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ORIGINAL ARTICLE

Molecular characteristics of thalassemia and hemoglobin variants in prenatal diagnosis program in northern Thailand

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Abstract

Molecular analysis of globin genes is an essential process for prenatal diagnosis (PND) of severe thalassemia. This study aimed to describe the molecular characteristics of thalassemia and hemoglobin (Hb) variants in PND program in northern Thailand. The type and frequency of globin gene mutations from 1290 couples at risk of fetal severe thalassemia diseases that were tested at Thalassemia Laboratory at Chiang Mai University from 2012 to 2017 were retrospectively reviewed. The PND program detected 444 (34.4%), 196 (15.2%) and 642 (49.8%) couples at risk of fetal Hb Bart's hydrops fetalis, beta-thalassemia major (BTM) and beta-thalassemia/Hb E disease, respectively. Coinheritance of more than one type of thalassemia was common and eight (0.6%) couples were at risk of two types of severe thalassemia. There were two types of alpha^0-thalassemia; 893 (99.7%) Southeast Asian and 3 (0.3%) Thai deletions. Twenty beta-globin gene mutations were found with 94.3% of beta^0-thalassemia. The codon 41/42 (−TTCT), codon 17 (*A*>*T*), IVS-I-1 (*G*>*T*) and codon 71/72 (+A) comprised 90% of beta-thalassemia mutations. The study shows a high percentage of couples at risk of fetal Hb Bart's hydrops fetalis and BTM. The percentage of beta^0-thalassemia is higher than those seen in other regions of Thailand.

Keywords Genotype · Globin gene · Hemoglobinopathies · Prenatal diagnosis · Thalassemia

Introduction

Thalassemia is a hereditary chronic hemolytic anemia that results from mutations of globin genes causing an imbalanced globin synthesis and ineffective erythropoiesis [1]. Thalassemia is common worldwide, and the prevalence is especially high among malaria-endemic areas, including Southeast Asia. In Thailand, the prevalence of alpha-thalassemia carriers and beta-thalassemia carriers is 20–30% and 3–9% of the population, respectively. Hemoglobin E (Hb E) is an Hb variant that is common in the region, with the prevalence of 10–53% in Thailand [2]. The three main types of severe thalassemia diseases in Thailand are Hb Bart's hydrops fetalis, beta-thalassemia major (BTM) and beta-thalassemia/Hb E disease (BE). Hb Bart's hydrops fetalis is caused by a homozygosity of alpha^0-thalassemia which results in severe fetal-onset hemolytic anemia and fetal hydrops. The affected fetus usually succumbs in utero. The condition is associated with maternal pre-eclampsia or eclampsia. Patients with BTM and severe BE usually present with chronic hemolytic anemia during infancy and require regular transfusion. Patients with these severe thalassemia diseases have a poor quality of life due to transfusion-dependency and complications related with chronic hypoxia and iron overload. Therefore, prenatal screening and diagnosis of severe thalassemia is generally offered [2, 3]. In Thailand, the national program for prenatal screening and diagnosis for Hb Bart's hydrops fetalis, BTM and BE has been established [4].

Wanapirak et al. reported that the overall prevalence of thalassemia carriers in pregnant women in northern Thailand was 25.4% which included 6.6% alpha^0-thalassemia, 3.7% beta-thalassemia, 11.6% Hb E carriers, 0.8% homozygous

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Hb E, and 2.7% of combination types of thalassemia [5]. When comparing to the other regions of Thailand, the northern region has a higher prevalence of alpha^0-thalassemia and beta-thalassemia carrier while prevalence of Hb E carrier is lower [2, 6–8]. With the high prevalence of northern Thai individuals carrying combination types of thalassemia, there are couples at risk of having fetuses with more than one severe thalassemia disease.

Regarding the molecular characteristics of beta-thalassemia in northern Thais, the information in the prenatal screening setting is lacking. All previous reports of beta-thalassemia mutations in northern Thailand were from patients with beta-thalassemia diseases which showed that the mutations consisted almost exclusively of beta^0-thalassemia [9, 10]. On the contrary, the studies in beta-thalassemia carriers in central and northeastern Thailand showed that beta$^+$-thalassemia comprises 10–20% of the beta-thalassemia mutations [11, 12]. The discrepancy likely resulted from the difference in the study populations with the higher percentage of severe mutations in patient population.

This study aimed to describe the molecular characteristics of thalassemia and Hb variants in prenatal diagnosis (PND) of severe thalassemia program in northern Thailand. The knowledge of molecular epidemiology of common and rare mutations in the population will be useful for planning the diagnostic tests for PND of thalassemia.

Materials and methods

The study was approved by the institutional research ethics board. The laboratory results of couples at risk of fetal severe thalassemia diseases including Hb Bart's hydrops fetalis, BTM and BE that were tested at the Thalassemia Laboratory, Department of Pediatrics, Faculty of Medicine at Chiang Mai University from 2012 to 2017 were retrospectively reviewed. The laboratory received parental blood samples and fetal chorionic villi or amniotic fluid or blood samples from the Chiang Mai University hospital and other PND centers in the northern region.

Following our model for prenatal control of severe thalassemia, couples coming for their first antenatal care visit were given genetic counseling about severe thalassemia disease. After an informed consent, blood will be taken from the pregnant woman for complete blood count and microcolumn Hb E screen. If the pregnant women had positive screen with the mean corpuscular volume (MCV) $\leq$ 80 fL or positive Hb E screen, the spouse would be screened. If they were a potential couple at risk of having a fetus with one of the severe thalassemia diseases, Hb analysis and polymerase chain reaction (PCR)-based analysis for Southeast Asian and Thai deletional alpha^0-thalassemia would be performed [4]. The Hb analysis was done by high-pressure liquid column chromatography (HPLC) using the Variant II HPLC system (Bio-Rad Laboratories, CA, USA) according to the manufacturer's recommendation. The identification of the Southeast Asian and Thai deletional alpha^0-thalassemia was performed by multiplex PCR with high-resolution melting (HRM) analysis (Supplementary data and supplementary Table 1). The diagnosis of beta-thalassemia carrier or Hb E carrier was made if the Hb analysis showed Hb AA_2 pattern with Hb A_2/E level between 3.6 and 10.0% and 10.1–35%, respectively. If both were beta-thalassemia carriers by the Hb A_2-level criteria, PCR with HRM analysis was performed to identify the type of mutations on beta-globin gene [13]. Primers for the detection of the 619 bp deletion were added to the PCR with HRM protocol (Supplementary Table 2). Unknown beta-thalassemia mutations were further searched for by the direct DNA sequencing method [10]. PND was done by chorionic villi sampling, amniocentesis, or cordocentesis, according to the gestational age. The couples at risk of fetal Hb Bart's hydrops fetalis were also given a choice of serial ultrasound for signs of fetal anemia, and if positive for ultrasound markers, the confirmation would be done by invasive fetal diagnosis. For parents with a diagnosis of Hb H disease by Hb analysis, alpha$^+$-thalassemia mutations were searched for in selected cases by a method previously described [9]. Detection of fetal Hb H disease was not included in the PND program and alpha$^+$-thalassemia mutations were not routinely searched for.

The number of couples at risk of fetal Hb Bart's hydrops fetalis, BTM and BE was collected. The types of alpha-globin and beta-globin mutations were analyzed and the frequencies of each mutation were recorded.

Results

There were 1290 couples at risk tested for PND of fetal severe thalassemia diseases, including 444 (34.4%), 196 (15.2%) and 642 (49.8%) couples at risk of fetal Hb Bart's hydrops fetalis, BTM and BE, respectively. Eight (0.6%) couples were at risk of having a fetus with more than one severe thalassemia; four couples were at risk of both Hb Bart's hydrops fetalis and beta-thalassemia disease, and four couples were at risk of both BTM and BE. There were 11 pairs of twins so the total number of fetuses was 1301. The PND program detected 358 (27.5%) fetuses with severe thalassemia; 118 fetuses with Hb Bart's hydrops fetalis, 56 BTM and 184 BE. The frequencies of each risk and the fetal outcome are summarized in Table 1 and Fig. 1.

Among 896 individuals who harbored alpha^0-thalassemia allele, 867 (96.8%) were alpha^0-thalassemia carrier and 29 (3.2%) had Hb H disease. Seventy-seven (8.6%) individuals who carried alpha^0-thalassemia also carried beta-thalassemia

Table 1 The frequencies of couples at risk of fetal severe thalassemia diseases

Risk of thalassemia	Number of couples at risk (%)	Number of fetuses (%)	Number of affected fetuses (%)
Alpha-thalassemia			
Hb Bart's hydrops fetalis	444 (34.4)	446 (34.3)	118 (33.0)
Beta-thalassemia			
BTM	196 (15.2)	198 (15.2)	56 (15.6)
BE	642 (49.8)	649 (49.9)	182 (50.8)
BTM and BE	4 (0.3)	4 (0.3)	1 BE (0.3)
Both alpha and beta-thalassemia			
Hb Bart's hydrops fetalis and BTM	1 (0.1)	1 (0.1)	0
Hb Bart's hydrops fetalis and BE	3 (0.2)	3 (0.2)	1 BE (0.3)
Total	1290 (100.0)	1301 (100.0)	358 (100.0)

**BE* beta-thalassemia/Hb E disease, *BTM* beta-thalassemia major

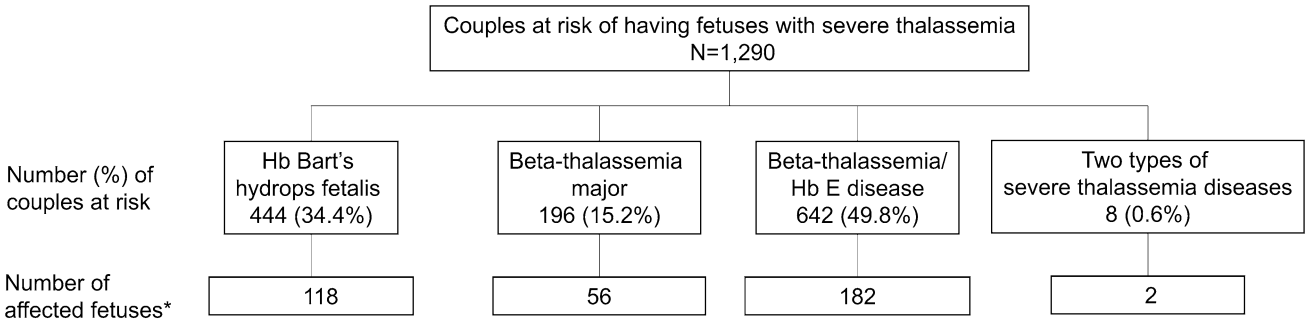

Fig. 1 The numbers of couples at risk of having fetuses with severe thalassemia and the numbers of affected fetuses according to the type of thalassemia disease. There were 11 pairs of twins so the total number of fetuses was 1301

Table 2 The frequencies of couples at risk for fetal Hb Bart's hydrops fetalis caused by two alpha-globin genotypes

Alpha-globin genotypes	No. of couples at risk (%)
Homozygous Southeast Asian deletion ($-^{SEA}/-^{SEA}$)	445 (99.3)
Compound heterozygous Southeast Asian/Thai deletions ($-^{SEA}/-^{THAI}$)	3 (0.7)
Total	448 (100.0)

or Hb E mutations. Regarding the type of alpha^0-thalassemia, 893 (99.7%) and 3 (0.3%) were the Southeast Asian and Thai deletions, respectively. The frequencies of couples at risk of fetal Hb Bart's hydrops fetalis caused by two alpha-globin genotypes are shown in Table 2.

Among 1692 individuals who were at risk of fetal BTM or BE, there were 1023 beta-thalassemia carriers, 587 Hb E carriers, 3 BTM, 5 beta^0/beta$^+$-thalassemia or beta^0-thalassemia/Hb variants, 29 BE, 44 homozygous Hb E and 1 AE Bart's CS disease. Eighteen beta-globin gene mutations were detected from 1023 beta-thalassemia carriers; 965 (94.3%) beta^0 or severe beta$^+$ mutations (13 mutations) and 58 (5.7%) mutations causing beta$^+$ or Hb variants (5 mutations). The four most common beta-thalassemia mutations were codon 41/42 (−TTCT) (43.0%), codon 17 (*A*>*T*) (32.5%), IVS-I-1 (*G*>*T*) (7.7%) and codon 71/72 (+A) (4.8%). The types of beta-globin gene mutations in 1023 beta-thalassemia carriers are summarized in Table 3. Eighty-one individuals had biallelic mutations of beta-globin gene; 37 BTM or BE and 44 homozygous Hb E. Two additional beta-globin gene mutations, 619 bp deletion and codon 136 (*G*>*A*) or Hb Hope, were detected from the individuals with biallelic mutations. The characteristics of the mutations are as shown in Table 4.

Discussion

The distribution of the thalassemia type in the identified couples at risk of fetal severe thalassemia diseases was approximately 2:1:3 for Hb Bart's hydrops

Table 3 The types of beta-globin gene mutations in 1023 beta-thalassemia carriers (not including Hb E carriers)

Types of beta-thalassemia	Beta-globin mutations	Number of beta-thalassemia carriers (%)
$Beta^0$ or severe $beta^+$-thalassemia	Codon 41/42 (−TTCT)	440 (43.0)
	Codon 17 (*A*>*T*)	332 (32.5)
	IVS I-1 (*G*>*T*)	79 (7.7)
	Codon 71/72 (+A)	49 (4.8)
	3.4 kb deletion	27 (2.6)
	Codon 35 (*C*>*A*)	13 (1.3)
	Codon 27/28 (+C)	9 (0.9)
	IVS II-654 (*C*>*T*)	5 (0.5)
	Codon 30 (*G*>*C*)	3 (0.3)
	IVS I-5 (*G*>*C*)	3 (0.3)
	Codon 41 (−C)	2 (0.2)
	Codon 132 (*A*>*T*)	2 (0.2)
	Codon 28 (−C)	1 (0.1)
$Beta^+$-thalassemia	nt −28 (*A*>*G*)	41 (4.0)
	nt −31 (A>G)	6 (0.6)
	nt −87 (*C*>*A*)	5 (0.5)
	Codon 19 (*A*>*G*) (Hb Malay)	3 (0.3)
	Codon 126 (*T*>*G*) (Hb Dhonburi)	3 (0.3)
Total		1023 (100.0)

Table 4 Beta-globin genotypes in 81 individuals with biallelic mutations

Types of beta-thalassemia	Beta-globin genotypes	Number
Biallelic beta-thalassemia mutations	IVS I-1 (*G*>*T*)/619 bp deletion	1
	Codon 41/42 (−TTCT)/IVS I-5 (*G*>*C*)	1
	Homozygous nt −28 (*A*>*G*)	1
	nt −31 (A>G)/IVS I-1 (*G*>*T*)	1
Beta-thalassemia/Hb E disease	Codon 41/42 (-TTCT)/codon 26 (*G*>*A*)	12
	Codon 17 (*A*>*T*)/codon 26 (*G*>*A*)	8
	nt −28 (*A*>*G*)/codon 26 (*G*>*A*)	2
	nt −31 (*A*>*G*)/codon 26 (*G*>*A*)	1
	Codon 27/28 (+C)/codon 26 (*G*>*A*)	1
	IVS I-1 (*G*>*T*)/codon 26 (*G*>*A*)	1
	Codon 71/72 (+A)/codon 26 (*G*>*A*)	1
Beta-thalassemia/other Hb variants	Codon 41/42 (−TTCT)/codon 136 (*G*>*A*) (Hb Hope)	3
	Codon 136 (*G*>*A*)/codon 26 (*G*>*A*)	3
	Codon 30 (*G*>*C*)/codon 136 (*G*>*A*)	
Homozygous Hb E	Codon 26 (*G*>*A*)/codon 26 (*G*>*A*)	44
Total		81

fetalis:BTM:BE. The percentage of beta-thalassemia diseases was consistent with the previously reported prevalence of thalassemia in pregnant women at our hospital in 2004 [5]. However, the percentage of couples at risk of Hb Bart's hydrops fetalis in this study was quite high which was more consistent with a recent report of the Southeast Asian deletional $alpha^0$-thalassemia carrier prevalence of 12.23% and Hb H disease (Southeast Asian and 3.7-kb deletions) of 2.82% in 638 pregnant women at our hospital [14].

As thalassemia is highly prevalent in the northern Thai population, the co-inheritance of more than one type of thalassemia can be seen. This study showed that 8 of 1290 couples (0.6%) were at risk of two types of severe thalassemia diseases. This finding underscores the importance of simultaneously testing for both alpha and beta-thalassemia, when the screening result is positive. Also, the percentage of affected fetus in this study was 27.5% (358 of 1301 fetuses) which was higher than an expected 25% by Hardy–Weinberg equilibrium. This finding could be explained by a number of parents with biallelic mutations of beta-globin gene, that when coupled with a partner with monoallelic mutation resulted in a 50% predicted chance of fetal severe thalassemia in each pregnancy.

Two large deletions causing alpha^0-thalassemia were identified. The findings that almost all deletions (99.7%) were Southeast Asian deletion and a small number (0.3%) were Thai deletion were comparable with the distribution seen in previous studies in Thailand, China and Taiwan [15–21]. In our practice, both deletions are sought for simultaneously by a multiplex PCR with HRM method. Li et al. suggested that due to the low frequency of the Thai deletion, PCR for Thai deletion can be performed sequentially in a screen-positive couple when one partner is diagnosed as a carrier of Southeast Asian deletion [19].

Twenty beta-globin mutations excluding an Hb E mutation were identified. There were 14 mutations causing beta^0/severe beta$^+$-thalassemia and 6 mutations causing beta$^+$-thalassemia. Among carriers of beta-thalassemia, 94.3% harbored beta^0-thalassemia mutations and 5.7% had beta$^+$-thalassemia mutations. When compared to the results from previous studies as summarized in Table 5, the findings differ from the two previous reports in BTM and BE patients from our hospital, and a report from central Thailand that the beta$^+$-thalassemia mutations in the current study were found in a higher percentage [9, 10, 22]. This is likely because of the difference in the studied population. However, the percentage of beta$^+$-thalassemia mutations were still much lower than those seen in northeastern Thailand, which reported a beta$^+$-thalassemia mutations prevalence of 23.1% among 849 beta-thalassemia carriers identified during prenatal screening, and in central Thailand with a beta$^+$-thalassemia mutations prevalence of 24.0% among 138 healthy beta-thalassemia carriers [11, 12]. This may again result from a selection bias because our study included only the couples at risk of fetal severe thalassemia diseases who underwent PND procedures. The couples at risk of fetal mild thalassemia diseases, such as a compound heterozygous beta$^+$-thalassemia from a promotor mutation and Hb E, usually receive routine antenatal care and do not undergo PND procedure, so such couples were not included in the current analysis.

The molecular characteristics of beta^0 and severe beta$^+$-thalassemia in northern Thailand were comparable to those in other parts of Thailand, except for the higher percentage of codon 41/42 (−TTCT) and codon 17 (*A*>*T*) mutations as shown in Table 5 [11, 12, 22–24]. The findings were consistent with the previous report of BTM and BE patients from our hospital [9, 10]. The percentage of IVS I-5 and IVS II-654 mutations were lower than those seen in other regions of Thailand. The combined percentage of the four most common mutations; codon 41/42 (−TTCT), codon 17 (*A*>*T*), IVS I-1 (*G*>*T*) and codon 71/72 (+A) was close to 90%, so we suggest that these four mutations be firstly tested in prenatal screening setting. If no mutations are detected, further tests to cover less common mutations can be performed.

The finding of codon 41/42 (−TTCT) as a predominant beta-globin mutation was similar to the molecular characteristics of beta-thalassemia in countries in Southeast Asia, China and Taiwan. Although the percentage of other mutations differs among populations [21, 25–30], the IVS I-5 is another predominant beta-globin mutation in China and Taiwan [21, 29, 30]. The codon 17 (*A*>*T*) is common in Laos and North Vietnam [25, 27].

Eighty-one parents with biallelic mutations of beta-globin gene were identified. Thirty-seven parents had biallelic beta-thalassemia mutations or beta-thalassemia/Hb E disease and 44 had homozygous Hb E. Our limitation was that the clinical information was unavailable. Most genotypes were expected to associate with mild clinical severity, but a few were homozygous beta^0-thalassemia and 23 were compound heterozygous beta^0-thalassemia/Hb E disease. This finding points out the complex genotypes that could be encountered in the prenatal screening and diagnosis practice.

This study had a limitation that the information of alpha$^+$-thalassemia was not available, as the PND program did not routinely test for alpha$^+$-thalassemia mutations. Alpha$^+$-thalassemia and Hb Constant Spring mutations were searched for in unusual cases of fetal anemia suspected to be from homozygous Hb Constant Spring or Hb H/Constant Spring disease [31–33].

In summary, the study shows a high percentage of couples at risk of fetal Hb Bart's hydrops fetalis and BTM in PND setting in northern Thailand. The percentage of beta^0-thalassemia is higher than those seen in other regions of Thailand. Complex genotypes are common which results in couples at risk of more than one type of fetal severe thalassemia. The information will be useful for planning a diagnostic protocol in prenatal screening and diagnosis of thalassemia in the population.

Table 5 Summary of beta-globin gene mutations identified in different regions of Thailand

Study	Thein et al. [23]	Viprakasit et al. [11]	Boonyawat et al. [22]	Laosombat et al. [24]	Yamsri et al. [12]	Sirichotiyakul et al. [10]	Charoenkwan et al. [9]	This study
Region	Central	Central	Central	Southern	Northeastern	Northern	Northern	Northern
Number of subjects	97	138	80	78	849	109	80	1023
Study population	79 BE, 19 BTM	Healthy beta-thal carriers	57 BE, 8 BTM, 15 beta-thal carriers	45 BE, 25 BTM, 8 beta-thal carriers	Beta-thal carriers	BTM	BE	Beta-thal carriers at risk for fetal severe thal
$Beta^0$ or severe β^+-thalassemia								
Codon 41/42 (−TTCT)	59 (50.9)	50 (36.3)	33 (37.5)	33 (32.0)	307 (36.2)	108 (49.5)	43 (53.8)	440 (43.0)
Codon 17 (*A>T*)	12(10.3)	24 (17.4)	23 (26.1)	11 (10.7)	215 (25.3)	75 (34.4)	27 (33.8)	332 (32.5)
IVS I-1 (*G>T*)	2 (1.7)	3 (2.2)	4 (4.5)	8 (7.8)	26 (3.1)	15 (6.9)	1 (1.2)	79 (7.7)
Codon 71/72 (+A)	1 (0.8)	6 (4.3)	2 (2.3)	1 (0.9)	36 (4.2)	13 (6.0)	3 (3.8)	49 (4.8)
3.4 kb deletion	–	1 (0.7)	4 (4.5)	–	19 (2.2)	–	1 (1.2)	27 (2.6)
Codon 35 (*C>A*)	3 (2.6)	2 (1.4)	4 (4.5)	–	4 (0.5)	–	–	13 (1.3)
Codon 27/28 (+C)	–	–	1 (1.1)	–	1 (0.1)	–	1 (1.2)	9 (0.9)
IVS II-654 (*C>T*)	13 (11.2)	8 (5.8)	6 (6.8)	1 (0.9)	19 (2.2)	1 (0.4)	1 (1.2)	5 (0.5)
Codon 30 (*G>C*)	–	–	–	–	–	–	–	3 (0.3)
IVS I-5 (*G>C*)	6 (5.2)	7 (5.1)	7 (8.0)	23 (22.3)	14 (1.7)	–	2 (2.5)	3 (0.3)
Codon 41 (−C)	–	–	–	1 (0.9)	–	–	–	2 (0.2)
Codon 132 (*A>T*)	–	–	–	–	–	–	–	2 (0.2)
Codon 28 (−C)	–	–	–	–	–	–	–	1 (0.1)
Initiation codon (*T>G*)	–	2 (1.4)	1 (1.1)	–	–	–	–	–
Codon 14/15 (+G)	1 (0.8)	–	–	–	–	–	–	–
Codon 15 (*G>A*)	–	–	1 (1.1)	–	–	–	–	–
Codon 16/17 (+G)	–	1 (0.7)	–	–	–	–	–	–
Codon 26 (*G>T*)	–	–	–	–	4 (0.5)	–	–	–
Codon 43 (*G>T*)	–	–	–	–	6 (0.7)	–	–	–
Codon 95 (+A)	–	–	–	–	1 (0.1)	–	–	–
Codon 123/125 (−8 bp)	–	1 (0.7)	1 (1.1)	–	–	–	–	–
619 bp deletion	–	–	–	–	–	–	1 (1.2)	–
$Beta^+$-thalassemia								
nt −28 (*A>G*)	12 (10.3)	20 (14.5)	–	8 (7.8)	191 (22.5)	3 (1.4)	–	41 (4.0)
nt −30 (*T>C*)	–	2 (1.4)	–	–	–	–	–	–
nt −31 (*A>G*)	–	4 (3.0)	–	–	1 (0.1)	1 (0.4)	–	6 (0.6)
nt −87 (*C>A*)	–	1 (0.7)	–	–	2 (0.2)	2 (1.0)	–	5 (0.5)
nt −86 (*C>G*)	1 (0.8)	–	–	–	–	–	–	–
nt −87 (*C>A*)	–	–	–	–	2 (0.2)	–	–	–
Codon 19 (*A>G*) (Hb Malay)	2 (1.7)	3 (2.2)	1 (1.1)	12 (11.7)	1 (0.1)	–	–	3 (0.3)

Table 5 (continued)

Study	Thein et al. [23]	Viprakasit et al. [11]	Boonyawat et al. [22]	Laosombat et al. [24]	Yamsri et al. [12]	Sirichotiyakul et al. [10]	Charoenkwan et al. [9]	This study
Codon 126 (*T*>*G*) (Hb Dhonburi)	–	3 (2.2)	–	–	–	–	–	3 (0.3)
Uncharacterized	4 (3.4)	–	–	5 (4.8)	–	–	–	–
Total number of beta-thalassemia alleles	116 (100.0)	138 (100.0)	88 (100.0)	103 (100.0)	849 (100.0)	218 (100.0)	80 (100.0)	1023 (100.0)

**BE* beta-thalassemia/Hb E disease, *beta-thal* beta-thalassemia, *BTM* beta-thalassemia major

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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