

RESEARCH ARTICLE

First report of the spectrum of δ -globin gene mutations among women of reproductive age in Fujian area—Discrimination of δ -thalassemia, α -thalassemia, and Iron Deficiency Anemia

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Abstract

Background: Low HbA₂ level is an underlying of δ -thalassemia, α -thalassemia, and IDA. Interactions of these disorders can generate a wide spectrum of phenotype, which will pose diagnostic conundrum for clinical assessment, carrier screening, and genetic counseling.

Methods: Subjects with HbA₂ levels below 2.0% with normal or reduced hematological parameters were recruited for further investigation. δ -globin gene mutations were identified by DNA sequencing of the HBD gene. Serum ferritin (SF) concentration was determined by the chemiluminescent microparticle immunoassay. The three common deletional α -thalassemia ($-\text{SEA}/\alpha\alpha$, $-\alpha^{3.7}/\alpha\alpha$, and $-\alpha^{4.2}/\alpha\alpha$) were detected using Gap-PCR, detection of the point mutations in the three nondeletional α -thalassemia ($\alpha^{\text{CS}}\alpha/\alpha\alpha$, $\alpha^{\text{QS}}\alpha/\alpha\alpha$, $\alpha^{\text{WS}}\alpha/\alpha\alpha$), and the 17 common β -thalassemia was performed using reverse dot blot hybridization (RDB).

Results: We had characterized the δ -globin gene mutations in 20 cases, revealing a frequency of 0.4% in the women of reproductive age (20/4 792). Two previously known mutations: $-77 T > C$ and $-30 T > C$ and 3 novel δ -globin gene defects: $-44G > A$, $CD87C > T$, and $CD134T > A$ were found. In the selected cases, we also found 85 cases confirmed with (51.2%, 85/166) IDA and 39 cases (23.5%, 39/166) with common α -thalassemia. Subjects with δ -thalassemia had statistically higher levels of Hb, MCV, and MCH compared with other two groups, whereas statistically lower levels of RDW were seen in δ -thalassemia group. What's more, statistically higher levels of SF were seen in δ -thalassemia group, compared with IDA groups.

Conclusion: We reported the spectrum of δ -thalassemia mutations for the first time with the frequency of 0.4% among women of reproductive age in Fujian area and found that $-77T > C$ mutation was the most common mutation, followed by $-30T > C$ mutation. What's more, 3 novel δ -globin gene defects: $-44G > A$, $CD87C > T$ and $CD134T > A$ were found. A thorough analysis of the hematological, electrophoretic

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characterization, and the level of SF was needed to suspect and further investigate the existence of IDA, α -thalassemia, and δ -thalassemia.

KEYWORDS

discrimination, IDA, molecular, α -thalassemia, δ -thalassemia

1 | INTRODUCTION

Anemia affects more than 500 million women of reproductive age (between 15 and 49 years) globally, and it is considered to be a major public health problem in developing countries.^{1,2} Iron deficiency anemia (IDA) has long been known to be the most common type of anemia in the world. Thalassemia is similar to IDA in terms of symptoms, and however, it is an inherited autosomal recessive blood disorder unlike IDA. According to the type of genetic defects, which result in reduced or absent synthesis of one or several globin peptide chains, thalassemia can be divided into α , β , and δ -thalassemia.³ Interactions of these disorders can generate a wide spectrum of phenotype, which will pose diagnostic conundrum for clinical assessment, carrier screening, and genetic counseling.

After the age of 2, hemoglobin A (HbA, $\alpha_2\beta_2$) is the main component of hemoglobin (Hb), accounting for more than 96% of total Hb, followed by hemoglobin A₂ (HbA₂, $\alpha_2\delta_2$) and fetal hemoglobin (HbF, $\alpha_2\gamma_2$). HbA₂ consists of two α chains and two δ chains ($\alpha_2\delta_2$) and comprises about 2.5%-3.5% of total hemoglobin (Hb) in normal individuals.⁴ Mutations that occur on the δ globin gene (HBD, MIM#142-000), located on the cluster of the beta-like globin genes on the chromosome 11, will affect the structure or the expression of the δ globin chain, leading to decreased HbA₂ levels or HbA₂ variants.⁵ Though HbA₂ is a type of Hb without an obvious clinical implication, but it plays an important role in screening program of thalassemia and iron deficiency.^{6,7} Increased HbA₂ level is considered as a classical diagnostic criterion of β thalassemia trait. However, in some situations, normal, borderline, or reduced HbA₂ level may still be associated with β thalassemia trait, which will make difficulty in the setting of genetic counseling, whereas a decreased HbA₂ level may lead to the suspicion of IDA, δ -thalassemia, and α -thalassemia. What's more, IDA needs to be confirmed by other biological parameters or by a therapeutic correction trial and δ -thalassemia, α -thalassemia needs DNA sequencing or other specific analysis.⁸⁻¹³

Fujian area, located along the southeastern coastal regions of China, adjacent to Guangdong Province, has a high prevalence of thalassemia.¹⁴ Thus, it is important to define the spectrum of δ -thalassemia. Nevertheless, so far, there are no reports specifically investigating the prevalence, molecular characterization, and phenotype of δ -thalassemia mutations in this region, which will aid in genetic counseling and prenatal diagnosis. Many reports showed that δ -thalassemia mutations decreased HbA₂ levels (2% in heterozygote or $\leq 0.6\%$ in homozygote subjects).^{13,15-18}

Therefore, we prospectively enrolled a cohort of Fujian subjects with HbA₂ levels below 2.0% and report for the first time the

spectrum of δ -thalassemia mutations, discrimination of α -thalassemia, and IDA in this cohort. Such a study may provide more data for genetic counseling and accurate prenatal diagnosis in this region to reduce the birth of babies with thalassemia major.

2 | MATERIALS AND METHODS

2.1 | Human subjects

A total of 4 792 women of reproductive age (between 15 and 49 years) in Fujian subjects were screened using capillary electrophoresis from January 2017 to December 2018, and 166 subjects with HbA₂ levels below 2.0% with normal or reduced hematological parameters (mean corpuscular volume (MCV) < 82.0 fL and mean corpuscular Hb (MCH) < 27.0 pg)^{13,15-18} at the Outpatient Department of Fujian Provincial Maternity and Children's Hospital were recruited for this study and further examined with red cell parameters and tested for thalassemia and ferritin level. The recruited subjects had a mean age of 33 years (range from 15 to 49 years) and came from 9 cities across the province. All subjects had no genetic relationship. This study was approved by the Ethics Review Committee of Fujian Province Maternity and Child Health Hospital. Written informed consent was obtained from all participants following a detailed description of the purpose of the study.

2.2 | Screening for δ -globin gene mutations

Peripheral blood samples were collected from 4 792 subjects and anticoagulated with EDTA-K2. Approximately 2 mL of the anticoagulated blood sample from 4 792 subjects was used for analysis of blood cell parameters on a Sysmex XN-2000 automatic hematology analyzer (Sysmex; Shanghai, China) with the reference value of MCV (mean corpuscular volume) ranging from 82 to 100 fL and MCH (mean corpuscular Hb) ranging from 27 to 34 pg, and the hemoglobin components and levels of 4 792 subjects were analyzed using an automated capillary electrophoresis system (CapillaryS 2, software version 6.2; Sebia, Paris, France) with the reference value of HbA₂ (hemoglobin A₂) ranging from 2.5% to 3.5%. Also, adult hemoglobin (HbA), fetal hemoglobin (HbF), and other hemoglobin variants such as hemoglobin E (HbE), hemoglobin S (HbS), hemoglobin C (HbC), hemoglobin O-Arab, and hemoglobin D would be scanned. Serum ferritin (SF) concentration, as a measure of iron status, was determined

by the chemiluminescent microparticle immunoassay (CMIA) (Abbott; ARCHITECT ci16200, USA). The diagnostic criteria for IDA were based on the World Health Organization (WHO) guidelines. Women with SF concentration of less than 15 ng per mL and Hb concentration of less than 120 g per L were confirmed as having IDA.² The three common deletional α -thalassemia ($--^{SEA}/\alpha\alpha$, $-\alpha^{3.7}/\alpha\alpha$, and $-\alpha^{4.2}/\alpha\alpha$) were detected using Gap-PCR with the thalassemia gene detection kit (Shenzhen Yishengtang Biological Products Co., Ltd)¹⁹ and detection of the point mutations in the three nondeletional α -thalassemia ($\alpha^{CS}/\alpha\alpha$, $\alpha^{QS}/\alpha\alpha$, $\alpha^{WS}/\alpha\alpha$), and the 17 common β -thalassemia was performed using reverse dot blot hybridization (RDB) with the thalassemia gene detection kit (Shenzhen Yishengtang Biological Products Co., Ltd) following the manufacturer's instructions.²⁰ Subjects without IDA and common thalassemia were suspected as δ -globin gene mutations carriers.

2.3 | Molecular analyses for δ -globin gene mutations

Genomic DNA was extracted from the peripheral blood samples using a genomic DNA isolation kit (Qiagen) following the manufacturer's instructions. δ -globin gene mutations were identified by DNA sequencing of the HBD gene as described by Liu, N, et al¹³ The sequencing data were compared with GenBank accession No. U01317, and nt changes were analyzed against the Globin Gene Server

database (<http://globin.cse.psu.edu>). For suspected rare types of α and β thalassemia, the full-length $\alpha 1$ -, $\alpha 2$ -, and β -globin genes were amplified using PCR assay and checked. The purified PCR products were subjected to direct sequencing with an ABI 3100 DNA Sequencer (Applied Biosystems).¹⁴

2.4 | Statistical Analysis

All data were entered into and managed using Microsoft Excel 2007 (Microsoft). Normality of the data was confirmed by the Kolmogorov-Smirnov test. Associations between hematological and electrophoretic characterizations and the variant genotypes were assessed by a nonparametric Kruskal-Wallis test. P values less than 0.05 were considered statistically significant. SPSS version 20.0 was used for statistical analysis.

3 | RESULTS

3.1 | Prevalence of δ -globin gene mutations

In this study, 166 subjects with HbA₂ levels below 2.0% with normal or reduced hematological parameters were recruited, and 42 subjects were conformed without IDA, common thalassemia, and rare thalassemia that were subsequently suspected as δ -globin

TABLE 1 Summary of the δ -globin gene mutations found in 20 cases. Mutations marked with an asterisk (*) are novel

No.	Age	HbA (%)	HbA ₂ (%)	HbF (%)	HBD mutation	HBD HUGO (Nomenclature)
1	28	98.1	0.8	1.1	-77 T > C/ 44G > A*	HBD:c.-127T > C/ HBD:c.-94G > A*
2	30	96.9	1.3	1.8	-77 T > C	HBD:c.-127T > C
3	36	98.4	1.6	0.0	-30 T > C	HBD:c.-80T > C
4	32	98.3	1.1	0.6	-77 T > C	HBD:C.-127T > C
5	28	98.3	1.2	0.5	-77 T > C	HBD:C.-127T > C
6	35	95.4	1.6	3.0	-77 T > C	HBD:C.-127T > C
7	28	96.7	1.4	1.9	-77 T > C	HBD:C.-127T > C
8	35	97.2	1.5	1.3	-77 T > C	HBD:C.-127T > C
9	29	98.0	1.6	0.4	-30 T > C	HBD:C.-80T > C
10	29	96.3	1.5	2.2	-77 T > C	HBD:C.-127T > C
11	30	97.6	1.3	1.1	-77 T > C/ CD87C > T*	HBD:C.-127T > C/ HBD:C.262C > T*
12	26	98.8	1.2	0.0	-77 T > C	HBD:C.-127T > C
13	25	96.0	1.4	2.6	-77 T > C	HBD:C.-127T > C
14	26	97.7	1.4	0.9	-77 T > C	HBD:C.-127T > C
15	29	94.2	1.4	4.4	-77 T > C	HBD:C.-127T > C
16	25	98.8	1.2	0.0	-77 T > C	HBD:C.-127T > C
17	29	98.8	1.2	0.0	CD134T > A*	HBD:C.404T > A*
18	34	96.8	1.4	1.8	-77 T > C	HBD:C.-127T > C
19	27	98.1	1.2	0.7	-77 T > C	HBD:C.-127T > C
20	26	96.6	1.3	2.1	-77 T > C	HBD:C.-127T > C

gene mutations carriers. By DNA sequencing of the HBD gene, we had characterized the δ -globin gene mutations in 20 cases, revealing a frequency of 47.6% (20/42) in the selected group and of 0.4% in the women of reproductive age (20/4792). The mean age at diagnosis was 29.3 ± 3.4 , range 25-36 years. The mean HbA₂ was $1.3 \pm 0.2\%$, range 0.8%-1.6% (Table 1). We detected two previously known mutations: -77 T > C and -30 T > C and 3 new δ -globin gene defects which had not yet been reported: -44G > A, CD87C > T, and CD134T > A (Table 1, Figure 1). Heterozygosity for the -77T > C mutation was the most common abnormality observed (15/20, 75.0%), followed by heterozygosity for -30T > C mutation (2/20, 10.0%), for CD134 (T > A) mutation (1/20, 5.0%), for compound heterozygotes of -77T > C mutation and -44G > A mutation (1/20, 5.0%), and for compound heterozygotes of -77T > C mutation and CD87C > T mutation (1/20, 5.0%). The mean HbA₂ levels of -77T > C mutation were $1.3 \pm 0.1\%$, and the HbA₂ levels of -30T > C mutation were 1.6%.

3.2 | Discrimination of δ -thalassemia, α -thalassemia, and IDA

In the selected group, 85 subjects were conformed with IDA and 39 were conformed with common α -thalassemia (including 31 cases with α^+/α^0 thalassemia (17 cases with $--^{SEA}/\alpha\alpha$, 5 cases with $-\alpha^{4.2}/\alpha\alpha$, 4 case with $\alpha^{CS}/\alpha\alpha$, 3 cases with $--\alpha^{3.7}/\alpha\alpha$ and 2 cases with $\alpha^{QS}/\alpha\alpha$) and 8 cases with HbH), without subjects with 17 common β thalassemia. Subjects with δ -thalassemia had statistically higher levels of Hb, MCV, and MCH compared with subjects with IDA and α -thalassemia ($P < .05$), whereas statistically lower levels of RDW were seen in δ -thalassemia group, compared with other groups ($P < .05$). Interestingly, statistically higher levels of HbA₂ were seen in δ -thalassemia group, compared with subjects with HbH, while statistically lower levels of HbA₂ were seen in δ -thalassemia group, compared with other groups ($P < .05$). What's more, higher levels of SF was seen in δ -thalassemia group, and α -thalassemia compared with IDA groups ($P < .05$). Lower levels of Hb, MCV, and MCH and higher levels of RDW were seen in IDA group, compared with α^+/α^0 thalassemia group ($P < .05$), which were adverse compared with HbH group ($P < .05$; Table 2).

4 | DISCUSSION

Low HbA₂ level is an underlying of IDA and α -thalassemia. However, if IDA and α -thalassemia are not present, then an associated δ -thalassemia can be suspected, because HbA₂ consists of two α chains and two δ chains ($\alpha_2\delta_2$) and if mutations that occur on the δ -globin gene will lead to a decreased HbA₂ levels or HbA₂ variants.^{4,6,7} Mutations that occur on the beta globin gene generally

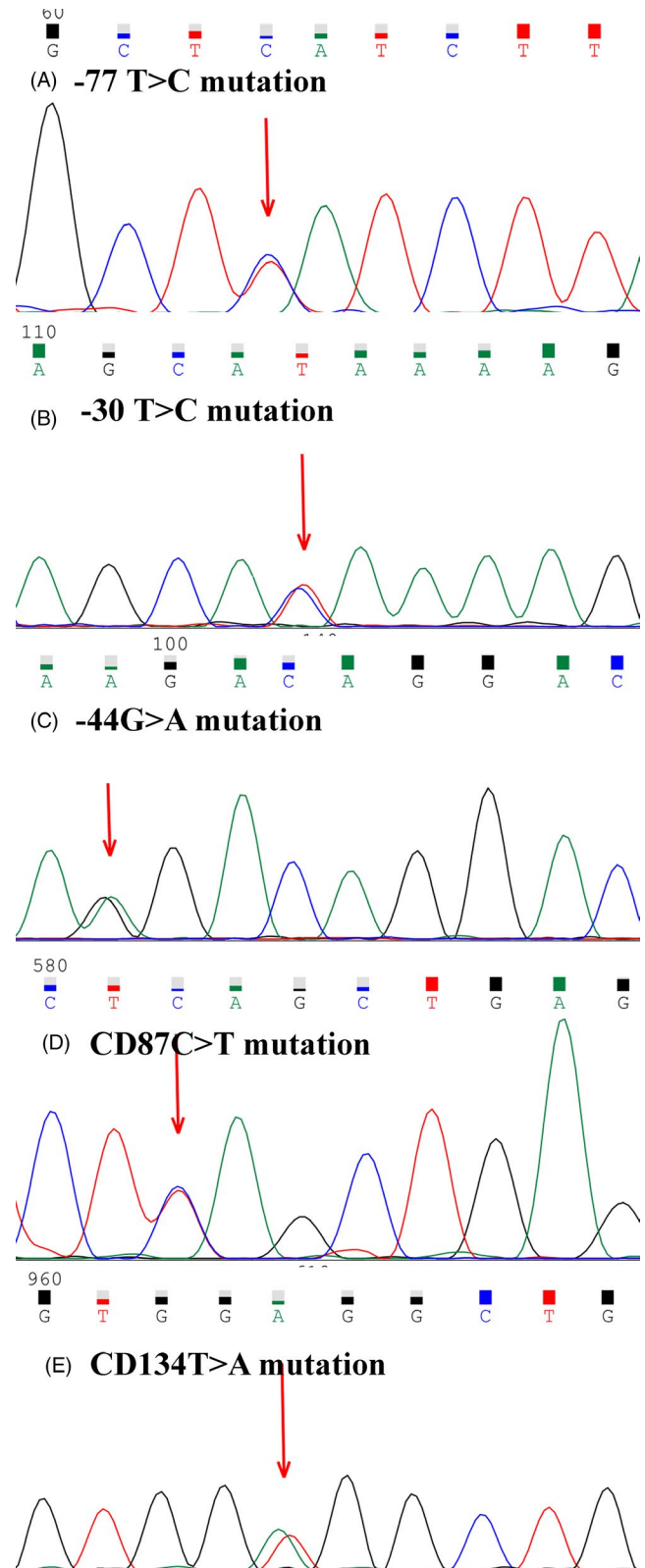


FIGURE 1 Sequence results of the 2 known and 3 novel δ -globin gene mutations, A, -77 T > C mutation; B, -30 T > C mutation; C, -44G > A mutation; D, CD87C > T mutation; E, CD134T > A mutation

TABLE 2 Discrimination of IDA, α -thalassemia, and δ -thalassemia by the hematological, electrophoretic characterization, and the level of SF (Mean \pm SD)

		Hb (g/L)	MCV (fL)	MCH (pg)	RDW (%)	HbA2 (%)	SF (ng/mL)
δ -thalassemia (n = 20)		123.8 \pm 13.1	82.6 \pm 4.1	28.6 \pm 2.4	14.1 \pm 3.8	1.3 \pm 0.2	80.9 \pm 82.2
IDA (n = 85)		86.3 \pm 13.5	70.6 \pm 6.7	21.2 \pm 3.1	19.4 \pm 3.6	1.9 \pm 0.2	5.5 \pm 2.7
α -thalassemia (n = 39)	α^+/α^0 thalassemia (n = 31)	105.6 \pm 18.9	72.3 \pm 6.9	23.4 \pm 3.2	15.6 \pm 2.7	1.8 \pm 0.2	73.9 \pm 62.5
	HbH (n = 8)	78.6 \pm 4.4	64.0 \pm 5.3	19.2 \pm 1.0	23.7 \pm 1.0	0.9 \pm 0.3	154.6 \pm 89.1
	total	101.5 \pm 20.0	71.0 \pm 7.4	22.8 \pm 3.4	16.8 \pm 3.9	1.7 \pm 0.5	84.7 \pm 71.5
P-value ^a	<.001*	<.001*	<.001*	<.001*	<.001*	<.001*	
P-value ^b	<.001*	<.001*	<.001*	<.001*	<.001*	.785	
P-value ^c	<.001*	<.001*	<.001*	<.001*	<.001*	.005*	
P-value ^d	<.001*	<.001*	<.001*	<.001*	.001*	.821	
P-value ^e	<.001*	.016*	<.001*	<.001*	.867	<.001*	
P-value ^f	.002*	<.001*	.002*	<.001*	<.001*	<.001*	
P-value ^g	<.001*	.542	.017*	<.001*	.046*	<.001*	
P-value ^h	<.001*	<.001*	<.001*	<.001*	<.001*	.003*	

^a Subjects with δ -thalassemia compared with were subjects with IDA.

^b Subjects with δ -thalassemia compared with were subjects with α^+/α^0 thalassemia.

^c Subjects with δ -thalassemia compared with were subjects with HbH.

^d Subjects with δ -thalassemia compared with were Subjects with α -thalassemia.

^e Subjects with IDA compared with were subjects with α^+/α^0 thalassemia.

^f Subjects with IDA compared with were subjects with HbH.

^g Subjects with IDA compared with were Subjects with α -thalassemia.

^h Subjects with α^+/α^0 thalassemia compared with were subjects with HbH.

* $P < .05$, Kruskal-Wallis test.

tend to an increased HbA₂ levels, but in some situations, β thalassemia traits are not associated with raised HbA₂. Furthermore, β thalassemia in combination with δ -thalassemia is described, as well as co-association with α -thalassemia, which are sources of diagnostic pitfalls in carrier screening and counseling.⁹⁻¹³

To date, 137 δ globin gene mutations have been reported, as summarized in <http://globin.cse.psu.edu/hbvar/menu.html> (accessed March 12, 2020). In this study, we reported the spectrum of δ -thalassemia mutations for the first time and found that $-77 T > C$ mutation (75.0%) was the most common mutation among women of reproductive age in Fujian subjects, followed by $-30 T > C$ mutation (10.0%), which was almost the same as the earlier report, nevertheless, with a little difference of the proportion of $-77 T > C$ mutation (63.2%) and $-30 T > C$ mutation (19.1%) in Guangdong subjects,¹³ which might be explained by the different selected subjects. Obviously, this was very different from the reports in other Asian population, such as in Iran,¹⁵ in Omani.¹⁶ What's more, we had characterized the δ -globin gene mutations in 20 cases, revealing a frequency of 0.4% in the women of reproductive age in Fujian subjects, with a mean HbA₂ of $1.3 \pm 0.2\%$, and the mean HbA₂ levels of $-77 T > C$ mutation were $1.3 \pm 0.1\%$ and the HbA₂ levels of $-30 T > C$ mutation were 1.6%, which was no distinct difference from the earlier report.¹³

Incidentally, 3 new δ -globin gene defects were found in our study, which had not yet been reported: $-44G > A$ (*HBD:c.-94G > A*), $CD87C > T$ (*HBD:c.262C > T*), and $CD134T > A$ (*HBD:c.404T > A*). The mutation of $-44G > A$ (*HBD:c.-94G > A*), was likely to cause δ^+ -thalassemia and the mutation of $CD134T > A$ (*HBD:c.404T > A*), resulted in the substitution of the amino acid valine by glutamic acid at codon 134, was likely to cause δ^+ - or δ^0 -thalassemia. What's more, the mutation of $CD87C > T$ (*HBD:c.262C > T*) resulted in a premature stop of δ -globin synthesis at codon 87 and might cause δ^0 thalassemia. Subjects with heterozygosity for $CD134T > A$ (*HBD:C.404T > A*) mutation and compound heterozygotes of $-77T > C$ mutation and $CD87C > T$ mutation showed the normal hematological parameters except for the reduction in HbA₂ level. The hematological parameters of the carrier with heterozygosity for $CD134T > A$ (*HBD:c.404T > A*) mutation was Hb 132 g/L, MCV 87.7 fL, MCH 29.5 pg, RDW 11.9%, HbA₂ 1.2%, and SF 130.2 ng/mL, and the carrier for compound heterozygotes of $-77T > C$ mutation and $CD87C > T$ mutation was Hb 128 g/L, MCV 91.1 fL, MCH 30.7 pg, RDW 13.6%, HbA₂ 1.3%, and SF 64.79 ng/mL. However, the carrier with compound heterozygotes of $-77T > C$ mutation and $-44G > A$ mutation showed a slightly decreased hematological parameters (Hb 115 g/L, MCV 79.2 fL, MCH 25.3 pg, RDW 15.3%, HbA₂ 0.8%, and SF 15.40 ng/mL), which might be due to the lower level of SF.

IDA and thalassemia were the most common types of anemia, which affected more than 500 million women of reproductive age (between 15 and 49 years) globally.^{1,2} With the reduced HbA₂ level, δ -thalassemia, IDA, and α -thalassemia were often confused. Moreover, the fundamental approach to preventing IDA was supplementing iron for individuals at high risk.²¹ Nevertheless, currently, detection of carriers with thalassemia and prenatal genetic diagnosis were the only effective interventions to prevent the birth of babies with thalassemia major and intermediate, due to lack of effective treatments for thalassemia major.²²

Therefore, effective discrimination was necessary. In this study, in 166 subjects with HbA₂ levels below 2.0% among the women of reproductive age in Fujian area, we found 20 cases (12.0%, 20/166) confirmed with δ -thalassemia, 85 cases (51.2%, 85/166) with IDA and 39 cases (23.5%, 39/166) with common α -thalassemia (including 31 cases with α^+ / α^0 thalassemia (17 cases with $-^{SEA}/\alpha\alpha$, 5 cases with $-\alpha^{4.2}/\alpha\alpha$, 4 case with $\alpha^{CS}/\alpha\alpha$, 3 cases with $-\alpha^{3.7}/\alpha\alpha$, and 2 cases with $\alpha^{QS}/\alpha\alpha$) and 8 cases with HbH), suggesting that IDA was the most common disease in the selected subjects, followed by common α -thalassemia, by δ -thalassemia. Further, we detected the hematological, electrophoretic characterization, and the level of SF of the diagnosed subjects, and the data showed that subjects with δ -thalassemia had statistically higher levels of Hb, MCV, and MCH than subjects with IDA and α -thalassemia ($P < .05$), whereas statistically lower levels of RDW were seen in δ -thalassemia group, compared with other groups ($P < .05$). Interestingly, statistically higher levels of HbA₂ were seen in δ -thalassemia group, compared with subjects with HbH, while statistically lower levels of HbA₂ were seen in δ -thalassemia group, compared with other groups ($P < .05$). What's more, higher levels of SF were seen in δ -thalassemia group and α -thalassemia group than IDA group ($P < .05$). Subjects with α^+ / α^0 thalassemia had higher levels of Hb, MCV, and MCH, inversely, lower levels of RDW, compared with subjects with IDA and HbH ($P < .05$), whereas higher levels of HbA₂ were seen in IDA group and α^+ / α^0 thalassemia group, compared with HbH group ($P < .05$). Reports in other studies had shown that an elevated RDW value and a decreased SF value were seen in IDA group,^{23,24} which was, partially, consistent with our data. This observation merited a clarification in that one with HbA₂ levels below 2.0% among the women of reproductive age in Fujian area, needed a thorough analysis of the hematological, electrophoretic characterization and the level of SF to suspect and further investigate the existence of IDA, α -thalassemia, and δ -thalassemia.

In this study, δ globin gene defect was not detected in 22 subjects with HbA₂ values lower than 2.0%, conformed without IDA and common thalassemia. For these cases, the reduced HbA₂ value could be due to rare mutations on α globin genes, or attribute to the mutations or integration artifacts not in the sequenced region or deletion mutations not identified by sequencing.

To the best of our knowledge, this study was first time to specifically deciphered the prevalence, molecular characterization, and phenotype of δ -thalassemia mutations among women of reproductive age in Fujian area. The presence of δ -thalassemia could lead to the misdiagnosis of β thalassemia, which was characterized

by increased HbA₂ level, as it produced a lower level of HbA₂. Therefore, the molecular screening of δ -globin gene mutations could be useful for the genetic counseling of at-risk couples from geographic areas in which thalassemia was common. Also, we had detected the hematological, electrophoretic characterization, and the level of SF of the confused cases and to some extent further clarified the issues. Since there were limited number of subjects among the three categories in this study, more works should be done in the future. In conclusion, to reduce the birth of babies with thalassemia major, the study of δ globin status was important and the data about the discrimination of δ -thalassemia, α -thalassemia, and IDA would provide more information for genetic counseling and accurate prenatal diagnosis.

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CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Liangpu Xu, Meihuan Chen, Hailong Huang, and Yuan Lin designed the study and prepared the manuscript. Lingji Chen, Na Lin, and Min Zhang collected the literature, collected the data, and prepared the manuscript. All authors approved the final manuscript.

ETHICAL APPROVAL

This study was proved by the Ethics Review Committee of Fujian Maternity and Child Health Hospital (2018-046).

PATIENT CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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