

Original Article

Spectrum of α -thalassemia mutations in Fujian province of South China

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Abstract: Alpha (α)-thalassemia (thal) is one of the most prevalent genetic diseases worldwide, which is especially common in tropical and subtropical districts. The aim of this study was to investigate the spectrum of α -thal in Fujian province of South China. A total of 27293 adults were continuously recruited from July 2008 to December 2015 in Fujian Provincial Maternity and Children's Hospital. Fasting venous blood samples were obtained, and detected by multiplex-polymerase chain reaction (multiplex-PCR) and reverse dot-blot hybridization. A total of 4687 cases of α -thal were found in 27293 outpatients, accounting for 17.17%. A total of six types of α -thal mutations were found, including $-\text{SEA}$ (3253/4687, 69.40%), $\alpha^{3.7}$ (leftward) (771/4867, 16.44%), $\alpha^{4.2}$ (leftward) (186/4867, 3.96%), $\alpha^{\text{QS}}\alpha$ (125/4867, 2.66%), $\alpha^{\text{WS}}\alpha$ (48/4867, 1.02%) and $\alpha^{\text{CS}}\alpha$ (65/4867, 1.38%). Clinical hematological features indicated that carriers of α -thal cases had more severe hypochromic microcytic anemia than non-thal individuals. The frequency of α -thal in Fujian province was similar with Guangdong, Guangxi, Hainan and Yunnan regions, but different with Guizhou and Chongqing. 217 α -thal cases were also found β -thal mutations, including IVS-2-654 (C \rightarrow T), CD41-42 (-TCTT), CD17 (A \rightarrow T), -28 (A \rightarrow G), CD27-28 (+C), CD26 (G \rightarrow A), CD71-72 (+A), CD43 (G \rightarrow T), -29 (A \rightarrow G), ATG \rightarrow AGG, IVS-1-1 (G \rightarrow T), IVS-1-5 (G \rightarrow T), codon 36 (-C), codon 30 (A \rightarrow G), +22 (G \rightarrow A) and codons 54-58 (-TTATGGGCAACCC). In conclusion, this is the first comprehensive mutation spectrum of α -thal mutations detected in Fujian province. The incidence of α -thal in Fujian province is high, and the gene variation spectrum is complex. Of these mutations, $-\text{SEA}$, $\alpha^{3.7}$ and $\alpha^{4.2}$ were the most prevalent α -thal gene mutation. Our results provide information regarding α -thal mutations on the Fujian province that may be useful for further genetic counseling, prenatal screening and clinical diagnosis of α -thal in this region.

Keywords: A-thal, mutations, Fujian province, anaemia

Introduction

Thalassemias are hereditary anemia syndromes occurring due to a deficit in the production of globin chains of hemoglobin (Hb) [1-3]. Alpha (α)-thalassemia (thal) is the most common type of thalassemia [4]. The term α -thal encompasses all of those conditions in which there are mutations in the production of α -globin chains of Hb [5, 6]. The α -globin gene cluster is located on the short arm of human chromosome 16, which includes $\alpha 1$, $\alpha 2$, and embryonic zeta globin genes. There are four copies of α -globin genes with two genes on each chromosome 16 ($\alpha\alpha/\alpha\alpha$). Deletion of both a genes on a single chromosome is called α^0 -thal (- / $\alpha\alpha$), and the one a gene deletion is called α^+ -thal (- $\alpha/\alpha\alpha$) [7]. Clinically there are four types of α -thal: silent carrier, α -thal trait,

α -thal intermedia (Hb H disease), and α -thal major (hydrops fetalis).

The severity of the clinical phenotype of α -thalassemia is diverse and depends on the copy number of α gene affected and the type of deletion or non-deletion mutation occurred [8]. Most silent carriers (one affected gene) are asymptomatic with normal hematological parameters. Individuals with α -thal trait (two affected genes) have mild anemia with the erythrocyte mean corpuscular volume (MCV) less than 80.0 fl, and the mean corpuscular hemoglobin (Hb) (MCH) below 27.0 pg. α -thal intermedia (three affected genes) causes mild-to-moderate anemia, and some affected individuals require regular transfusion. α -thal major (four affected genes) is the most severe type, and the individuals with this status are either

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Table 1. Clinical characteristics of participants

Group	Male	Female
Cases	11478	15815
Age (years)	43.25 \pm 2.78	42.55 \pm 3.01
BMI ratio (kg/m ²)	20.79 \pm 3.34	29.78 \pm 2.86
RBC (10 ¹² /L)	5.23 \pm 0.46	4.72 \pm 0.53
Hb (g/L)	145.38 \pm 12.57	124.03 \pm 10.44
MCV (fL)	82.66 \pm 6.69	80.34 \pm 6.67
MCH (pg)	28.88 \pm 3.21	28.42 \pm 3.17

Data are shown as mean \pm standard deviation (SD). BMI: body mass index; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin.

stillborn or die soon. The most clinical phenotype of individuals with α -thal is mild and recognized during a routine complete blood count. Increasing evidence has shown that about 7.0% of populations of the world are carriers of α -globin gene mutation [1, 3, 6].

Fujian province of the People's Republic of China (PRC) is situated in the southeastern part of China, facing Taiwan across the Taiwan Straits, and adjacent to provinces with high thal morbidity [9]. However, there is no comprehensive studies of α -thal were available for Fujian province. A detailed mutation spectrum of α -thal in this area is essential and urgently needed. In the present study, a total of 27293 adults from all over Fujian province were analyzed. The goal of this study was to identify and expand the mutational spectrum in this region in order to facilitate its molecular diagnosis, genetic counseling, and prenatal diagnosis (PND).

Materials and methods

Ethics approval

The study was approved by the Ethic Committees of Fujian Provincial Maternity and Children's Hospital (Fuzhou, China). Consent forms was signed from all patients and conducted in accordance with the regulations of the Declaration of Helsinki.

Clinical samples

The current study was conducted between July 2008 to December 2015 at Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternal and Children Health Hospital (Fuzhou, China). A total of 27293

adults were randomly selected from all counties across Fujian Province; there were 11478 males and 15815 females in the study. The clinical characteristics of samples are shown in **Table 1**. Fasting venous blood sample of each participant was obtained and taken in EDTA-contained tubes (Qiagen Inc., Valencia, CA, USA).

Blood routine examination

Red blood cell (RBC) indices were determined by using automated cell counter (XS-800i; Sysmex Co. Ltd., Japan). The levels of HbA, A2, and HbF were analyzed on the Bio-Rad Variant II HPLC system (HPLC, VARIAN™, Bio-Rad, USA). α -thal diagnosis is based on microcytosis (mean corpuscular volume (MCV) 80.0 fl, mean corpuscular Hb (MCH) 27.0 pg) and normal lower Hb A2 level (2.5%). These RBC indices may also show other low values: Hb (512.0 g/dL in females and 514.0 g/dL in males) and mean corpuscular Hb concentration (MCHC) 531.0 g/dL.

DNA extraction

DNA was extracted from whole blood cells by using the QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany). The concentration of DNA was determined by using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA was subsequently stored at -20°C for further use.

In this study, PCR was performed for detection of deletions, and a concentration of DNA around 80 ng/ml was required.

Gap-PCR

Gap-PCR was used to detect 3 common missing ($-\alpha^{SEA}$, $-\alpha^{3.7}$ and $-\alpha^{4.2}$) of α -globin gene cluster [10, 11]. Gap-PCR conditions were performed as follows: each 50 μ L reaction contained 200 mM of each dNTP, 1.5 mM MgCl₂, 1xQ-solution (Qiagen GmbH), 2.5 U HotStarTaq DNA polymerase in supplied reaction buffer (Qiagen GmbH), 100-200 ng of genomic DNA, and 16 different primers at various concentrations. Reactions were conducted in a T3 thermal cycler (Biometra GmbH, Germany), with an initial 15 minutes denaturation at 96°C, followed by 30 cycles at 95°C denaturation for 30 seconds, 60°C annealing for 90°C seconds, and extension at 72°C for 2 min and 15 seconds. A

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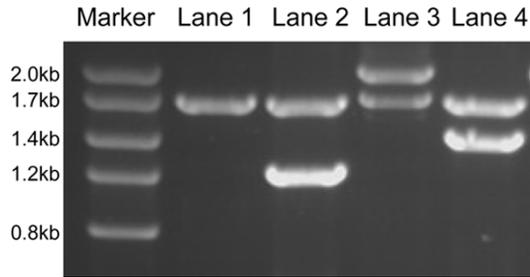


Figure 1. Gel electrophoresis of PCR amplifying results showed three deletional genotype of α -thal mutations. Lane 1: Normal; Lanes 2: Southeast Asia deletion (genotype of $-\text{SEA}$); Lane 3: Leftward deletion (genotype of $\alpha^{3.7}$); Lanes 4: Leftward deletion (genotype of $\alpha^{4.2}$).

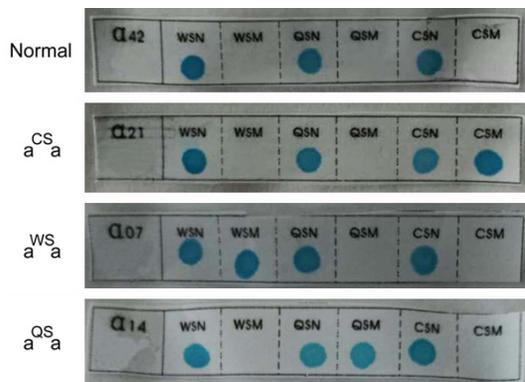


Figure 2. Reverse dot-blot hybridization showed three non-deletional genotype ($\alpha^{\text{CS}}\alpha$, $\alpha^{\text{WS}}\alpha$ and $\alpha^{\text{QS}}\alpha$) of α -thal mutations.

Table 2. α -thalassemia mutations identified in Fujian province

Mutation	Number	Ratio (%)
$-\text{SEA}$	3253	69.40%
$\alpha^{3.7}$ (leftward)	771	16.44%
$\alpha^{4.2}$ (leftward)	186	3.96%
$\alpha^{\text{QS}}\alpha$	125	2.66%
$\alpha^{\text{WS}}\alpha$	48	1.02%
$\alpha^{\text{CS}}\alpha$	65	1.38%

final extension at 72°C for 5 minutes. 1.5% agarose gel electrophoresis was applied to detect PCR amplified results.

Reverse dot-blot hybridization

Reverse dot-blot hybridization was used for the three nondeletional types of α -thal mutations [Hb Constant Spring (Hb CS) or $\alpha 142$, Term→Gln, TAA>CAA ($\alpha 2$), Hb Quong Sze (Hb QS) or $\alpha 109$ (H8) Leu→Pro, CTG>CCG ($\alpha 2$) and

Hb Westmead or $\alpha 122$ (H5) His→Gln, CAC>CAG ($\alpha 2$)] and β -thal mutations. Samples with incomplete genotype findings from the multiplex PCR method were further analyzed by direct DNA sequencing of the entire α -globin genes in an ABI PRISM 3130 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The PCR products were purified by using QIA quick (Qiagen, Germany) PCR purification kit. Then, the sequenced samples were precipitated with ethanol-sodium acetate precipitation and used for Sanger sequencing [12, 13]. After sequencing, the data were analyzed using DNA sequencing analysis version 5.2 software (Invitrogen, CA, USA).

Statistical analysis

SPSS software version 19.0 (SPSS, Chicago, IL, USA) was applied for statistical analysis. Data are represented as the mean \pm standard deviation (SD). One-way ANOVA (One-way analysis of variance) or Student's t-test was used to analyze the differences between groups. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

General clinical data

A total of 27293 adults were continuously recruited from July 2008 to December 2015 in Fujian Provincial Maternity and Children's Hospital. Fasting venous blood samples were obtained and were determined. The clinical data of male and female groups are shown in **Table 1**. Statistical analysis revealed that there was no significant difference in age, sex, body mass index (BMI), body mass index (RBC), hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the male and female groups.

α -thalassemia mutations identified in Fujian province

α -thalassemia mutations were detected by using Gap-PCR and reverse dot-blot hybridization. The gel electrophoresis of Gap-PCR amplifying results are shown in **Figure 1**. Non-deletional types of α -thal mutations are shown in **Figure 2**.

4687 cases of α -thal were found in 27293 outpatients, accounting for 17.17%. As shown

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Table 3. Hematological features of different α -thal genotypes

Genotype	Sex	Case	Hb (g/L)	MCV (fL)	MCH (pg)
--SEA/ $\alpha^{3.7}$	Male	21	92.01 \pm 12.71	54.44 \pm 8.60	17.23 \pm 2.11
	Female	43	80.25 \pm 8.73	63.09 \pm 7.75	18.36 \pm 1.54
--SEA/ $\alpha^{4.2}$	Male	7	91.23 \pm 16.50	64.41 \pm 8.78	18.59 \pm 4.03
	Female	23	81.32 \pm 7.45	62.81 \pm 6.93	18.99 \pm 3.32
--SEA/ $\alpha^{CS}\alpha$	Male	8	80.35 \pm 9.14	70.33 \pm 7.41	18.46 \pm 1.78
	Female	10	68.21 \pm 13.25	75.93 \pm 2.82	20.64 \pm 0.95
--SEA/ $\alpha^{QS}\alpha$	Male	3	82.57 \pm 24.74	65.71 \pm 15.86	18.92 \pm 3.53
	Female	2	69.56 \pm 9.27	74.01 \pm 7.05	18.24 \pm 1.67
--SEA/ $\alpha^{WS}\alpha$	Female	2	104.06 \pm 6.41	61.77 \pm 0.12	20.26 \pm 0.14

α -thal: alpha-thalassemia.

in **Table 2**, a total of six types of α -thal mutations were found, including --SEA (3253/4687, 69.40%), $\alpha^{3.7}$ (leftward) (771/4867, 16.44%), $\alpha^{4.2}$ (leftward) (186/4867, 3.96%), $\alpha^{QS}\alpha$ (125/4867, 2.66%), $\alpha^{WS}\alpha$ (48/4867, 1.02%) and $\alpha^{CS}\alpha$ (65/4867, 1.38%). 217 α -thal cases were also found β -thal mutations, including IVS-2-654 (C \rightarrow T), CD41-42 (-TCTT), CD17 (A \rightarrow T), -28 (A \rightarrow G), CD27-28 (+C), CD26 (G \rightarrow A), CD71-72 (+A), CD43 (G \rightarrow T), -29 (A \rightarrow G), ATG \rightarrow AGG, IVS-1-1 (G \rightarrow T), IVS-1-5 (G \rightarrow T), codon 36 (-C), codon 30 (A \rightarrow G), +22 (G \rightarrow A) and codons 54-58 (-TTATGGGCAACCC).

Hematological features of different α -thal genotypes

As shown in **Table 3**, clinical hematological features indicated that carriers of α -thal cases had more severe hypochromic microcytic anemia than non-thal individuals. There was no significant difference in the hemoglobin content between in non-deletional genotype group (--SEA/ $\alpha^{CS}\alpha$, --SEA/ $\alpha^{QS}\alpha$, --SEA/ $\alpha^{WS}\alpha$) and in deletional genotype group (--SEA/ $\alpha^{3.7}$ and --SEA/ $\alpha^{4.2}$), the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) in non-deletional genotype group was higher than that in deletional genotype group ($P < 0.05$). However, when the number of --SEA/ $\alpha^{WS}\alpha$ cases were excluded, the Hb content in non-deletional genotype group was significantly lower than that in deletional genotype group, and the difference of MCV and MCH between the two groups did not change significantly ($P < 0.05$).

Compared with the mutation of α -thal in neighboring provinces of Fujian province

The incidence of α -thal in Fujian province was high, and the gene variation spectrum was

complex. The mutation frequencies were compared to the distribution characteristics of six neighboring provinces of Fujian province, including Guangdong, Guangxi, Hainan, Yunnan, Guizhou and Chongqing. As shown in **Table 4**, the frequency of α -thal was similar with Guangdong, Guangxi, Hainan and Yunnan regions, but different with Guizhou and Chongqing ($P < 0.05$).

Discussion

Although α -thal is one of the most common genetic disorders worldwide, different populations or ethnic groups often have different prevalence and spectrum of α -thal [14]. The molecular characteristics of α -thal in most South China regions have been elucidated. The provinces with the highest prevalence of α -thal are Guangxi and Guangdong, with the prevalence rates being 17.55% and 8.53%, respectively [15, 16]. In the Chinese population, α -thal is most frequently caused by deletions of one or both a genes. Three common deletions, the Southeast Asian deletion (--SEA), $\alpha^{3.7}$ and $\alpha^{4.2}$, account for more than 92.0% of all the mutation alleles in Chinese carriers and patients, and no more than 8.0% of α -thal is caused by non-deletional mutations, with the three most common types of alleles being $\alpha^{CS}\alpha$, $\alpha^{QS}\alpha$ and $\alpha^{WS}\alpha$ [17]. However, the prevalence and mutation spectrum of α -thal in Fujian province, a mountainous area in Southwestern China, are still unknown. The estimated population of Fujian province was around 36.7 million in 2010. Therefore, it is important to determine the prevalence and mutation spectrum of α -thal for the control of this disease in this region.

In this study, we performed the first molecular epidemiological survey of α -thal in Fujian province. A total of 27293 adults were continuously recruited from July 2008 to December 2015 in Fujian Provincial Maternity and Children's Hospital. The results revealed that the carrier rate of α -thal in Fujian Province is 17.17%. A previous study reported that the carrier frequency of β -thal was 1.51% in Fujian Province [9]. The current study was an initial screening of

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Table 4. Compared with the mutation of α -thal in neighboring provinces of Fujian province

Mutations	Fujian (%)	Guangxi (%)	Guangdong (%)	Hainan (%)	Chongqing (%)	Yunnan (%)	Guizhou (%)
--SEA	69.40	68.50	70.54	44.21	15.25	59.2	16.78
$\alpha^{3.7}$ (leftward)	16.44	16.36	13.40	29.58	50.85	19.0	48.23
$\alpha^{4.2}$ (leftward)	3.96	8.40	5.98	26.21	8.47	6.34	4.76
$\alpha^{QS}\alpha$	2.66	0.62	1.66	-	1.69	-	-
$\alpha^{WS}\alpha$	1.02	3.09	2.88	-	-	-	-
$\alpha^{CS}\alpha$	1.38	3.03	5.54	-	15.52	-	14.21

α -thal mutations by using Gap-PCR and reverse dot-blot hybridization. In comparison with neighboring provinces, the frequency of α -thal carriers in Fujian Province (17.11%) was similar with Guangdong, Guangxi, Hainan and Yunnan regions, but different with Guizhou and Chongqing [18-21].

Due to the limited number of α -thal carriers detected in 4687 umbilical cord blood samples, the spectrum of α -thal mutations derived from these data may not be complete. In order to perfectly reflect the actual spectrum of α -thal mutations in Fujian province, the results of genetic testing of 4687 α -thal patients or carriers were analyzed retrospectively. A total six types of α -thal mutations were found, including --SEA (3253/4687, 69.40%), $\alpha^{3.7}$ (leftward) (771/4867, 16.44%), $\alpha^{4.2}$ (leftward) (186/4867, 3.96%), $\alpha^{QS}\alpha$ (125/4867, 2.66%), $\alpha^{WS}\alpha$ (48/4867, 1.02%) and $\alpha^{CS}\alpha$ (65/4867, 1.38%). Clinical hematological features indicated that carriers of α -thal cases had more severe hypochromic microcytic anemia than non-thal individuals. There was no significant difference in the hemoglobin content between in non-deletional genotype group (--SEA/ $\alpha^{CS}\alpha$, --SEA/ $\alpha^{QS}\alpha$, --SEA/ $\alpha^{WS}\alpha$) and in deletional genotype group (--SEA/ $\alpha^{3.7}$ and --SEA/ $\alpha^{4.2}$), the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) in non-deletional genotype group was higher than that in deletional genotype group. This frequency was consistent with that reported in Guangxi (68.50%) [22] and in Yunnan (59.2%) [19], but lower than that in Hong Kong (89.0%) [17]. As a result, --SEA is the most common mutation of α -thal in South China with varying allele frequencies in different regions, except Chongqing and Guizhou, where the most common mutation is $\alpha^{3.7}$. Three non-deletional mutations were detected in this study. The allele frequency of Hb $\alpha^{CS}\alpha$ (1.38%) was similar to those reported in Guangdong (2.09%) and Guangxi (3.03%) but much lower than that in Chongqing (15.52%) and Guizhou (14.21%).

Precise characteristics and quantification risks of α -thal in different populations is necessary to develop policies for the diagnosis and management of α -thal, especially in prenatal diagnosis (PND) of Hb Bart's hydrops fetalis, and long-term management of Hb H disease [8]. Currently, there are no programs on management and prenatal testing of α -thal in Fujian province due to the lack of epidemiological data of this disorder in this region. In this study, the average carrier rate was 1.73% (95% confidence interval (95% CI) 1.13 to 1.94%) for --SEA, 1.64% (95% CI 1.23 to 2.59) for α -thal caused by only one affected α -globin gene. Based on the data issued by the local government, the annual number of births is about 500,000 in Fujian province. Thus, the expected annual number of hydrops fetalis and Hb H disease is 95 (95% CI 43 to 158) and 133 (95% CI 81 to 245), respectively. The results of this study will be useful in genetic counseling and PND service of α -thal in Fujian province.

In conclusion, this study reveals α -thal mutations in the Fujian province of South China. The incidence of α -thal in Fujian province is high, and the gene variation spectrum is complex. Of these mutations, --SEA, $\alpha^{3.7}$ and $\alpha^{4.2}$ are the most prevalent α -thal gene mutation. In addition, data based on clinical hematological variable analysis indicates that the severity of hypochromic microcytic anemia is associated with the genotype of α -thal. Our results provide evidence that may be useful for further genetic counseling, prenatal screening and clinical diagnosis of α -thal in this region.

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Disclosure of conflict of interest

None.

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