Prevalence and Molecular Spectrum of α- and β-Globin Gene Mutations in Hainan, China

Zhen Wang
The First Affiliated Hospital of Hainan Medical University
Wenye Sun
The First Affiliated Hospital of Hainan Medical University
Huaye Chen
The First Affiliated Hospital of Hainan University
Yongfang Zhang
The First Affiliated Hospital of Hainan Medical University
Fei Wang
The First Affiliated Hospital of Hainan Medical University
Yanhua Huang
The First Affiliated Hospital of Hainan Medical University
Hongjian Chen
The First Affiliated Hospital of Hainan Medical University
Yao Zhou
The First Affiliated Hospital of Hainan Medical University
Xixi Zhou
The First Affiliated Hospital of Hainan Medical University
Qi Li
The First Affiliated Hospital of Hainan Medical University
Yanlin Ma (mayanlinma@hotmail.com)

Research

Keywords: Thalassemia, Gene mutation, Prenatal diagnosis, Genetic diagnosis, Hainan Province

Posted Date: June 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32882/v1

Abstract

Background: Thalassemia is one of the most prevalent inherited single gene diseases. Prevention of β-thalassemia through prenatal diagnosis is the one of the most effective and direct approach to control the spread of this life-threatening disease. This study aims to determine the prenatal diagnosis of α-thalassemia and β-thalassemia in 3049 families among eighteen regions of Hainan Province using molecular diagnosis.

Methods: This study enrolled a total of 3049 couples and their fetuses at The First Affiliated Hospital of Hainan Medical University from January 2004 to March 2020. Genomic DNA was extracted from peripheral blood of the couples and villus, amniotic fluid, or fetal cord blood of fetuses. DNA-based diagnosis was performed using polymerase chain reaction.

Results: Here, the most commonly detected mutation of α-thalassemia was a South-East Asian deletion (31.53%), followed by $-\alpha^{4.2}/\alpha\alpha$ (11.15%), $-\alpha^{3.7}/\alpha\alpha$ (11.02%). The most common mutation for β-thalassemia was CD41/42, followed by -28, accounting for 30.27% and 2.56%, respectively. The regions with the highest prevalence were the coastal regions and the regions with the lowest prevalence were Wenchang, Lingao and Ding'an. We also examined thalassemia gene mutations in Han people and other minority groups and found that the most common gene mutations in different ethnic were not homogeneous. Prenatal diagnosis showed 556 normal, 118 α-thalassemia hydrops and 161 β-thalassemia major fetuses.

Conclusion: Our findings provide important information for clinical genetic counseling of prenatal diagnosis for thalassemia major in Hainan Province.

Background

Thalassemia, also referred to as Mediterranean anemia, is an autosomal inherited defect caused by the mutations in the α or β globin gene. Thalassemia is categorized into two major types, α- and β- thalassemia according to the mutations that occur in these globin genes, either of which can be further subdivided into another two forms, the α^0 and α^+ thalassemia and β^0 and β^+ thalassemia, respectively. The heterozygous form of α^+ thalassemia or α^0 thalassemia may not have symptoms of anemia, while the compound heterozygous form for α^+ thalassemia and α^0 thalassemia usually causes hemoglobin H. The homozygous state for α^0 thalassemia causes Hemoglobin Bart's, which is lethal in utero or soon after birth. The heterozygous form of β-thalassemia, which is called β-thalassemia minor, usually shows asymptomatic microcellular anemia, while others are silent carriers. In contrast, the homozygous or compound heterozygous form of β mutations, also named the β-thalassemia major and intermediate respectively, can cause severe anemia and patients carrying these mutations need transfusion for life, which poses financial burden to their families and society.

The high frequency of inherited hemoglobin variants is present in tropical and sub-tropical areas such as Mediterranean countries and Southeast Asia [1]. In China, the prevalence of α-thalassemia, β-thalassemia and α- and β-thalassemia ranges from 1.20 ~ 19.87%, 0.53 ~ 6.84% and 0.08 ~ 1.22%, respectively [2]. Southern China including Guangxi, Guangdong, Fujian and Hainan Provinces are the high incidence areas of the disease, and the spectrum of the mutations of these areas have been examined previously [3–5].

However, the distribution of thalassemia is not homogeneous. A huge variation of the spectrum of thalassemia gene mutations has been reported in different ethnic populations and related geographical regions. For example, the most common mutation of β-thalassemia in Northeastern Iranian is IVS-I-5 [6], which differs from CD41/42 found in Thailand [7] and CD26 in North Vietnam [8]. Even in any given country, the spectrum of thalassemia gene mutations is diverse in different geographical regions. In China, for instance, CD17 is the most frequent genotype in Baise Region [9], while IVS-II-654 has the highest prevalence in Fuzhou [5]. In Hainan, thalassemia is highly prevalent, and the frequency and spectrum of α- and β- thalassemia mutations among ethnic groups are not identical. The incidence of thalassemia of Li people is higher (65.27%) than that in Han people (23.12%) [10]. In addition, Li people shows high frequencies of $-\alpha^{4.2}$ and $-\alpha^{3.4}$ but very low frequencies of —SEA/αα [10]. On the contrary, ––SEA/αα has been reported to be highly prevalent in Han people in Hainan [10]. Therefore, the spectrum of thalassemia gene mutations appears to be region- and ethnic- dependent.

As thalassemia is an inherited autosomal recessive disease and has serious impacts on the quality of patient's life, couples who are both heterozygous for thalassemia gene have 25% probability to have a child with thalassemia major. Presently, prevention programs like molecular diagnostics, genetic counselling, and prenatal diagnosis have achieved great success in preventing the occurrence of thalassemia major, as demonstrated by a decline in the birth rate of thalassemia major in some countries such as Iran, Pakistan, Thailand and China [11–13]. Currently, prenatal diagnosis of thalassemia by molecular biology technology has been widely used in China and significant for thalassemia prevention. A previous study has provided important information about prenatal diagnosis of thalassemia in Han and Li people in Hainan [14]. However, few studies have shown geographical distribution and family-based prenatal diagnosis of thalassemia gene mutations in Hainan Province. Furthermore, the main aim of a thalassemia prevention and control program is to prevent the birth of infants carrying thalassemia major. Hence, it is necessary to enroll pregnant women and their husbands in the study cohort.

In this study, we analyzed α-and β-thalassemia genotypes in the fetal specimen collected from 3049 pregnant women using molecular prenatal diagnosis in our Prenatal Diagnosis Center between January 2004 and March 2020 in Hainan. In addition, we conducted a large-scale familial investigation in eighteen regions of Hainan Province. The aims of this study were to reveal the detailed geographical and ethnic distribution and familial prevalence of thalassemia, and provide scientific basis for thalassemia prevention and control in the province, and construct a detailed frequency map of the spectrum of thalassemia mutations in Hainan. This study will provide comprehensive data of thalassemia's prevalence in Hainan, which will significantly contribute to its control and management, genetic counseling and prenatal diagnosis.

Methods

Subjects

This study included pregnant women and their husbands who registered for a program of diagnosis of thalassemia at The First Affiliated Hospital of Hainan Medical University in Hainan Province between January 2004 and March 2020. All couples had resided in Hainan Province, and came from eighteen regions including Haikou, Wuzhishan, Lingao, Ding'an, Sanya, Danzhou, Qionghai, Dongfang, Tunchang, Chengmai, Changjiang, Baisha, Baoting, Lingshui, Dongfang, Wanning, Ledong and Qiongzhong. This study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliated Hospital of Hainan Medical University.

Samples Collection And Pretreatment

Depending on the gestational age of the fetus, chorionic villus sampling (CVS) at 10–12 weeks, amniotic fluid at 16–21 weeks and cord blood at 18–28 weeks of gestation were collected. All procedures were performed under the guidance of ultrasonography. Peripheral blood from parents and umbilical cord blood from the fetus were collected via venipuncture of an antecubital vein using Ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. The villus samples were isolated under a dissecting microscope. Amniocentesis fluid cells were isolated by centrifugation at 2000 rpm for 2 minutes. Genomic DNA of peripheral blood of both parents, chorionic villus, amniotic fluid and umbilical cord blood were extracted by Tiangen DNA extract kit (Tiangen Biotech CO., LTD., China) following the manufacturer's instructions.

Molecular Diagnosis Of α- And β-thalassemia

Gap-polymerase chain reaction (gap-PCR), the reverse dot blot (RDB) system (Shenzhen Yaneng Biotechnology Co., Ltd., China) and flow-through hybridization technology (Hybribio Limited, China) were used to determine mutations including deletional α- and β-globin mutations. All mutations identified are presented in Table 1.

PCR for detection of nondeletion of α-thalassemia and β-thalassemia mutations was performed according to the following protocol: 55 °C for 5 minutes, initial denaturation at 94 °C for 10 minutes, 94 °C for 1 minutes, 55 °C for 30 seconds, 72 °C for 30 seconds at 35 cycles of amplification, and 72℃ 5 minutes. PCR for deletion of α-thalassemia was performed according to the following protocol: 55 °C for 5 minutes, initial denaturation at 94 °C for 10 minutes, 96 °C for 5 minutes, 98 °C for 45 seconds, 65 °C for 90 seconds, 72 °C for 3 minutes at 10 cycles of amplification, 98 °C for 30 seconds, 65 °C for 45 seconds, 72 °C for 3 minutes at 25 cycles of amplification and then 72℃ 10 minutes. Flow-through hybridization was carried out according to the recommended protocol.

Table 1
Identified mutations in this study

	Gene	Mutation	HGVS name
α-globin deletions	$-\alpha^{4.2}/\alpha\alpha$	$-\alpha^{4.2}$	HGVS not attributable
	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{3.7}$	NG_000006.1: g.34164_37967del3804
	−−SEA/αα	−−(SEA)	NG_000006.1: g.26264_45564del19301
α-globin mutations	$-\alpha^{WS}/\alpha\alpha$	Hb Westmead	HBA2: c.369C > G
	$-\alpha^{QS}/\alpha\alpha$	Hb Quong Sze	HBA2: c.377T > C
	$-\alpha^{CS}/\alpha\alpha$	Hb Constant Spring (Hb CS)	HBA2: c.427T > C
β-globin mutations	CD41/42	Codons 41/42 (-TTCT)	HBB: c. 124_127delTTCT
	-28	-28 (A->C)	HBB: c.-78A > C
	IVS-II-654	IVS-II-654 (C->T)	HBB: c.316-197C > T
	CD17	Codon 17 (A->T)	HBB: c.52A > T
	CD71/72	Codons 71/72 (+ A)	HBB: c.216_217insA
	CD26	Codons26(GAG > AAG)	HBB: c.79G > A
	-50	-50(G->A)	HBB: c.-100G > A
	-29	-29 (A->G)	HBB: c.-79A > G
	Int	Initiation codon ATG->AGG	HBB: c.2T > G
	IVS-I-1	IVS-I-1 (G->T)	HBB: c.92 + 1G > T
	CD43	Codon 43 (G->T)	HBB: c.130G > T
	CD27/28	Codons 27/28 (+ C)	HBB: c.84_85insC
	Cap	5'UTR; +43 to + 40 (-AAAC) beta+	HBB: c.-11_-8delAAAC
	CD14/15	Codons 14/15 (+ G)	HBB: c.45_46insGa
	-32	-32 (C->A)	HBB: c.-82C A
	-30	-30 (T->C)	HBB: c.-80T > C
	IVS-I-5	IVS-I-5 (G->C)	HBB: c.92 + 5G > C
	CD31	Codon 31 (-C)	HBB: c.94delC

Table 2
Common - and -thalassemia mutations in Hainan Province

αβ				
	genotype		n	Ratio (%)
	Total		6098	
α-thalassemia	−−SEA/αα	α^0/α	1923	31.53
	$-\alpha^{4.2}/\alpha\alpha$	α^+/α	680	11.15
	$-\alpha^{3.7}/\alpha\alpha$	α^+/α	672	11.02
	$-\alpha^{WS}/\alpha\alpha$	α^+/α	334	5.48
	$-\alpha^{3.7}/-\alpha^{4.2}$	α^+/α^+	231	3.79
	others		1083	17.76
	total		4923	80.73
β-thalassemia	CD41/42	β^0/β^N	1846	30.27
	-28	β^+/β^N	156	2.56
	IVS-II-654	β^0/β^N	62	1.02
	CD17	β^0/β^N	48	0.79
	CD71/72	β^0/β^N	41	0.67
	others		72	1.18
	total		2225	36.49
Both α- and β-thalassemia	$-\alpha^{3.7}/\alpha\alpha$, CD41/42	$\alpha^+/\alpha\beta^0/\beta^N$	274	4.49
	$-\alpha^{4.2}/\alpha\alpha$, CD41/42	$\alpha^+/\alpha\beta^0/\beta^N$	259	4.25
	$-\alpha^{WS}/\alpha\alpha$, CD41/42	$\alpha^+/\alpha\beta^0/\beta^N$	143	2.35
	$-\alpha^{3.7}/-\alpha^{4.2}$, CD41/42	$\alpha^+/\alpha^+\beta^0/\beta^N$	102	1.67
	−−SEA/αα, CD41/42	$\alpha^0/\alpha\beta^0/\beta^N$	93	1.53
	others		451	7.40
	total		1322	20.99

Result

Prevalence of α- and β-globin gene mutations among couples in Hainan Province

We first examined 3049 couples of Hainan Province and diagnosed 1) 4923 cases as carrying α- thalassemia, accounting for 80.73%, with the mutation rates of. 31.53% for −−SEA/αα deletions, 11.15% for $-\alpha^{4.2}$ deletions and 11.02% for $-\alpha^{3.7}$ deletions, respectively (Table 2); 2) 2225 cases as carrying β-thalassemia, accounting for 34.69% of the total number of patients, with the mutation rates of 30.27% for CD41/42, 2.56% and 1.02% for − 28 and IVS-II-654, respectively; 3) 1322 cases as carrying compound α- and β-thalassemia, accounting for 20.99% of the total number of patients; and 4) among them, the five most frequent types were $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$, $-\alpha^{WS}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{4.2}$ and −−SEA/αα, with all accompanied by CD41/42, accounting for 4.49%, 4.25%, 2.35%, 1.67% and 1.53% of all the subjects, respectively.

Geographical distribution of α- and β-thalassemia gene mutations in eighteen regions of Hainan

Characterization of thalassemia mutations in the risk couples had been performed before the fetal samples were collected. During the 17-year period (2004–2020), a total of 3,049 subjects were screened for thalassemia at Prenatal Diagnosis Center of The First Affiliated Hospital of Hainan Medical University. The rates of α-thalassemia carrier in 3,049 couples of Hainan varied between 1.38% and 13.63% in eighteen regions. The rate was higher in coastal region (Haikou, Sanya, Lingshui, Danzhou, Ledong, Fig. 1A). The β-thalassemia carrier showed less variation, ranging from 0.67–4.39% in eighteen regions, which was higher in the coastal regions (Sanya, Haikou, Lingshui, Dongfang, Ledong, Fig. 1B). The rate of α- and β-thalassemia carrier presented less variation in eighteen regions, ranging from 0.23–3.1%. The distributed status was similar to that of β-thalassemia carriers (Fig. 1C). We then analyzed the prevalence of α- and β-thalassemia mutations in eighteen regions of Hainan Province. The geographical distribution of α-thalassemia mutations is shown in Table 3. A significantly higher frequency of the —SEA/αα mutation was found in the general population of Hainan Province. Twenty-four types of α-thalassemia were identified in eighteen regions of Hainan Province. −−SEA/αα, $-\alpha^{4.2}/\alpha\alpha$ and $-\alpha^{3.7}/\alpha\alpha$ are the most frequent α- thalassemia types in Haikou, Wenchang, Wuzhishan, Lingao,

Ding'an, Sanya, Danzhou, Qionghai, Dongfang, Tunchang, Chengmai, Changjiang, Baisha, Baoting, Lingshui and Dongfang, while the most common genotypes were − $\alpha^{4.2}$/αα, −$\alpha^{3.7}$/αα and −α^{WS}/αα in Wanning and Qiongzhong, six mutations (−α^{QS}/−−SEA, −α^{QS}/-α^{QS}, −α^{CS}/-α^{WS}, −$\alpha^{4.2}$/-α^{CS}, −α^{CS}/−−SEA, −$\alpha^{3.7}$/-α^{CS}) were observed at a frequency of less than 1% in eighteen regions. In general, the percentage of α-thalassemia mutation genes were in a similar tendency in different regions.

The geographical distribution of β-thalassemia mutations is shown in Table 4. Sixteen different point mutations of β-thalassemia were identified. CD41/42 (> 50%) was the most common mutation in eighteen regions, and five mutations (Int, CD17, IVS-I-1, CD43, CD27/28, Cap, CD14/15) were observed at a frequency of less than 1%.

Fifty-eight types of compound gene mutations were detected. The specific phenotype and frequency are shown in Table S1 (Supplementary Information). 1,319 cases carried both α and β-globin gene mutations and, among them, the seven most frequent types were − $\alpha^{4.2}$/αα, −$\alpha^{3.7}$/αα, α^{WS}/αα, −−SEA/αα, −$\alpha^{3.7}$/-$\alpha^{4.2}$, −$\alpha^{3.7}$/-α^{WS}, −$\alpha^{3.7}$/-$\alpha^{3.7}$ and −$\alpha^{4.2}$/-$\alpha^{4.2}$, all of which were accompanied by CD41/42 in eighteen regions of Hainan Province.

Table 3
Distribution of -thalassemia mutations in Hainan province

α												
Genotype	Haikou	Wenchang	Wuzhishan	Lingao	Ding'an	Sanya	Danzhou	Qionghai	Dongfang	Wanning	Tunchang	Chengm
	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)
−−SEA/αα	55.60	64.57	17.73	53.85	45.24	25.04	43.21	63.85	27.43	39.83	55.79	62.87
-$\alpha^{4.2}$/αα	12.64	6.30	14.18	13.08	19.05	16.70	16.90	10.33	19.47	14.94	7.89	7.19
-$\alpha^{3.7}$/αα	14.32	11.81	19.86	11.54	10.71	13.63	9.70	6.57	19.47	10.37	12.11	13.17
-α^{WS}/αα	5.17	6.30	12.06	6.92	8.33	5.79	5.82	6.57	7.08	11.20	4.21	4.19
-$\alpha^{3.7}$/-$\alpha^{4.2}$	2.05	2.36	6.38	0.77	—	7.50	4.16	0.94	4.87	1.24	1.05	4.79
-$\alpha^{3.7}$/−−SEA	1.56	1.57	0.71	0.77	4.76	4.43	3.88	3.76	1.77	1.66	0.53	3.59
-$\alpha^{3.7}$/-α^{WS}	0.48	3.94	6.38	1.54	—	2.04	1.39	0.94	3.54	3.32	1.05	0.60
-$\alpha^{3.7}$/-$\alpha^{3.7}$	0.84	0.79	2.13	0.77	2.38	4.26	1.11	0.94	3.54	1.66	1.58	0.60
-$\alpha^{4.2}$/-α^{WS}	0.60	0.79	5.67	—	2.38	4.60	0.28	0.47	3.10	2.49	1.05	0.60
-$\alpha^{4.2}$/−−SEA	0.84	—	1.42	2.31	—	3.41	4.43	—	2.65	2.90	4.74	—
-α^{QS}/αα	2.53	0.79	2.84	3.08	2.38	1.87	4.43	1.41	—	3.73	2.63	1.80
-$\alpha^{4.2}$/-$\alpha^{4.2}$	0.96	0.79	—	3.08	1.19	4.77	1.66	1.88	2.21	0.41	0.53	—
-α^{WS}/−−SEA	0.12	—	0.71	0.77	2.38	1.02	1.11	0.94	0.88	1.66	1.58	0.60
-$\alpha^{3.7}$/-α^{QS}	—	—	0.71	0.77	1.19	1.87	0.28	—	0.44	0.83	1.05	—
-$\alpha^{4.2}$/-α^{QS}	0.24	—	4.96	0.77	—	1.19	0.83	0.47	—	1.66	1.58	—
-α^{WS}/-α^{WS}	0.36	—	2.84	—	—	0.17	0.55	—	2.21	1.24	0.53	—
-α^{CS}/αα	1.44	—	—	—	—	0.51	0.28	—	0.44	0.41	1.58	—
−α^{QS}/-α^{WS}	0.12	—	0.71	—	—	1.19	—	—	0.88	—	—	—
-α^{QS}/−−SEA	0.12	—	—	—	—	—	—	0.94	—	—	—	—
-α^{QS}/-α^{QS}	—	—	—	—	—	—	—	—	—	—	—	—
-α^{CS}/-α^{WS}	—	—	—	—	—	—	—	—	—	0.41	—	—
-$\alpha^{4.2}$/-α^{CS}	—	—	0.71	—	—	—	—	—	—	—	—	—
-α^{CS}/−−SEA	—	—	—	—	—	—	—	—	—	—	0.53	—
-$\alpha^{3.7}$/-α^{CS}	—	—	—	—	—	—	—	—	—	—	—	—
Total	831	127	141	130	84	587	361	213	226	241	190	167

Table 4
Distribution of -thalassemia mutations in Hainan province

β												
Genotype	Haikou	Wenchang	Wuzhishan	Lingao	Ding'an	Sanya	Danzhou	Qionghai	Dongfang	Wanning	Tunchang	Chengm
	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)	Ratio (%)
CD41/42	74.00	67.80	96.30	65.85	66.67	89.93	76.81	68.69	89.29	86.08	74.65	57.14
-28	13.60	6.78	2.78	24.39	23.81	2.24	10.14	15.15	6.55	5.06	16.90	21.43
IVS-II-654	4.00	10.17	—	2.44	4.76	2.24	5.07	1.01	0.60	2.53	—	8.33
CD17	4.00	1.69	—	4.88	4.76	1.49	1.45	8.08	1.19	1.27	2.82	3.57
CD71/72	2.00	3.39	0.93	2.44	—	1.12	4.35	2.02	1.19	2.53	2.82	2.38
CD26	2.00	8.47	—	—	—	0.75	—	2.02	—	1.27	1.41	2.38
-50	—	—	—	—	—	0.37	0.72	—	1.19	—	1.41	2.38
-29	0.40	—	—	—	—	0.75	—	1.01	—	—	—	—
Int	—	1.69	—	—	—	—	—	1.01	—	—	—	2.38
IVS-I-1	—	—	—	—	—	—	—	1.01	—	1.27	—	—
CD43	—	—	—	—	—	0.75	0.72	—	—	—	—	—
CD27/28	—	—	—	—	—	0.37	—	—	—	—	—	—
Cap	—	—	—	—	—	—	0.72	—	—	—	—	—
CD14/15	—	—	—	—	—	—	—	—	—	—	—	—
Total	250	59	108	41	42	268	138	99	168	79	71	84

Spectrum of α- and β-thalassemia mutations identified in Li and Han people of Hainan Province

Apart from geographical factors, ethnic difference also plays an important role in gene diversity. Since Hainan Province is a place whose population is mainly composed of Han and other minority groups, which generates diverse genotypes. We then analyzed the frequency and spectrum of α- and β- thalassemia mutations of the Han people in Hainan Province and compared these findings with the minority. A total of 4,914 cases were screened. As is shown in Table 5, twenty-four α-globin genotypes were found in 4,597 cases of α-thalassemia, with —SEA/αα, $-\alpha^{4.2}$/αα and $-\alpha^{3.7}$/αα being the three most frequent α-thalassemia types among the Han and Li people, accounting for 57.50%, 12.37% and 11.26% in Han people and 13.43%, 16.43%, 16.76% in Li people, respectively. SEA/αα and $-\alpha^{3.7}$/αα are the two most frequent in Miao and Zhuang people, accounting for 62.75% and 13.73% in Miao and 55.88% and 14.71% in Zhuang people.

Surprisingly, CD41/42 was the dominant mutation of β- thalassemia among the Han, Li, Miao, Zhuang people, accounting for 70.03%, 94.84%, 83.64%, and 56.25%, respectively. (Table 6).

Fifty-eight types of compound gene mutations were detected (Table S2, Supplementary Information). The specific phenotypes and frequency are shown in Table 6. The three most frequent types in Han people were $-\alpha^{3.7}$/αα, $-\alpha^{4.2}$/αα and $-\alpha^{WS}$, all of which were accompanied by CD41/42, accounting for 19.48%, 16.29% and 16.86% respectively. The three most frequent types in Li people were $-\alpha^{3.7}$/αα, $-\alpha^{4.2}$/αα and —SEA/αα, all of which were accompanied by CD41/42, accounting for 21.45%, 21.29% and 11.28%, respectively. On the contrary, the patients who carried compound gene mutations in Miao and Zhuang were less than 1% of the total subjects.

Table 5
Spectrum of -thalassemia mutations of Han people and minority in Hainan Province

α								
	Han		Li		Miao		Zhuang	
	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)
−−SEA/αα	1557	57.50	278	13.43	64	62.75	19	55.88
$-\alpha^{4.2}/\alpha\alpha$	335	12.37	340	16.43	4	3.92	1	2.94
$-\alpha^{3.7}/\alpha\alpha$	305	11.26	347	16.76	14	13.73	5	14.71
$-\alpha^{WS}/\alpha\alpha$	149	5.50	177	8.55	5	4.90	2	5.88
$-\alpha^{3.7}/-\alpha^{4.2}$	42	1.55	184	8.89	3	2.94	1	2.94
$-\alpha^{3.7}/$−−SEA	62	2.29	73	3.53	5	4.90	1	2.94
$-\alpha^{3.7}/-\alpha^{WS}$	22	0.81	104	5.02	—	—	—	—
$-\alpha^{3.7}/-\alpha^{3.7}$	18	0.66	106	5.12	—	—	1	2.94
$-\alpha^{4.2}/-\alpha^{WS}$	11	0.41	106	5.12	—	—	—	—
$-\alpha^{4.2}/$−−SEA	56	2.07	56	2.71	3	2.94	1	2.94
$-\alpha^{QS}/\alpha\alpha$	65	2.40	46	2.22	—	—	1	2.94
$-\alpha^{4.2}/-\alpha^{4.2}$	20	0.74	86	4.15	—	—	—	—
$-\alpha^{WS}/$−−SEA	18	0.66	39	1.88	3	2.94	—	—
$-\alpha^{3.7}/-\alpha^{QS}$	4	0.15	42	2.03	—	—	—	—
$-\alpha^{4.2}/-\alpha^{QS}$	10	0.37	31	1.50	—	—	—	—
$-\alpha^{WS}/-\alpha^{WS}$	9	0.33	23	1.11	—	—	—	—
$-\alpha^{CS}/\alpha\alpha$	19	0.70	4	0.19	1	0.98	2	5.88
$-\alpha^{QS}/-\alpha^{WS}$	2	0.07	18	0.87	—	—	—	—
$-\alpha^{QS}/$−−SEA	3	0.11	5	0.24	—	—	—	—
$-\alpha^{QS}/-\alpha^{QS}$	—	—	2	0.10	—	—	—	—
$-\alpha^{CS}/$−−SEA	1	0.04	—	—	—	—	—	—
$-\alpha^{CS}/-\alpha^{WS}$	—	—	1	0.05	—	—	—	—
$-\alpha^{4.2}/-\alpha^{CS}$	—	—	1	0.05	—	—	—	—
$-\alpha^{3.7}/-\alpha^{CS}$	—	—	1	0.05	—	—	—	—
Total	2708		2070		102		34	

Table 6
Spectrum of -thalassemia mutations of Han people and minority in Hainan Province

β								
Genotype	Han		Li		Miao		Zhuang	
	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)
CD41/42	694	70.03	1103	94.84	46	83.64	9	56.25
-28	135	13.62	20	1.72	—	—	1	6.25
IVS-II-654	55	5.55	6	0.52	1	1.82	—	—
CD17	33	3.15	7	0.60	5	9.09	5	31.25
CD71/72	32	3.23	9	0.77	1	1.82	—	—
CD26	17	1.72	7	0.60	—	—	1	6.25
-50	7	0.71	9	0.77	—	—	—	—
-29	7	0.71	1	0.09	—	—	—	—
Int	4	0.40	—	—	—	—	—	—
IVS-I-1	1	0.10	—	—	2	3.64	—	—
CD43	3	0.30	—	—	—	—	—	—
CD14/15	1	0.10	—	—	—	—	—	—
IVS-II-654//IVS-II-654	1	0.10	—	—	—	—	—	—
Cap	—	—	1	0.09	—	—	—	—
CD27/28	1	0.10	—	—	—	—	—	—
Total	991		1163		55		16	

Prenatal Diagnosis

The frequencies of thalassemia genotypes obtained from 3,049 prenatal diagnosis in Hainan Province are shown in Table 7. Among these 3,049 cases of α-thalassemia, 3.87% of the fetus were α-thalassemia hydrops, 70.58% were α-thalassemia carriers,5.28% were β-thalassemia major, 26.76% were β-thalassemia carriers, and 18.24% were normal. The spectrum of α-thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province are shown in Table 8. The most frequent genotype was —SEA/αα, accounting for 21.28% of total fetuses, and is followed, in order of frequency, by the mutations $-\alpha^{4.2}/\alpha\alpha$ and $-\alpha^{3.7}/\alpha\alpha$, accounting for 15.45% and 14.89%, respectively. The top three α-globin gene mutations in the fetuses were the same to those observed in the pregnant women and husbands.

For the β-thalassemia mutations, the most frequent genotype was CD41-42 (42.84%), followed by CD41/42/CD41-42, -28, CD71/72 and − 28/CD41/42, accounting for 15.53%, 5.01%, 3.42% and 2.93%, respectively. In the pregnant women and husbands, the most common mutation was CD41/42, followed by IVS-II-65, CD17 and − 28 in parents (Table 9).

A total of 485 genotypes were both α and β-thalassemia carriers. The top three frequent types were $-\alpha^{3.7}/\alpha\alpha$ (17.53%), $-\alpha^{4.2}/\alpha\alpha$ (16.91%) and $-\alpha^{WS}/\alpha\alpha$ (8.87%), all of which were accompanied by CD41/42. The top three α- and β-combination globin gene mutations in the fetuses were the same to those observed in the pregnant women and husbands (Table S3, Supplementary Information).

Table 7
Genotypes of thalassemia identified by prenatal diagnosis in Hainan Province

Variable	One of parent was carrier		Both parents were carriers		Both parents were normal		Total	Ratio (%)
	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)		
α-thalassemia hydrops	2	0.81	116	4.16	—	—	118	3.87
α-thalassemia carrier	110	44.53	2042	73.22	—	—	2152	70.58
β-thalassemia major	1	0.40	160	5.74	—	—	161	5.28
β-thalassemia carrier	39	15.79	781	28.00	—	—	816	26.76
α + β-thalassemia carrier	9	3.64	476	17.07	—	—	485	15.91
Normal	98	39.68	445	15.96	13	100	556	18.24
Total	247		2789		13		3049	

Table 8
Spectrum of -thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province

α						
Genotype	pregnant women		Husbands		fetus	
	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)
−−SEA/αα	946	37.27	977	32.15	460	21.28
$-\alpha^{4.2}/\alpha\alpha$	367	14.46	313	10.30	334	15.45
$-\alpha^{3.7}/\alpha\alpha$	360	14.18	312	10.27	322	14.89
$-\alpha^{WS}/\alpha\alpha$	166	6.54	168	5.53	160	7.40
$-\alpha^{3.7}/-\alpha^{4.2}$	130	5.12	101	3.32	76	3.52
$-\alpha^{3.7}/-\alpha^{WS}$	75	2.96	51	1.68	42	1.94
$-\alpha^{3.7}$/−−SEA	72	2.84	69	2.27	139	6.43
$-\alpha^{3.7}/-\alpha^{3.7}$	68	2.68	57	1.88	52	2.41
$-\alpha^{4.2}$/−−SEA	66	2.60	50	1.65	162	7.49
$-\alpha^{4.2}/-\alpha^{WS}$	66	2.60	51	1.68	39	1.80
$-\alpha^{QS}/\alpha\alpha$	53	2.09	59	1.94	39	1.80
$-\alpha^{4.2}/-\alpha^{4.2}$	46	1.81	60	1.97	45	2.08
$-\alpha^{3.7}/-\alpha^{QS}$	28	1.10	18	0.59	23	1.06
$-\alpha^{WS}$/−−SEA	25	0.99	35	1.15	63	2.91
$-\alpha^{4.2}/-\alpha^{QS}$	21	0.83	20	0.66	17	0.79
$-\alpha^{CS}/\alpha\alpha$	19	0.75	8	0.26	8	0.37
$-\alpha^{WS}/-\alpha^{WS}$	11	0.43	21	0.69	18	0.83
$-\alpha^{QS}/-\alpha^{WS}$	11	0.43	9	0.30	17	0.79
$-\alpha^{QS}$/−−SEA	3	0.12	5	0.16	15	0.69
$-\alpha^{CS}$/−−SEA	1	0.04	—	—	8	0.37
$-\alpha^{QS}/-\alpha^{QS}$	1	0.04	1	0.03	1	0.05
$-\alpha^{3.7}/-\alpha^{CS}$	1	0.04	—	—	1	0.05
$-\alpha^{4.2}/-\alpha^{CS}$	1	0.04	—	—	1	0.05
$-\alpha^{CS}/-\alpha^{WS}$	1	0.04	—	—	1	0.05
−−SEA/−−SEA	—	—	—	—	119	5.50
Total	2538		3039		2162	

Table 9
Spectrum of -thalassemia genotypes among pregnant women, their husbands and fetuses in Hainan Province

β						
Genotypes	pregnant women		Husbands		Fetus	
	N	Ratio (%)	N	Ratio (%)	N	Ratio (%)
CD41/42	976	85.54	876	80.81	551	67.36
IVS-II-654	29	2.54	34	3.14	1	0.12
CD17	23	2.02	28	2.58	6	0.73
CD26	13	1.14	12	1.11	6	0.73
-50	6	0.53	10	0.92	—	—
-29	5	0.44	3	0.28	1	0.12
IVS-I-1	2	0.18	1	0.09	3	0.37
CD27/28	1	0.09	—	—	1	0.12
CD14/15	1	0.09	—	—	1	0.12
CD41/42/CD41/42	—	—	—	—	127	15.53
-28	—	—	71	6.55	41	5.01
CD71/72	—	—	41	3.78	28	3.42
-28/CD41/42	—	—	—	—	24	2.93
CD41/42/IVS-II-654	—	—	—	—	5	0.61
CD41/42/-50	—	—	—	—	4	0.49
-28/IVS-II-654	—	—	—	—	4	0.49
-28/-28	—	—	—	—	4	0.49
CD17CD41/42	—	—	—	—	4	0.49
IVS-II-654/IVS-II-654	—	—	1	0.09	1	0.12
CD26/CD26	—	—	—	—	1	0.12
CD41/42/CD26	—	—	—	—	1	0.12
CD43	—	—	3	0.28	2	0.24
Int	—	—	4	0.37	1	0.12
Cap	—	—	1	0.09	1	0.12
Total	1141		1084		818	

Discussion

Previous studies have shown the prevalence and molecular spectrum of α- and β-thalassemia mutations in Hainan Province, but those studies had limited sample size and information [14, 10]. This study was the first to provide a large scale, sampling of the eighteen regions of Hainan Province. Also, we analyzed the family-based sampling, as we knew that the couples who carried the same type of thalassemia mutations had high risk to have a moderate or severe thalassemia fetus. These pregnant women and their husbands are critical subjects of thalassemia intervention, which will help to prevent birth of moderate to severe thalassemia fetus. Our center is the earliest established Prenatal Diagnosis Center in Hainan province, and nearly all carriers of thalassemia in the whole province come here for prenatal diagnosis. Hence, our study presented the prevalence and molecular variations of α- and β-globin gene mutations in Hainan Province.

Our data indicated a high prevalence of thalassemia in Hainan province. The frequencies of α-thalassemia and β-thalassemia were 80.73% and 34.69% in the total subjects of our study, respectively. We also found a high prevalence of α-and β-thalassemia carrier status. 2,538 (83.24%) pregnant women, 3,039 (99.67%) husbands and 2,162 (70.90%) fetuses were α-thalassemia carriers. The frequencies for β-thalassemia alone were 1141 (37.42%) in pregnant women, 1084 (35.55%) in husbands, 818 (26.83%) in fetuses. Finally, the frequencies of α-and β-thalassemia together were 689 (22.60%) in pregnant women, 1080 (35.42%) in husbands and 485 (15.91%) in fetuses. The frequency of α-thalassemia shown in our study were higher than that reported in other provinces of China, such as Guangdong[3], Fujian[5], Guangxi[4], Sichuan[15] and the frequency of β-thalassemia reported were lower than that reported in Fujian[5], but higher than that in Guangdong[3], Guangxi[4] and Sichuan[15]. In addition, the frequencies obtained from this study were higher than that reported in other countries/regions of the world, such as the north of Southwest Iran[16], Thailand[17, 7], and Laos [18] and lower than that reported in Pakistan[13].

In this study, the regions with the highest prevalence of α-thalassemia was Haikou, followed by the Sanya, Lingshui, Danzhou, Dongfang. The three regions with the highest prevalence of β-thalassemia mutations were Sanya, Lingshui and Dongfang. The lowest prevalence α-and β-thalassemia was Lingao, Wenchang, Ding'an. In our study, the prevalence of thalassemia carrier status in Sanya City was lower than that reported by Li [19]. Among α-thalassemia genotypes,—SEA/αα, which is consistent with the previous research by Sanya City and Ding'an County of Hainan Province, Sichuan, Fujian, Guangdong and Guangxi [19, 20, 15, 5, 3, 9], but different from that of Lingshui Autonomous County of Hainan Province[21], accounts for the highest frequence in the total couples of eighteen regions. Comparing with other countries, the most frequent α-thalassemia mutations are different from those reported Thailand [17], and Iran [6]. Among the β-thalassemia genotypes, CD41/42, consistent with the previous study that reported to be the most frequent genotype in Sanya City, Lingshui and Ding'an County of Hainan Province, Yunnan [22], Guangdong [3], accounts for the greatest proportion among couples of eighteen regions. These findings may be explained with the special location of Hainan Province which is located on a small island in southeast China. Comparing with other countries, the results are consistent with that in Thailand [7] but different from those in Pakistan [23], Syria [24], Iran [6], Vietnam [8] and India [25].

Race difference may also play a significant role in gene difference, so we don't exclude racial diversity. As population of Hainan Province is composed of Han and the minority ethnic group such as Li, Miao, Zhuang, so we also analyzed thalassemia genotype of couples based on their nations. Interestingly, —SEA/αα and CD41/42 are the most frequent thalassemia genotype in Han, Li, Miao and Zhuang people, different from that in an early study [10], whose dominant genotype is $-\alpha^{4.2}/\alpha\alpha$. This result may be explained by continued migration or the screened population we selected.

Due to geography, culture, customs, and other reasons, the proportion of inter-marriages between some regions of Hainan Province and other regions is relatively small, which consequently led to an overlapping distribution of thalassemia genes and

resulting in a high number of thalassemia carriers. Prenatal diagnosis is one of the most effective and direct method to prevent thalassemia major. In this study, we showed the results of prenatal diagnosis in our regions from 2004 to 2020, all participants were lived in Hainan Province. This study is the first systematic family-based prenatal diagnosis of thalassemia in the Hainan Province.

The findings of fetal diagnosis presented 9.15% for thalassemia major, 18.26% for the normal type. Compared with one parent being a carrier, the parents that both were carriers were more likely to have a child with thalassemia major or carrier. Also, the couples who carried the same thalassemia genotype had a high risk to have a fetus with thalassemia major. Interestingly, —SEA/αα and CD41/42 were found to be the most common types of thalassemia of prenatal diagnosis, which were also the most predominant mutations of the full set of couples in our study, couples with such thalassemia genotype as —SEA/αα or CD41/42 had 1/4 chance of producing a fetus with Hb Bart's or β-thalassemia major. We identified 118 (3.88%) fetuses with Bart's hydrops syndrome and 161(5.29%) fetuses with β-thalassemia major. However, we were not able to provide the information of the total number of terminated pregnancies, as some couples were referred from the other hospitals for molecular testing and prenatal diagnosis, and we could not follow all the affected pregnancies.

At present, Hainan Province has established several Prenatal Diagnosis Center and Health Department which has initiated free screening program since 2011. Other organizations such Li Ka-shing Health Poverty Alleviation fund projects and Prevention and Control Committee and Volunteers Association of Hainan Province, will help the prevention and diagnosis of thalassemia. We also recommend further studies on the factors that can affect the accessibility of thalassemia interventions to provide a scientific basis for government decision-making.

Conclusion

The gene types of α- and β-thalassemia in Hainan Province in China are characterized by a wide range of distribution, high carrier rate, genetic diversity, genetic heterogeneity, geographical and ethnic differences. Therefore, it is necessary to detect the thalassemia genes in the suspected population in this area so as to provide clinical genetic counseling and prenatal diagnosis of for thalassemia major in Hainan Province.

Abbreviations

PCR
polymerase chain reaction; SEA:Southeast Asia

Declarations

Acknowledgments

The authors would like to appreciate all patients who consented to disclose their medical records. Their cooperation throw light upon our data collection and statistical analysis. We are also thankful to Jun Wang from Texas Heart Institute for improvement of the manuscript.

Authors' contributions

Zhen Wang and Wenye Sun conducted the laboratory work, data analysis and

manuscript draft writing. Huaye Chen, Yongfang Zhang, Fei Wang, Yanhua Huang, Hongjian Chen, Yao Zhou, Xixi Zhou assisted in clinical information collection, interpretation and to improve the manuscript. Qi Li and Yanlin Ma designed the study plan and supervised the overall project. All authors read, critically reviewed the draft and approved the final version.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional file.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital of Hainan Medical University and all subjects gave their written informed consent before study participation.

Consent for publication

All the participants described in this article provided written informed consent for participation in the study and for publication of the results. All the authors have read and approved the paper for publication

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by Major Science and Technology Project of Hainan

Province (No. ZDKJ2017007), International Science and Technology Cooperation Program of China (No. 2014DFA30180), Natural Science Foundation of Hainan Province (No.2019CXTD408), National Natural Science Foundation of China (No. 81060016, 31140021, 81260032, 81460034, 81660433), and Hainan Provincial Department of Science and Technology (No. YJJC20120007, 2012-GH009, ZDZX2013003 and KJHZ2014-11)

Author details

[1]Hainan Provincial Key Laboratory for Human Reproductive Medicine and Genetic Research, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University. [2]Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Medical University. [3]Hainan Provincial Clinical Research Center for thalassemia, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University. [4]Haikou Key Laboratory for Preservation of Human Genetic Resource, The First Affiliated Hospital of Hainan Medical University, Hainan Medical University.

References

1. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155–67.
2. Lai K, Huang G, Su L, He Y. The prevalence of thalassemia in mainland China: evidence from epidemiological surveys. Scientific reports. 2017;7(1):920.
3. Yin A, Li B, Luo M, Xu L, Wu L, Zhang L, Ma Y, Chen T, Gao S, Liang J, Guo H, Qin D, Wang J, Yuan T, Wang Y, Huang WW, He WF, Zhang Y, Liu C, Xia S, Chen Q, Zhao Q, Zhang X. The prevalence and molecular spectrum of α- and β-globin gene mutations in 14,332 families of Guangdong Province, China. PloS one. 2014;9(2):e89855.
4. He S, Li J, Li DM, Yi S, Lu X, Luo Y, Liang Y, Feng C, Chen B, Zheng C, Qiu X. Molecular characterization of α- and β-thalassemia in the Yulin region of Southern China. Gene. 2018;655:61–4.
5. Xu C, Liao B, Qi Y, Huangfu Z, Chen J, Chen Y. Analysis of Gene Mutation Types of α- and β-Thalassemia in Fuzhou, Fujian Province in China. Hemoglobin. 2018;42(3):143–7.
6. Jaripour ME, Hayatigolkhatmi K, Iranmanesh V, Zand FK, Badiei Z, Farhangi H, Ghasemi A, Banihashem A, Esfehani RJ, Sadr-Nabavi A. Prevalence of β-Thalassemia Mutations among Northeastern Iranian Population and their Impacts on Hematological Indices and Application of Prenatal Diagnosis, a Seven-Years Study. Mediterranean journal of hematology infectious diseases. 2018;10(1):e2018042.
7. Singha K, Taweenan W, Fucharoen G, Fucharoen S. Erythrocyte indices in a large cohort of β-thalassemia carrier: Implication for population screening in an area with high prevalence and heterogeneity of thalassemia. International journal of laboratory hematology. 2019;41(4):513–8.
8. Vo LTT, Nguyen TT, Le HX, Le HTT. Analysis of Common β-Thalassemia Mutations in North Vietnam. Hemoglobin. 2018;42(1):16–22.
9. He S, Qin Q, Yi S, Wei Y, Lin L, Chen S, Deng J, Xu X, Zheng C, Chen B. Prevalence and genetic analysis of α- and β-thalassemia in Baise region, a multi-ethnic region in southern China. Gene. 2017;619:71–5.
10. Yao H, Chen X, Lin L, Wu C, Fu X, Wang H, Yao Z, Chen W, Huang L, Tang R, Rao R, Wang S, Ding Y. The spectrum of α- and β-thalassemia mutations of the Li people in Hainan Province of China. Blood Cells Mol Dis. 2014;53(1–2):16–20.
11. Sargolzaie N, Montazer Zohour M, Ayubi E, Shahraki F. Relationship Between Social Determinants of Health and the Thalassemia Prenatal Diagnosis Test in Zahedan, South Eastern Iran. Hemoglobin. 2018;42(4):231–5.
12. Nopparatana C, Nopparatana C, Saechan V, Karnchanaopas S, Srewaradachpisal K. Prenatal diagnosis of α- and β-thalassemias in southern Thailand. Int J Hematol. 2020;111(2):284–92.
13. Kanwal S, Bukhari S, Perveen S. Molecular genetics and prenatal diagnosis of beta thalassemia to control transfusion dependent births in carrier Pakistani couples. JPMA The Journal of the Pakistan Medical Association. 2017;67(7):1030–4.
14. Liang C, Chen X-y, Gao X, Chen H-j, Jin Y-x, Zhou Y, Li M-h, Wang W-c, Lu W-y, Huang Y-h, Wang J, Li Q, Ma Y-l. Spectrum of thalassemia mutations in fetuses of Han and Li ethinicities in Hainan province, China. Asian Pacific Journal of Tropical Medicine. 2019;12(12):537–44.
15. Yu X, Yang LY, Yang HT, Liu CG, Cao DC, Shen W, Yang H, Zhan XF, Li J, Xue BR, Lin M. Molecular Epidemiological Investigation of Thalassemia in the Chengdu Region, Sichuan Province, Southwest China. Hemoglobin. 2015;39(6):393–7.
16. Nezhad FH, Nezhad KH, Choghakabodi PM, Keikhaei B. Prevalence and Genetic Analysis of α- and β-Thalassemia and Sickle Cell Anemia in Southwest Iran. Journal of epidemiology global health. 2018;8(3–4):189–95.

17. Pharephan S, Sirivatanapa P, Makonkawkeyoon S, Tuntiwechapikul W, Makonkawkeyoon L. Prevalence of α-thalassaemia genotypes in pregnant women in northern Thailand. Indian J Med Res. 2016;143(3):315–22.
18. Phanmany S, Chanprasert S, Munkongdee T, Svasti S, Leecharoenkiat K. Molecular prevalence of thalassemia and hemoglobinopathies among the Lao Loum Group in the Lao People's Democratic Republic. International journal of laboratory hematology. 2019;41(5):650–6.
19. Li M, Xiang SH, Ding Y, Liu WW, Xu YY, Bo J. [Genotype Analysis of Patients with Thalassemia in Sanya Area of Hainan Province in China]. Zhongguo shi yan xue ye xue za zhi. 2018;26(4):1146–50.
20. Tu ZH, Wang J, Hu JJ, Zhao LQ, Ran HL, Wang AG, Li YJ, Ma N, Wu Z, Zhou Z. [Genetic Screening of Thalassemia among the Couples of Childbearing Age in Ding'an County of Hainan Province and Its Analysis]. Zhongguo shi yan xue ye xue za zhi. 2019;27(5):1592–5.
21. Tu ZH, Zhou Z, Wu WX, Wang XP, Zhou YZ, Huang CD, Ma N, Ruan HL, Zhao LQ, Wang J. [Analysis of Genetic Screening in Couples of Reproductive Age for Thalassemia in Lingshui Li Autonomous County of Hainan Province]. Zhongguo shi yan xue ye xue za zhi. 2019;27(4):1227–31.
22. Zhang J, He J, Mao X, Zeng X, Chen H, Su J, Zhu B. Haematological and electrophoretic characterisation of β-thalassaemia in Yunnan province of Southwestern China. BMJ open. 2017;7(1):e013367.
23. Muhammad R, Shakeel M, Rehman SU, Lodhi MA. Population-Based Genetic Study of β-Thalassemia Mutations in Mardan Division, Khyber Pakhtunkhwa Province, Pakistan. Hemoglobin. 2017;41(2):104–9.
24. Murad H, Moasses F, Dabboul A, Mukhalalaty Y, Bakoor AO, Al-Achkar W, Jarjour RA. Geographical distribution of β-globin gene mutations in Syria. Hematology (Amsterdam Netherlands). 2018;23(9):697–704.
25. Shah PS, Shah ND, Ray HSP, Khatri NB, Vaghasia KK, Raval RJ, Shah SC, Rao MV. Mutation analysis of β-thalassemia in East-Western Indian population: a recent molecular approach. The application of clinical genetics. 2017;10:27–35.

Figures

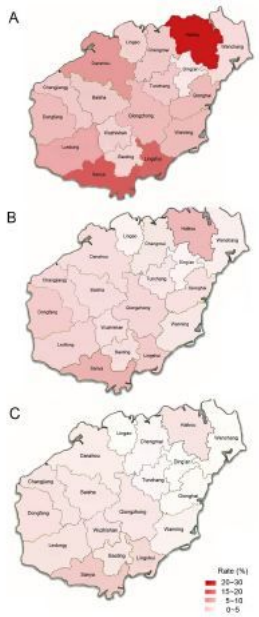

Figure 1

Frequencies of carrier status among couples in the eighteen regions of Hainan Province, China. A. α-thalassemia only, B. β-thalassemia, C. α- and β-thalassemia. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- additonalfileTableS1S3.docx