cell count (RBC), Hb, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), which were measured using a Sysmex XN-9000 automated hematology analyzer (Sysmex Corporation). Serum samples (5 ml) were collected into a serum separator tube with inert gel for the measurement of SI, TIBC, SF, TS, FOL and VITB12 levels, which were determined using an ADVIA 1800 clinical chemistry analyzer (Siemens Healthineers).

Genetic testing. Genomic DNA was first extracted and then quantified to ensure its concentration ranged from 50-150 ng/μl, with an OD260/280 ratio between 1.7 and 1.9. Gap-polymerase chain reaction (gap-PCR) was applied to detect three common α-thalassemia deletions, namely--SEA, -α3.7 and -α4.2. Reverse dot blot (RDB) was used to identify non-deletion α-thalassemia mutations (αWS, αQS and αCS) and 17 β-thalassemia mutations (CD41-2, βE, CD43, CD71-72, IVS-II-654, -28, -29, -30, -32, CD14-15, CD27-28, CD31, IVS-I-1, IVS-I-5, CAP, CD17 and the initiation codon). The complete specifications of all tested mutations, including their Human Genome Variation Society (HGVS) nomenclature and functional impacts, are presented in Table I. Cases were considered discordant if the hematological indices (such as MCV <80 fl, MCH <27 pg) suggested a thalassemia trait, but the initial gap-PCR/RDB results were negative, or if genotype-phenotype inconsistencies were observed (for example severe anemia with mild genotype). Such cases were prioritized for Sanger sequencing and research-grade gap-PCR (Shenzhen Yaneng Bioscience) to confirm rare genotypes.

Clinical phenotype classification. Based on consensus guidelines (3,4), β-thalassemia phenotypes were classified into βTT, βTI, and βTM. α-thalassemia was categorized as α+ trait, αTT, αTI, αTM and rare genotypes.

Statistical analysis. Microsoft Excel was utilized to input and organize the data. Carrier rates and proportions are presented as percentages (%). For the carrier population data, the direct counting method was employed, with the number of detected cases denoted as 'n'. Hematological and biochemical

Table I. Common thalassemia mutations assessed and their molecular features.

Traditional name	Gene	Transcript	HGVS nomenclature	Common name	Type	Functional impact
31M	HBB	NM_000518.5	c.94delC (p.Arg32fs)	CD31(-C)	β^0	Translation affected
CapM	HBB	NM_000518.5	c.-11_-8delAAAC	CAP+43/+40 (-AAAC)	β^+	Transcription affected
41-42M	HBB	NM_000518.5	c.126_129delCTTT (p.Phe42Leufs*19)	CD 41/42 (-TTCT)	β^0	Translation affected
43M	HBB	NM_000518.5	c.130G>T (p.Glu44*)	CD 43 (G>T)	β^0	Translation affected
71-72M	HBB	NM_000518.5	c.216_217insA (p.Ala73Serfs*13)	CD 71/72 (+A)	β^0	Translation affected
IntM	HBB	NM_000518.5	c.2T>G (p.Met1Arg)	Init CD ATG>AGG	β^0	Translation affected
654M	HBB	NM_000518.5	c.316-197C>T	IVS2-654C>T	β^+	RNA processing affected
14-15M	HBB	NM_000518.5	c.45_46insG (p.Trp15Glyfs*2)	CD 14/15 (+G)	β^0	Translation affected
17M	HBB	NM_000518.5	c.52A>T (p.Lys18*)	CD 17 (AAG>TAG)	β^0	Translation affected
-28M	HBB	NM_000518.5	c.-78A>G	-28A>G	β^+	Transcription affected
-29M	HBB	NM_000518.5	c.-79A>G	-29A>G	β^+	Transcription affected
26M (βEM)	HBB	NM_000518.5	c.79G>A (p.Glu27Lys)	CD 26 GAG>AAG [Glu>Lys]	β^0	RNA processing affected
-30M	HBB	NM_000518.5	c.-80T>C	-30T>C	β^0/β^+	Transcription affected
-32M	HBB	NM_000518.5	c.-82C>A	-32 (C>A)	β^+	Transcription affected
27/28M	HBB	NM_000518.5	c.84_85insC (p.Leu28Profs*22)	CD 27/28 (+C)	β^0	Translation affected
IVS-I-1M	HBB	NM_000518.5	c.92+1G>T	IVS 1-1 (G>T)	β^0	RNA processing affected
IVS-1-5M	HBB	NM_000518.5	c.92+5G>C	IVS1-5G>C	β^+	RNA processing affected
--SEA	HBA1+ HBA2	NC_000016.9	g.215400_ 234700del19300	--SEA	α^0	α^0 deletion type
-α4.2	HBA2	NC_000016.9	g.219817_ 224074del4258	-α4.2	α^+	α^+ deletion type
-α3.7	HBA1 HBA2	NC_000016.9	g.223300_ 227103del3804	-α3.7	α^+	α^+ deletion type
αWS	HBA2	NM_000517.6	c.369C>G (p.His123Gln)	αWS	α^+	Translation affected
αQS	HBA2	NM_000517.6	c.377T>C (p.Leu126Pro)	αQS	α^0	Translation affected
αCS	HBA2	NM_000517.6	c.427T> (p.Trp143Gln)	αCS	α^0	Translation affected

The table comprehensively summarizes common α- and β-thalassemia mutations with details on traditional names, associated genes (*HBB* for β-thalassemia; *HBA1*/*HBA2* for α-thalassemia), transcript references, HGVS nomenclature, common abbreviations, mutation types, and functional impacts, where β^0-thalassemia denotes mutations causing complete absence of β-chain production, β^+-thalassemia indicates mutations with reduced β-chain synthesis, α^+-thalassemia refers to impaired expression of a single α-globin gene (via deletion or point mutation), and α^0-thalassemia represents absent expression of both α-globin genes on one allele (typically due to large deletions). The table further categorizes variants as deletional or non-deletional to facilitate genotype-phenotype correlations.

Table II. Prevalence and genotype distribution of thalassemia.

Clinical Phenotype	Genotype	Reproductive age group			Pediatric group			Statistics
		n	Proportions (%)	Carriage rate (%)	n	Proportions (%)	Carriage rate (%)	χ^2 and P-value
α^+ trait		86	43.43	3.30	18	26.87	4.42	1.32, 0.25
	$-\alpha^{3.7}/\alpha\alpha$	73	36.87	2.80	16	23.88	3.93	
	$-\alpha^{4.2}/\alpha\alpha$	9	4.55	0.35	2	2.99	0.49	
	$\alpha^{WS}\alpha/\alpha\alpha$	4	2.02	0.15	0	0.00	0.00	
αTT		99	50.00	3.80	48	71.64	11.79	48.41, <0.01[a]
	$^{--SEA}/\alpha\alpha$	93	46.97	3.57	43	64.18	10.57	
	$\alpha^{CS}\alpha/\alpha\alpha$	3	1.52	0.12	2	2.99	0.49	
	$\alpha^{QS}\alpha/\alpha\alpha$	1	0.51	0.04	2	2.99	0.49	
	$-\alpha^{3.7}/-\alpha^{4.2}$	1	0.51	0.04	1	1.49	0.25	
	$\alpha^{QS}\alpha/-\alpha^{3.7}$	1	0.51	0.04	0	0.00	0.00	
αTI		7	3.54	0.27	1	1.49	0.25	0.01, 0.93
	$^{--SEA}/-\alpha^{3.7}$	4	2.02	0.15	1	1.49	0.25	
	$\alpha^{CS}\alpha/^{--SEA}$	2	1.01	0.08	0	0.00	0.00	
	$^{--SEA}/-\alpha^{4.2}$	1	0.51	0.04	0	0.00	0.00	
Rare Genotype	$^{HK\alpha\alpha}/\alpha\alpha$	6	3.03	0.23	0	0.00	0.00	0.94, 0.33
	$^{HK\alpha\alpha}/-\alpha^{3.7}$	5	2.53	0.19	0	0.00	0.00	
	$^{HK\alpha\alpha}/^{--SEA}$	1	0.51	0.04	0	0.00	0.00	
Total		198	100	7.60	67	100	16.46	34.41, 4.46×10^{-9}[a]

[a]P<0.05 indicates a statistically significant difference between the reproductive-age and pediatric groups. αTT, α-thalassemia trait; αTI, α-thalassemia intermedia.

indicators were characterized using the mean ± standard deviation (SD). Statistical analyses were performed using Prism 9.0 (Dotmatics) and SPSS 13.0 (SPSS, Inc.) software. To compare continuous variables, t-tests were applied. For categorical variables, the chi-squared test was utilized for assessment. P<0.05 was considered to indicate a statistically significant difference.

Results

Prevalence and genotype distribution. The thalassemia carrier rate was 14.94% (389/2604) in the individuals of reproductive age and 37.35% (152/407) in the pediatric population. The carrier rates for α-thalassemia, β-thalassemia and αβ-thalassemia were 7.60% (198/2604), 7.68% (200/2604) and 0.35% (9/2604) in the individuals of reproductive age, respectively and 16.46% (67/407), 21.38% (87/407) and 0.49% (2/407) in the pediatric group, respectively (Tables II-IV).

The overall population carrier rate was 17.97% (541/3011), with the carrier rates for α-thalassemia and β-thalassemia being 8.8% (265/3011) and 9.53% (287/3011), respectively.

Predominant genotypes for thalassemia. The predominant genotypes observed in both groups were--SEA/αα and -α3.7/αα. The αTT phenotype was most common, accounting for 71.64% (48/67) in the pediatric group, which was significantly higher than that in the individuals of reproductive age [50.00% (99/198)] (P<0.01) (Table II).

The dominant genotypes in both groups were β654, β41-42 and β17. The βTT phenotype accounted for 98.00 and 96.55% of cases in the individuals of reproductive age and pediatric populations, respectively (Table III). A total of 11 cases of αβ-thalassemia were identified, presenting eight distinct genotypes (Table IV).

Regional genotype comparisons. The α-thalassemia genotype distribution in Hubei (--SEA/αα, -α3.7/αα-α4.2/αα) was consistent with that of neighboring regions (Hunan, Jiangxi, Chongqing and Guizhou); however, it differed from high-prevalence regions (Yunnan, Guangxi and Hainan) (7-14) (Fig. 1A). Similarly, the primary β-thalassemia genotypes (βN/β654 and βN/β41-42) in Hubei were similar to those in Hunan and Jiangxi, although they diverged from other regions (Fig. 1B).

Characteristics of hematological and anemia-related biochemical data among different clinical phenotype groups. Significantly reduced levels of RBC, Hb, MCV and MCH (P<0.05) were observed across all clinical phenotypes, apart from rare subtypes. Notably, the reductions in Hb and MCH parameters were more marked compared to those in RBC and MCV measurements (Fig. 2). Concurrently, thalassemia carriers exhibited significantly elevated SF and

Table III. Distribution and frequency of β-thalassemia genotype.

Clinical phenotype	Genotype	Reproductive-age group (n=2604) n	Reproductive-age group (n=2604) Proportions (%)	Reproductive-age group (n=2604) Carriage rate (%)	Pediatric group (n=407) n	Pediatric group (n=407) Proportions (%)	Pediatric group (n=407) Carriage rate (%)	Statistics χ^2 and P-value
βTT		200	98.00	7.53	86	96.55	20.64	74.07, <0.01[a]
	β^N/β^{654}	97	48.50	3.73	41	47.13	10.07	
	β^N/β^{41-42}	55	27.50	2.11	20	22.99	4.91	
	β^N/β^{17}	24	12.00	0.92	10	11.49	2.46	
	β^N/β^{-28}	9	4.50	0.35	3	3.45	0.74	
	β^N/β^E	6	3.00	0.23	3	3.45	0.74	
	β^N/β^{71-72}	2	1.00	0.08	3	3.45	0.74	
	β^N/β^{-29}	1	0.50	0.04	1	1.15	0.25	
	β^N/β^{43}	1	0.50	0.04	2	2.30	0.49	
	β^N/β^{CAP}	1	0.50	0.04	0	0.00	0.00	
	$\beta^N/\beta^{IVS-I-1}$	0	0.00	0.00	1	1.15	0.25	
	β^N/β^{27-28}	3	1.50	0.12	2	2.30	0.49	
	β^N/β^{14-15}	1	0.50	0.04	0	0.00	0.00	
βTI		0	0.00	0.00	1	1.15	0.25	6.40, 0.011[a]
	β^{654}/β^{654}	0	0.00	0.00	1	1.15	0.25	
Total		200	100	7.68	87	100	21.38	76.56, <0.01[a]

[a]P<0.05 (reproductive-age group vs. pediatric group). βTT, β-thalassemia trait; βTI, β-thalassemia intermediate.

Table IV. Distribution and frequency of αβ-thalassemia genotypes.

Genotype		Reproductive Age Group n	Reproductive Age Group Proportions (%)	Reproductive Age Group Carriage rate (%)	Pediatric Group n	Pediatric Group Proportions (%)	Pediatric Group Carriage rate (%)	Statistics χ^2 and P-value
α-Thalassemia	β-Thalassemia							
$^{--SEA}/\alpha\alpha$	β^N/β^{654}	2	22.22	0.08	0	0.00	0.00	
$^{--SEA}/\alpha\alpha$	$\beta^N/\beta E$	1	11.11	0.04	1	50.00	0.25	
$^{--SEA}/\alpha\alpha$	β^N/β^{17}	1	11.11	0.04	1	50.00	0.25	
$^{--SEA}/\alpha\alpha$	β^N/β^{-28}	1	11.11	0.04	0	0.00	0.00	
$-\alpha^{3.7}/\alpha\alpha$	β^N/β^{654}	1	11.11	0.04	0	0.00	0.00	
$-\alpha^{3.7}/\alpha\alpha$	β^N/β^{CAP}	1	11.11	0.04	0	0.00	0.00	
$-\alpha^{3.7}/\alpha\alpha$	β^N/β^{654}	1	11.11	0.04	0	0.00	0.00	
$-\alpha^{3.7}/\alpha\alpha$	β^N/β^{654}	1	11.11	0.04	0	0.00	0.00	
Total		9	100	0.35	2	100	0.49	0.21, 0.65

vitamin B12 levels (P<0.05 for both biomarkers), as detailed in Table V.

Discussion

Thalassemia, an inherited hematologic disorder, is characterized by a broad spectrum of genotypic variations and diverse clinical manifestations. Its high morbidity and substantial disease burden pose significant public health challenges. In China, the disease exhibits marked regional heterogeneity, with particularly high prevalence rates observed in southern provinces, including Hainan and Guangdong. Notably, the implementation of comprehensive prenatal screening programs in these high-endemic regions has effectively reduced the incidence of severe thalassemia cases among newborns, demonstrating the importance of preventive strategies.

The present large-scale genetic screening study provides comprehensive epidemiological data on thalassemia in Hubei Province, revealing several critical findings. The observed carrier rate of 14.94% among individuals of reproductive age aligns with regional reports from Tongji Hospital (19.08%)

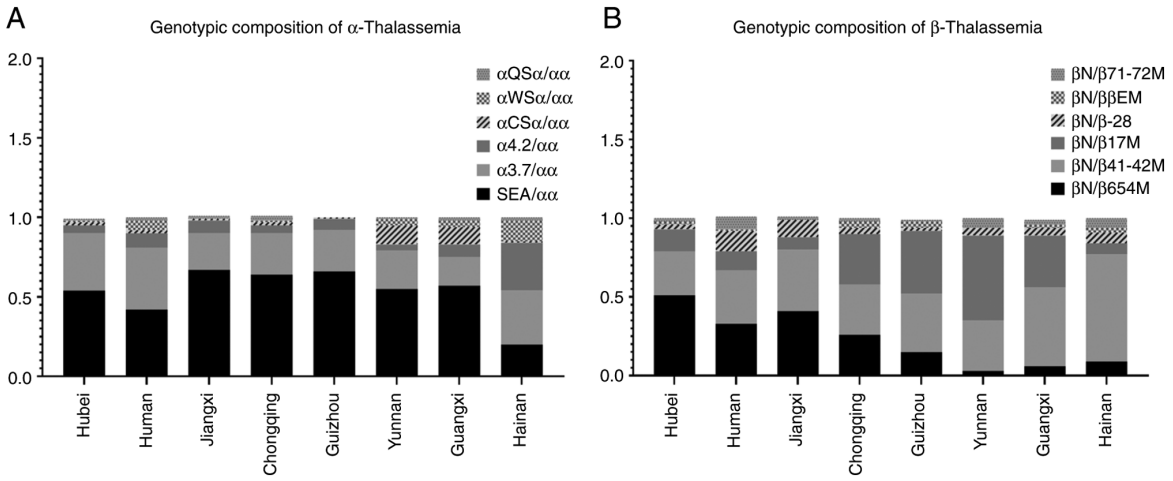

Figure 1. Genotypic composition of α-/β-thalassemia across geographic regions. (A) Depiction of the most prevalent genotypic distribution of α-thalassemia, including deletion variants (--SEA/αα, -α3.7/αα, -α4.2/αα) and non-deletion variants (αWSα/αα, αQSα/αα, αCSα/αα) in Hubei and other high-prevalence regions (Hunan, Jiangxi, Chongqing, Guizhou, Yunnan, Guangxi, Hainan). (B) Illustratrion of the dominant genotypic composition of β-thalassemia, featuring mutations β654, β41-42, β17, β-28, βE and β71-72M in the same regions, with βN denoting wild-type alleles. Bar heights reflect the relative frequency of each genotype, demonstrating that the genotypes in Hubei align with neighboring provinces (Hunan, Jiangxi, Chongqing and Guizhou), but differ from southern high-prevalence areas (Yunnan, Guangxi and Hainan).

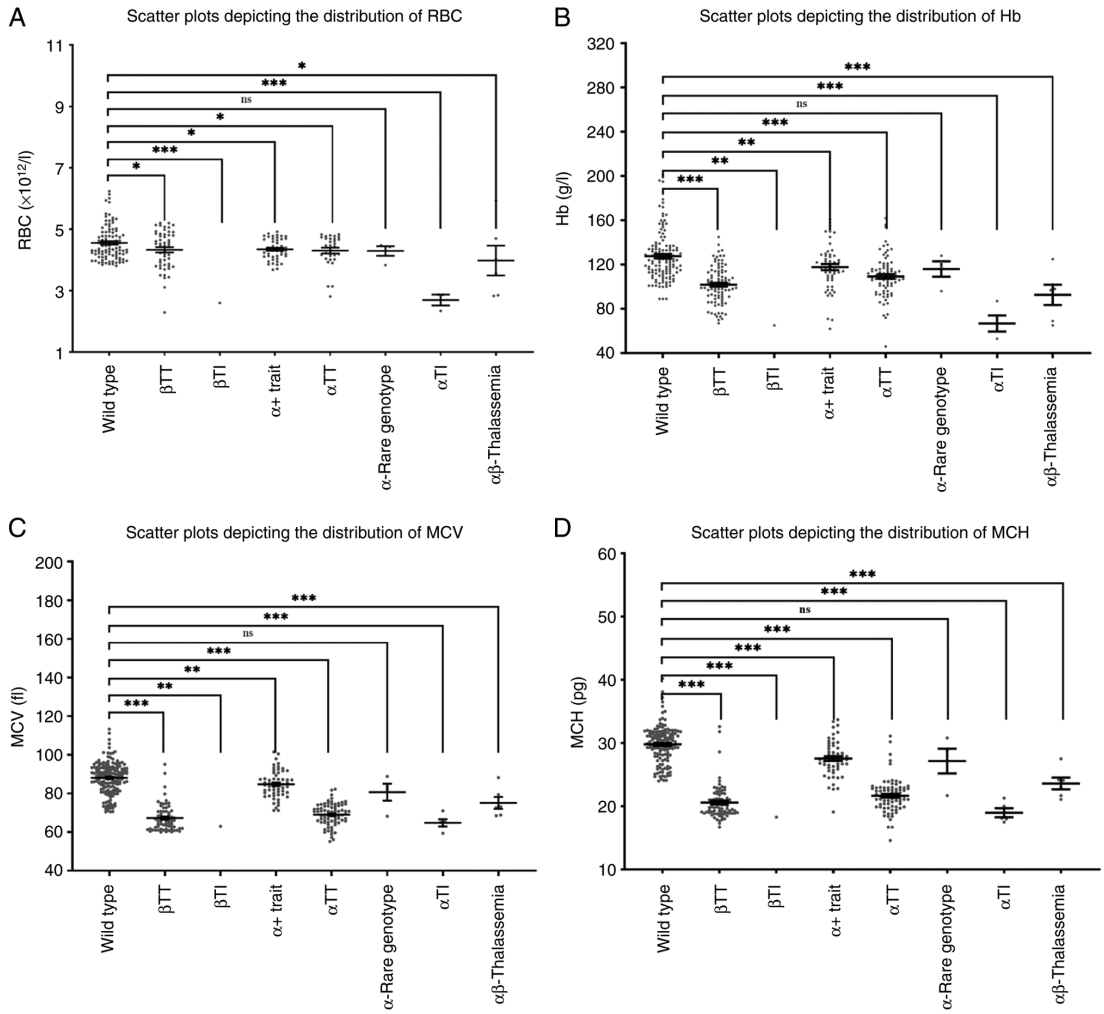

Figure 2. Distribution of RBC, Hb, MCV and MCH values across thalassemia clinical phenotypes. (A-D) Scatter plots illustrating individual data points with group medians for (A) RBC (x10^{12}/l), (B) Hb (g/l), (C) MCV (fl) and (D) MCH (pg) across thalassemia phenotypes, including βTT, βTI, αTT, αTI and αβ-thalassemia compared to wild-type controls, with symbols indicating statistical significance vs. wild type (ns, not significant; *P≤0.05, **P≤0.01 and ***P≤0.001) and hematological parameters differentiating phenotypes from controls and correlating with clinical severity. RBC, red blood cell count; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; βTT, β-thalassemia trait; βTI, β-thalassemia intermediate; αTT, α-thalassemia trait; αTI, α-thalassemia intermedia.

Table V. Variations in iron and folate metabolism in patients with thalassemia (mean ± SD, N).

Phenotype classification biochemical indicators	SF (ng/ml)	FOL (ng/ml)	VITB12 (pg/ml)	SI (μmol/l)	TIBC (μmol/l)	TS (%)
Wild type	68.94±20.35, 112	21.80±3.70, 78	287.90±27.06, 89	12.85±1.73, 64	49.63±3.01, 96	25.06±3.97, 82
α^+ trait	74.13±32.21, 18	12.28±2.49, 12	398.60±44.47, 11	13.98±3.10, 10	69.40±6.08, 12	23.47±4.55, 12
αTT	90.13±22.20, 40	14.00±1.10, 31	468.30±44.73, 31	22.38±4.40, 26	59.95±2.89, 33	26.25±3.13, 32
αTI	313.5±96.34, 6	13.41±6.05, 3	458.00±50.06, 3	24.02±7.33, 5	39.20±8.78, 4	64.08±11.20, 4
Rare genotypes	73.13±23.11, 3	10.67±4.04, 3	471.70±106.50, 3	18.33±3.41, 3	64.60±9.62, 3	30.99±9.14, 3
βTT	176.00±30.82, 58	23.76±9.12, 54	521.20±44.33, 54	18.97±1.28, 41	65.26±5.48, 59	29.74±2.00, 54
βTI	465.40±0.00, 1	19.24±0.00, 1	527.00±0.00, 1	16.40±0.00, 1	32.80±0.00, 1	50.00±0.00, 1
αβ-Thalassemia	69.25±5.65, 2	14.94±1.22, 3	432.30±40.99, 3	10.77±2.87, 3	51.87±3.24, 3	20.34±4.63, 3
χ^2 and P-value	2.95, 0.01[a]	0.26, 0.97	4.14, 0.00[b]	1.73, 0.11	1.95, 0.06	1.41, 0.21

[a]P<0.05 and [b]P<0.01. αTT, α-thalassemia trait; αTI, α-thalassemia intermedia; βTT, β-thalassemia trait; βTI, β-thalassemia intermediate; SF, serum ferritin; FOL, folate; VITB12, vitamin B12; SI, serum iron; TIBC, total iron binding capacity; TS, transferrin saturation.

and Changsha DIAN Medical Laboratory (26.18%) (15), while demonstrating some variation from other published data (16). This discrepancy may reflect differences in study populations or methodological approaches, such as variations in ethnic composition, geographical origins and sampling methods, as well as methodological differences in genetic testing and data analysis.

The present study positions Hubei as an emerging thalassemia-endemic region, with an overall carrier rate of 17.97%, particularly among pediatric populations (37.35%). The high pediatric carrier rate approaches that of traditional high-burden regions, indicating potential inadequacies in current screening programs (15,17-20). Given that the pediatric subjects in the present study had specific clinical indications related to thalassemia, it is likely that the actual carrier rate in the general pediatric population is lower, although this may still be concerning. This highlights the urgent need to strengthen prenatal prevention strategies, including expanding screening coverage, improving screening methods and enhancing genetic counseling services.

The genotypic distribution patterns observed in the present study are consistent with both intraprovincial reports within Hubei and inter-provincial data from neighboring regions (21-23). This suggests relative genetic homogeneity across the province and shared genetic-environmental influences with adjacent regions. The predominance of --SEA/αα and CD41-42 mutations aligns with established genetic drift patterns in central China, likely reflecting historical population migrations from southern endemic provinces where these mutations are highly prevalent (6). Understanding these genetic patterns is crucial for predicting disease prevalence, developing targeted prevention strategies and providing accurate genetic counseling.

The findings of the present study strongly support the primary thalassemia screening strategy in China, which combines phenotypic screening techniques by using RBC indices (RBC, Hb, MCV and MCH) with hemoglobin analysis. These parameters demonstrate a strong association with genetic diagnoses across all clinical phenotypes, rendering them effective initial screening tools. However, due to the potential for false negatives in routine blood tests, especially in high-prevalence regions, it is advisable to directly conduct universal genetic testing in prenatal care (24). This approach can improve the accuracy of diagnosis and enable early intervention, reducing the risk of severe thalassemia in offspring.

Notably, the present study reveals a previously unreported positive association between the thalassemia carrier status and elevated serum vitamin B12 levels (χ^2=4.14, P<0.01). This unexpected finding may be related to compensatory erythropoiesis, where the body increases the utilization of vitamin B12 as a substrate for hemoglobin synthesis in response to impaired hemoglobin production. However, considering the cross-sectional design of the present study and the limited sample size for this specific analysis, future research with larger cohorts and longitudinal measurements of vitamin B12 metabolism is required to confirm this association and clarify the underlying mechanisms.

In conclusion, the present study establishes Hubei as an emerging thalassemia-endemic region in central China, with pediatric carrier rates approaching those of traditionally high-burden areas. These findings emphasize the urgent need for targeted public health interventions, including enhanced screening programs, optimized genetic counseling services and region-specific prevention strategies (14,25). Although Hubei has implemented prenatal and newborn screening initiatives, the observed regional genotypic heterogeneity, such as distinct profiles compared to southern provinces, highlights the necessity for tailored screening algorithms. The identification of a substantial carrier rate among individuals of reproductive age (14.94%) and the utility of key hematological markers (Hb, MCH) provide actionable insight to refine local screening protocols, thereby reducing diagnostic gaps and improving the cost-effectiveness of existing programs. Integrating hematological and genetic screening approaches remains critical for effective thalassemia control

in Hubei, with the potential to mitigate disease burden and enhance population health outcomes. Notably, the higher carrier rate in pediatric subjects (37.35%) may be attributed to selection bias inherent in hospital-based sampling, as these individuals were recruited based on clinical indicators, such as anemia and jaundice, or a family history of thalassemia, factors strongly associated with increased thalassemia risk. This recruitment strategy likely overestimates the true population-level prevalence compared to community-based screening. Future studies utilizing representative community samples are therefore warranted to validate these estimates and provide a more accurate assessment of the regional thalassemia burden.

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Availability of data and materials

The data generated in the present study are not publicly available due restrictions that apply to the availability of these data, which were used under the license from the Ethics Committee of Renmin Hospital of Wuhan University, but may be requested from the corresponding author.

Authors' contributions

WY made a substantial contribution to the acquisition of data by performing all laboratory experiments for thalassemia diagnosis. YL substantially contributed to the data acquisition by performing clinical examinations and compiling the hematological and biochemical profiles. JW was responsible for reviewing and validating the data analysis results, as well as drafting the introduction and discussion sections of the manuscript. TY and YZ confirm the authenticity of all the raw data and conducted data analysis. Additionally, TY and YZ took primary responsibility for drafting the initial versions of the methodology, the results sections and all tables of the manuscript. JW designed the study and edited the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval for the present study was obtained from the Medical Ethics Committee of Renmin Hospital of Wuhan University (approval no. WDRY2023-K175; Wuhan, China). This retrospective study utilized anonymized clinical laboratory data from routine patient care at Renmin Hospital of Wuhan University. Individual informed consent was waived by the Ethics Committee of Renmin Hospital of Wuhan University, as the study involved no additional interventions, and all data were analyzed in a de-identified manner.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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