Genotype Spectrum and Hematological Features of β–Thalassemia with or without Different Forms of α-Thalassemia in Shenzhen

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Abstract

Background: The effects of different forms of α−thalassemia on β-thalassemia phenotype has not been clearly described thus far.

Methods: Genotype spectrum and hematological features of 873 female diagnosed as β-thalassemia carriers with or without different forms of α-thalassemia was retrospectively analyzed.

Results: Thirteen kinds of genotypes were found in the 755 β-thalassemia carriers, including four kinds of β^+-thalassemia, eight kinds of β^0 -thalassemia and one kind of β^E-thalassemia. The values of hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) decreased and red blood cell (RBC) increased in the order of β^E, β^+- and β^0 group ($p<0.05$). Nine genotypes were determined from the 43 carriers with concurrent α- and β^+-thalassemia and seventeen genotypes were determined from the 75 carriers with concurrent α- and β^0 -thalassemia. Significant higher Hb, MCV and MCH values were noted in β^+−thalassemia or β^0−thalassemia co-inherited with α^+-thalassemia or α^0-thalassemia as compared to the only β^+−thalassemia or β^0−thalassemia heterozygosity with normal alpha globin gene ($p<0.05$). Moreover, the values of Hb, MCV and MCH increased much more when β^0−thalassemia co-inheritance of α^0-thalassemia than that of α^+-thalassemia ($p<0.05$).

Conclusion: The β-thalassemia presented diverse molecular heterogeneity and hypochromic microcytosis at various degrees. Co-inherited with α−thalassemia could alleviate phenotype of anemic in β^+- or β^0-thalassemia, and β^0-thalassemia demonstrated milder phenotype with two deletion or mutation in α-globin gene than one.

Background

Thalassemia is an autosomal recessive hereditary diseases, it is widely distributed in the tropical and subtropical areas including Mediterranean region, Middle East, Indian subcontinent, east and southeast Asia [1]. It is characterized by decreased or absent of α or β-globin chain synthesis that resulting in ineffective erythropoiesis and excessive peripheral hemolysis. There are two main types of thalassemia, α-thalassemia and β-thalassemia. The clinical manifestations range widely from mild hypochromic anemia to lifelong transfusion-dependent anemia [2, 3]. Most of fetuses with hemoglobin Bart's Hydrops fetalis died in the middle or late period of pregnancy or just after birth. β−thalassemia major presented with severe anemia from infancy and it is usually treated by regular blood transfusion, iron therapy and hematopoietic stem cell transplantation [4]. It is an effective way to avoid the birth of children with severe thalassemia by prenatal diagnosis of fetal gene, early detection and termination of pregnancy.

According to slight reduction or complete absence of β-globin chain, β-thalassemia is divided into two types, β^+- and β^0- thalassemia [5]. Because of the complex interaction of several genetic modifiers and environment, different clinical manifestations were presented even for the same genotype [6]. Several modifiers (including the severity of β-thalassaemia mutation, concomitant α-thalassemia and HbF levels)

have strong impacts on ameliorating the clinical severity of β-thalassemia [7]. Carries co-inheritance of different types of α-thalassaemia could ameliorate the phenotype of β-thalassemia at various degrees, which could result in β−thalassemia ignored and is a huge challenge for the screening program. It would be important to detect the genotype and phenotype characterizations of β-thalassemia in high prevalence regions. Previous studies in China were mainly focus on the molecular epidemiological investigation of β-thalassemia carriers, but limited data were available about the effects of different forms of α−thalassemia on β-thalassemia phenotype in a cohort of carriers [8–11]. In this study, we prospectively evaluated a cohort of 873 β-thalassemia carriers in order to elucidate the molecular heterogeneity and interaction of α−thalassemia on clinical severity of β-thalassemia.

Methods

Study Population

A total of 898 females who were diagnosed as β-thalassemia carriers by DNA analysis due to abnormal hematological parameters or hemoglobin A_2 (HbA_2) when attending at antenatal or premarital outpatient were included in Shenzhen Baoan Women's and Children's Hospital in Guangdong Province, China. The age of β-thalassemia carriers ranged from 18 to 45 years. The genotype and hematological parameter of all the subjects were analyzed retrospectively. Informed consent was signed by each participant. This study was approved by the Ethics Committee of Shenzhen Baoan Women's and Children's Hospital, Jinan University.

Hematological Measurements

For each participant, 2 ml of peripheral blood sample was collected into EDTA-K_2 anti-coagulated tube and sent for hematological analyses within 3 hours. By using automatic LH750 blood cell analyzer (Beckman Coulter, USA), hematological parameters including red blood cell (RBC), hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured according to the manufacturer’s procedures.

Dna Analysis

2 ml of peripheral blood samples were collected into sodium citrate anti-coagulated tubes for gene detection. Genomic DNA was extracted from peripheral blood with commercially available kit (Yaneng Biotech, Shenzhen, China) according to the manufacturer’s procedures. The DNA concentration was determined by NanoDrop spectrophotometry (Thermo Scientific, USA). Three nondeletional mutations of α-thalassemia and seventeen common nondeletional mutations of β-thalassemia were detected by reverse dot-blot hybridization after being amplified by the polymerase chain reaction (PCR),and three deletional mutations were carried out by gap PCR (Yaneng Biotech, Shenzhen, China).

The three deletional mutations of α-thalassemia included the rightward deletion (-$a^{3.7}$), the leftward deletion (-$a^{4.2}$) and the Southeast-Asian deletion (--^{SEA}), and three nondeletional mutations of α-thalassemia contained Hb Constant Spring (Hb CS; *HBA2*: c.427 T□C), Hb Quong Sze (Hb QS; *HBA2*: c.377 T□C) and Hb Westmead (Hb WS; *HBA2*:c.369C□G).The seventeen nondeletional mutations of β-thalassemia described as follows: codons 41–42(-TCTT), *HBB c.*126_129delCTTT; codons 43(G□T), *HBB c.*130G□T; IVS-Ⅱ-654 (C→T), *HBB c.*316–197 C□T); -28(A□G), *HBB c.*–78A□G; -29(A→G), *HBB c.*-79A□G; -30(T□C), *HBB c.*-80 T□C; -32(C□A), *HBB c.*-82 C□A; codons 71–72(+ A), *HBB c.*216_217insA; codons 26(G□A), *HBB c.*79G□A; codons 17(A□T), *HBB c.*52A□T; codons 31(-C), *HBB c.*94delC; codons 14–15(+ G), *HBB c.*45_46insG; codons 27–28(+ C), HBB c.84_85insC; IVS-Ⅰ-1(G□T), HBB c.92 + 1G□T; IVS-Ⅱ-5 (G□C), *HBB c.*92 + 5G□C; CAP + 40 − 30(-AAAC), *HBB c.*-11_-8del AAAC and Initiation codon (T□G), *HBB c.*2T□G.

Statistical analysis

Statistical analyses were conducted with SSPS 12.0 software. Mean and standard deviation were used to describe the distribution of hematological parameters of the subjects. One-Way analysis of variance (ANOVA) was used to test the difference of RBC, Hb, MCV and MCH among groups with different genotypes. P-value□0.05 was considered as statistically significant.

Results

Genotypes and hematological parameters of β- thalassemia carriers

873 suspected carriers were confirmed to be β-thalassemia by DNA analysis, of which 755 were β-thalassemia carriers, 43 were α- and β^+-thalassemia carriers and 75 were concurrent α- and β^0–thalassemia carriers. In total, thirteen kinds of different genotypes were found in the 755 β-thalassemia heterozygosity including four genotypes of β^+-thalassemia (313), eight genotypes of β^0-thalassemia (421) and one type of β^E-thalassemia (21). The most common two genotype of β^+ -thalassemia was β^{CD654}/β^N (69.3%, 217/313) and β^{-28}/β^N (27.8%, 87/313), followed by β^{-29}/β^N (1.9%, 6/313) and β^{CAP}/β^N (0.96%, 3/313). Meanwhile, the most common two genotypes of β^0-thalassemia were $\beta^{CD41-42}/\beta^N$ (63.4%, 267/421) and β^{CD17}/β^N (27.8%, 117/421), and the remaining six genotypes identified with low frequencies included $\beta^{CD71-72}/\beta^N$ (3.3%, 14/421), $\beta^{CD27/28}/\beta^N$ (2.1%, 9/421), β^{CD43}/β^N (1.7%,7/421), $\beta^{CD14-15}/\beta^N$ (0.71%,3/421), $\beta^{IVS-I-IM}/\beta^N$ (0.71%, 3/421) and β^{Int}/β^N (0.24%, 1/421).

The values of Hb, MCV and MCH decreased and RBC increased in the order of β^E, β^+- and β^0-group ($P<$ 0.05, Table 1). The hematological parameters of the different genotypes of β^+- and β^0-thalassemia carriers were summarized in Table 2. In most of cases, β^+- and β^0-thalassemia manifested typical hypochromic microcytosis with low Hb, MCV and MHC values. However, we didn't do statistical analysis for each kind of mutations in β^+- or β^0-thalassemia group because of insufficient samples number in some genotypes.

Table 1

Hematological parameters of β^E, β^+- and β^0- thalassemia

Genotype	N	RBC(× 10^{12}/L)	Hb(g/L)	MCV(fL)	MCH(pg)
β^E	21	4.3 ± 0.4 ^{bc}	112.5 ± 10.4 ^{bc}	79.6 ± 4.4 ^{bc}	26.1 ± 1.3 ^{bc}
β^+	313	4.7 ± 0.6 ^{ac}	100.1 ± 10.7 ^{ac}	67.8 ± 5.0 ^{ac}	21.3 ± 1.7 ^{ac}
β^0	421	4.8 ± 0.6 ^{ab}	97.2 ± 9.5 ^{ab}	64.9 ± 3.8 ^{ab}	20.3 ± 1.2 ^{ab}

^aP,^bP and ^cP meaned significant differences respectively from β^E group, β^+ group and β^0 group.

Table 2

Hematological parameters of different genotypes of β^0 and β^+-thalassemia

Genotype		N	RBC (× 10^{12}/L)	Hb (g/L)	MCV (fL)	MCH (pg)
β^+ (313)	$\beta^{IVS-\square-654}/\beta^N$	217	4.7 ± 0.6	96.8 ± 9.5	65.9 ± 3.5	20.6 ± 1.1
	β^{-28}/β^N	87	4.7 ± 0.5	107.6 ± 9.4	71.5 ± 3.7	22.7 ± 1.1
	β^{-29}/β^N	6	4.7 ± 0.6	109.8 ± 9.1	71.6 ± 3.0	23.2 ± 0.8
	β^{CAP}/β^N	3	3.4 ± 0.3	104.0 ± 4.6	92.8 ± 2.1	30.3 ± 1.6
β^0 (421)	$\beta^{CD41-42}/\beta^N$	267	4.8 ± 0.6	97.2 ± 9.3	65.5 ± 3.9	20.4 ± 1.2
	β^{CD17}/β^N	117	4.8 ± 0.6	98.0 ± 9.9	64.4 ± 3.2	20.2 ± 1.1
	$\beta^{CD71-72}/\beta^N$	14	4.7 ± 0.3	96.6 ± 6.2	64.9 ± 3.8	20.4 ± 1.0
	$\beta^{CD27/28}/\beta^N$	9	4.6 ± 0.9	91.7 ± 11.0	64.7 ± 5.9	20.2 ± 2.0
	β^{CD43}/β^N	7	4.3 ± 0.5	92.6 ± 8.1	67.8 ± 4.1	21.4 ± 1.1
	$\beta^{CD14-15}/\beta^N$	3	4.5 ± 0.14	97.0 ± 1.0	66.5 ± 0.9	21.0 ± 0.7
	$\beta^{IVS-I-IM}/\beta^N$	3	4.8 ± 0.8	100.3 ± 16.3	67.7 ± 5.2	20.9 ± 1.2
	β^{Int}/β^N	1	4.1	76.0	59.5	18.7

Genotypes And Hematological Parameters Of β-thalassemia Combined With α-thalassemia

As shown in Table 3, nine genotypes were determined from the 43 carriers with concurrent α- and β^+-thalassemia. The most common genotype was $\beta^{IVS-\square-654}/\beta^N$ simultaneously with $--^{SEA}/\alpha\alpha$ that accounted

for 39.5% (17/43). The hematological parameters for β^+-thalassemia combined groups were summarized in Table 4. Significant higher Hb, MCV and MCH values were noted in β^+–thalassemia co-inherited with α^+-thalassemia or α^0-thalassemia as compared to the only β^+–thalassemia heterozygosity with normal alpha globin gene (control) (p□0.05). There were no significant differences observed of Hb, MCV and MCH values between β^+–thalassemia co-inherited with α^+-thalassemia and α^0-thalassemia (p□0.05). Moreover, there were no significant differences of RBC value among the three groups (p□0.05).

Table 3

The genotype spectrum of β^+-thalassemia combined with α-thalassemia

β^+/β^N	α^+-thalassemia (22)				α^0-thalassemia (21)
	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{4.2}/\alpha\alpha$	$\alpha\alpha^{WS}/\alpha\alpha$	$\alpha\alpha^{CS}/\alpha\alpha$	$--^{SEA}/\alpha\alpha$
$\beta^{IVS-□-654}/\beta^N$	3	4	1	0	17
β^{-28}/β^N	6	5	2	1	4

Table 4

Hematological parameters of β^+-thalassemia heterozygote combined with different genotypes of α-thalassemia

β^+	N	RBC ($\times 10^{12}$/L)	Hb (g/L)	MCV (fL)	MCH (pg)
α^0-thalassemia	21	4.9 ± 0.4	110.3 ± 7.9[a]	71.9 ± 3.7[a]	22.7 ± 0.9[a]
α^+-thalassemia	22	4.6 ± 0.4	107.2 ± 10.0[a]	72.6 ± 5.0[a]	22.9 ± 1.7[a]
Control	313	4.7 ± 0.6	100.1 ± 10.7	67.8 ± 5.0	21.3 ± 1.7
[a]significant differences from control group.					

Genotypes and hematological parameters of β^0-thalassemia combined with α-thalassemia

Seventeen genotypes were observed from the 75 carriers with concurrent α- and β^0 –thalassemia in Table 5. The most common combined genotype was $\beta^{CD41-42}/\beta^N$ simultaneously with $--^{SEA}/\alpha\alpha$ that accounted for 30.7% (23/75). The hematological parameters for β^0-thalassemia combined groups were summarized in Table 6. The average values of Hb, MCV and MCH decreased in the order of β^0–thalassemia co-inheritance of α^0-thalassemia group, α^+-thalassemia group and control group (p□0.05). However, there were no significant differences of RBC value among the three groups (p□0.05).

Table 5

The genotype spectrum of β^0-thalassemia combined with α-thalassemia

β^0 /β^N Mutations	α^+-thalassemia(43)				α^0-thalassemia (32)
	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{4.2}/\alpha\alpha$	$\alpha\alpha^{WS}/\alpha\alpha$	$\alpha\alpha^{CS}/\alpha\alpha$	$--^{SEA}/\alpha\alpha$
$\beta^{CD41-42}/\beta^N$	13	4	7	2	23
β^{CD17}/β^N	4	3	2	0	6
$\beta^{CD71-72}/\beta^N$	2	0	1	1	2
β^{CD43}/β^N	0	0	0	0	1
$\beta^{CD27/28}/\beta^N$	1	1	0	0	0
$\beta^{CD14-15}/\beta^N$	0	2	0	0	0

Table 6

Hematological parameters of β^0-thalassemia heterozygote combined with different genotypes of α-thalassemia

β^0	N	RBC(× 10^{12}/L)	Hb(g/L)	MCV(fL)	MCH(pg)
α^0-thalassemia	32	4.7 ± 0.5	106.4 ± 8.7 ^{bc}	71.6 ± 4.3 ^{bc}	22.6 ± 1.5 ^{bc}
α^+-thalassemia	43	4.8 ± 0.5	101.0 ± 8.6 ^{ac}	66.8 ± 3.5 ^{ac}	21.1 ± 0.9 ^{ac}
control	421	4.8 ± 0.6	97.2 ± 9.5 ^{ab}	64.9 ± 3.8 ^{ab}	20.3 ± 1.2 ^{ab}

^{a}P,^{b}P and ^{c}P meaned significant differences respectively from α^0-thalassemia group, α^+-thalassemia group and control group.

Discussion

We reported 13 genotypes and compared the hematological phenotypes of 873 female β-thalassemia carriers in Shenzhen, an immigrant city of Guangdong province, the south of China. In this study, the most common four genotypes were $\beta^{CD41-42}/\beta^N$, $\beta^{IVS-Ⅱ-654}/\beta^N$, β^{CD17}/β^N and β^{-28}/β^N. The highest frequency of β-thalassemia in Guangdong province were $\beta^{CD41-42}/\beta^N$, $\beta^{IVS-Ⅱ-654}/\beta^N$, β^{-28}/β^N and β^{CD17}/β^N [10], in Guangxi province were $\beta^{CD41-42}/\beta^N$, β^{CD17}/β^N, β^{-28}/β^N and $\beta^{IVS-Ⅱ-654}/\beta^N$ [12], in Fujian province were $\beta^{IVS-Ⅱ-654}/\beta^N$, $\beta^{CD41-42}/\beta^N$, β^{CD17}/β^N and β^{-28}/β^N [8], and in Chengdu Region were $\beta^{CD41-42}/\beta^N$, β^{CD17}/β^N, $\beta^{IVS-Ⅱ-654}/\beta^N$and β^{-28}/β^N [13]. However, the most common β-thalassemia in Yunnan was β^E/β^N, followed by β^{CD17}/β^N, $\beta^{CD41-42}/\beta^N$, $\beta^{IVS-Ⅱ-654}/\beta^N$ and β^{-28}/β^N [14], while $\beta^{CD41-42}/\beta^N$ was the only type of β-thalassemia in Li ethic people of Hainan province [15]. This data suggest high genetic heterogeneity and region-specific prevalence of β-thalassemia genotype in China.

In this study, the values of Hb, MCV and MHC decreased and RBC increased in the order of β^{E}, β^{+}- and β^{0}-thalassemia group. A previously study in Thai people also reported that β^{+}-thalassemia was associated with relatively milder phenotype than β^{0}-thalassemia according to hematological characteristics [16], which was consistent with our results. It's worth noting that β^{E}-thalassemia manifested borderline RBC, Hb, MCV and MCH values which were nearly close to normal values. However, the clinical manifestations varied widely from mild form of thalassemia intermedia to severe thalassemia major when β^{E}-thalassemia co-inherited with other type of β-thalassemia [17]. Some severe patients would present with hepatosplenomegaly, severe anemia, skeletal disease and need receive intermittent or regular blood transfusions [18]. For this reason, HbA_2 parameter should be considered in case of β^{E}/β^{N} being ignored in the β-thalassemia screening in high risk couples. In most of cases, β^{+}- and β^{0}-thalassemia manifested typical anemia characteristic. However, some kind of mutations such as β^{-28}/β^{N}, β^{-29}/β^{N} and β^{CAP}/β^{N} manifested mild phenotypes could also compromise carrier screening. It is well known that more than 200 kinds of β-thalassemia mutations have been recognized and the predominant modifier of thalassemia severity is depending on the severity of β-thalassaemia mutation [5]. But we didn't do statistical analysis for each kind of mutations because of limited numbers of certain genotypes. We will collected more samples in each type in order to obtain comprehensive data in future studies.

Among the 873 β-thalassemia carriers, 118 β-thalassemia carriers were co-inheritance of α-thalassemia, indicating the high frequency in Shenzhen. The overall prevalence of β-thalassemia co-inherited with α–thalassemia in mainland China is about 0.48% [19], but the general prevalence in Guangdong Province is 8.1% [9]. A total of 26 different genotypes were detected, and the most common two genotypes were $\beta^{IVS-\square-654}/\beta^{N}$ with $--^{SEA}/\alpha\alpha$ and $\beta^{CD41-42}/\beta^{N}$ with $--^{SEA}/\alpha\alpha$. This pattern is in accordance with the finding that $--^{SEA}/\alpha\alpha$ was the most common genotype of α–thalassemia in Guangdong Province [10].

The major form of hemoglobin tetramer in normal red blood cells in adult is HbA, which consists of two α and β chains, mutation or defect in the α-or β-globin chain results in imbalanced globin chain synthesis and ineffective erythropoiesis. Absolute excess production of α-chain could increase the imbalance state and exacerbate the clinical course of β-thalassaemia [20, 21]. Conversely, decrease of α chain or increase of other substitution of β-chain such as γ-chain could reduce chain imbalance of hemoglobin in β-thalassemia [22]. This could be supported by our results that significant higher Hb, MCV and MCH values in β^{+}–thalassemia or β^{0}–thalassemia co-inherited with α–thalassemia were detected compared to the only β^{+}–thalassemia or β^{0}–thalassemia heterozygosity with normal alpha globin gene. It meaned obviously that β-thalassemia co-inherited of α–thalassemia demonstrated slighter phenotype as compared to β-thalassemia heterozygote could be misdiagnosed in clinical screening. The phenotype severity of β-thalassaemia depending on the variable extent of α or β-globin chain imbalance. It is known that α^{+}-thalassemia is characterized with one a-globin gene deleted or reduced and α^{0}-thalassemia with two, which could have different influences on clinical characteristics of β-thalassaemia. In this study, we observed that β^{0}-thalassemia demonstrated milder phenotype with α^{0}-thalassemia compared with α^{+}-thalassemia, but was not detected in β^{+}–thalassemia group. A Study in Thailand reported that α-

thalassemia ameliorated the clinical severity of β–thalassemia, but β^{+}–thalassemia coexistent of α^{0}-thalassemia showed no significant improvement of MCV and MCH campared with pure β-thalassemia heterozygote [23], which was different from our results. This could be explained by the fact that only the β^{-28}/β^{N} genotype were contained in β^{+}–thalassemia group and merely five samples were concluded in α^{0}-thalassemia group, and the epidemic genotypes in Thailand are also different from China.

Conclusions

In summary, most genotypes of β-thalassemia present typical hypochromic microcytosis and co-inheritance of α–thalassemia could alleviate phenotype of anemic in β^{+}- or β^{0}-thalassemia at various degrees. Although β–thalassemia carries co-inherited with α–thalassemia ameliorated clinical severity, they are still at risk of having babies with severe thalassemia such as Hemoglobin-H disease, hemoglobin Bart's Hydrops fetalis or β–thalassemia major when married with thalassemia heterozygote. Accurate diagnosis is essential to prevent β-thalassaemia carriers with mild phenotype or borderline hematological parameters be ignored. Therefore, our results are meaningful for carrier screening, genetic counseling and clinical outcome predicting.

Abbreviations

Hb
hemoglobin; MCV:mean corpuscular volume; MCH:mean corpuscular hemoglobin; RBC
red blood cell; HbA2
hemoglobin A2; PCR:polymerase chain reaction; Hb CS:Hb Constant Spring; Hb QS:Hb Quong Sze; Hb WS:Hb Westmead; ANOVA:One-Way analysis of variance.

Declarations

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Availability of data and materials

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

Authors' contributions

Likuan Xiong conceived and designed the study. Xu Chen, Zehao Yan, Guangxing Mai and Kai Shen performed experimental operation. Mingyue Luo collected the data, performed the statistical analyses

and wrote the manuscript. All authors have read, revised, and approved the final manuscript.

Ethics approval and consent to participate

Informed consent was signed by each participant. This study was approved by the Ethics Committee of Shenzhen Baoan Women's and Children's Hospital, Jinan University.

Consent for publication

Not applicable.

Competing interests

All authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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