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Clinical characteristics, laboratory features and genetic profile of hemoglobin E (HBB:c.79 G > A)/β (nucleotide -28 A > G) (HBB:c.-78 A > G) -thalassemia subjects identified from community- and hospital-recruited cohorts

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ABSTRACT

Despite several existing laboratory-based studies of hemoglobin (Hb) E (HBB:c.79 G > A)/ β (nucleotide (NT) -28 A > G) (HBB:c.-78 A > G) -thalassemia, no reports have ever provided clinical severity information as well as dependency of blood transfusion. Previously, a comparative study of community- and hospital-recruited Hb E/β-thalassemia subjects was conducted in the lower northern Thailand between June 2020 and December 2021. A mobile medical team visited each community hospital on-site, collecting clinical severity parameters, and conducting Hb and DNA analyses. The control included Hb E/β-thalassemia patients undergoing transfusions. Subgroup study of adult Hb E/β (NT -28 A > G) -thalassemia subjects was subsequently conducted. Additional pediatric individuals were recruited from prenatal diagnosis databases. Twenty adult and nine pediatric subjects were enrolled; all were classified as having mild disease severity. Twenty-two individuals (75.9 %) were asymptomatic. Six adults (20.7 %) required blood transfusion. The mean Hb level of subjects without transfusion (23 [79.3 %]) was 10.77 ± 1.10 g/dL. Hb analysis revealed a distinct EFA pattern with low Hb F fraction. The positive impact of genetic modifiers could not be statistically demonstrated except rs7482144-*Xmn*I. These findings could provide essential information for parents carrying fetuses with Hb E/β (NT -28 A > G) -thalassemia.

1. Introduction

Couples at risk of carrying fetuses with hemoglobin (Hb) E (HBB: c.79 G > A)/β-thalassemia are common in Thailand and other Southeast Asian countries [1,2]. The clinical phenotype of Hb E/β-thalassemia is significantly influenced by molecular genetics, particularly the mutation characteristic of β-globin gene *in trans* to Hb E mutation [3–5]. More than 200 β-thalassemia mutations have been reported worldwide which comprise of β^{++}-, β^{+}- and β^{0}-thalassemia alleles, distinguished by varying levels of β-globin messenger RNA synthesis [5]. Studies conducted in Thailand have identified six common β-globin gene mutations: codon 41/42 (-TTCT); codon 17 (A > T); nucleotide (NT) -28 (A > G); IVS II-654 (C > T); IVS I-1 (G > C); and codon 19 (A > G), with respect of their frequencies, accounting for 85 % of all β-thalassemia mutations in the country [6–8]. Therefore, according to the prevalence of these mutations, association of β-thalassemia with NT -28 (A > G) (HBB:c.-78 A > G) mutation and Hb E is the most common form of Hb E/β^{+}-thalassemia in Thailand [6–8].

Previous studies of Hb E/β^{+}-thalassemia, including Hb E/β (NT -28 A > G) -thalassemia, have been conducted in different regions of Thailand. A large cohort study at the northeastern thalassemia center compared the hematologic and molecular genetic features between 177 Hb E/β^{+}-thalassemia and 94 Hb E/β^{0}-thalassemia patients. In their findings, over 80 % of Hb E/β^{+}-thalassemia subjects carried the NT -28 (A > G) mutation; they exhibited mild to moderate anemia, low mean corpuscular volume (MCV), and slightly elevated Hb F levels [8].

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Despite several existing laboratory-based studies of Hb E/β (NT -28 A > G) -thalassemia, all of the reports still lack clinical information regarding disease severity and dependency of blood transfusion to confirm their clinical prognostication using for prenatal genetic counseling. Due to the reported mild to moderate anemia of Hb E/β (NT -28 A > G) -thalassemia, studies conducted with community-recruited subjects are the only way to demonstrate authentic phenotypes.

The objective of this study was to describe the clinical characteristics, laboratory findings, and genetic profile of individuals with Hb E/β (NT -28 A > G) -thalassemia enrolled from a community setting. Hopefully, our findings could provide essential information for parents carrying fetuses with Hb E/β (NT -28 A > G) -thalassemia.

2. Material and methods

This study was conducted as part of a previous prospective study comparing genetic modifiers between community- and hospital-recruited individuals with Hb E/β-thalassemia in lower northern Thailand between June 2020 and December 2021 [9]. One hundred and two adult participants were husbands or wives diagnosed with Hb E/β thalassemia identified from a hospital database of couples at risk for having a baby with thalassemia who attended antenatal care at community hospital. A mobile medical team visited each community hospital on-site to collect clinical severity information and blood samples. One hundred and four adult patients with Hb E/β-thalassemia currently being treated in university/provincial hospitals during the same recruitment period comprised the control group. Subgroup study of Hb E/β (NT -28 A > G) -thalassemia subjects identified from community- and hospital-recruited cohorts was subsequently conducted. Additional pediatric individuals with Hb E/β (NT -28 A > G) -thalassemia, identified from a prenatal diagnosis program conducted at the Thalassemia Research Unit of Naresuan University Hospital located in the same region, were recruited.

The Mahidol severity score was employed to categorize clinical severity using clinical parameters including age at presentation and first transfusion, transfusion requirement, spleen size, growth development percentile, and pre-transfusion Hb levels. The scoring system classified disease severity as mild (score 0–3.5), moderate (4–7), or severe (7.5–10) [10]. Requirement for blood transfusion was defined by the frequency of transfusion to maintain patient quality of life: regular (every three weeks to three months); occasional (every four months to once a year); and rare (none or once in several years) [10]. Hematological parameters were assessed using an automated blood cell counter (XN-10; Sysmex Corporation, Kobe, Japan). Diagnosis was confirmed for all subjects using automated capillary zone electrophoresis (Minicap Flex; Sebia, Lisses, France) for Hb analysis. An Hb F fraction of less than 5 % was defined as no Hb F level elevation. The β-globin mutation was identified by real-time PCR and high-resolution melting analysis, and coinherited deletional α-globin mutation ($--^{SEA}$, $--^{THAI}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$) and Hb Constant Spring mutation were detected by gap PCR and amplification refractory mutation systems (ARMS) PCR, respectively [11]. Tetra-primer ARMS-PCR was developed to detect three single nucleotide polymorphisms (SNP): rs766432 (*BCL11A*); rs9399137 (*HBS1L-MYB*); and rs7482144-*Xmn*I (*Gγ-globin*) [9]. For the present study, anti-3.7 α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$) was added to the set of genetic modifiers using multiplex PCR assay described elsewhere [12]. The study was approved by the Institutional Ethical Committee of Naresuan University (approval number 393/2019). Written informed consent was obtained from all subjects before entering the study.

Demographic data, clinical severity information, and hematological parameters were presented for adult and pediatric populations. Clinical and hematological parameters were compared between groups characterized by EA and EFA patterns from Hb analysis, and between groups with different forms of α-thalassemia coinheritance, using Fisher's exact test for categorical data. The Man-Whitney *U* test, F-test in ANOVA, and Kruskal-Wallis test were chosen for continuous variables. Multiple linear regression models were used to determine the relationship between potential genetic modifiers and clinical severity scores as well as Hb levels among all subjects. The statistical significance level was established at $p < 0.05$. All data were analyzed using STATA version 14.0 (StataCorp, College Station, TX, USA).

3. Results

Twenty-nine subjects diagnosed with Hb E/β (NT -28 A > G) -thalassemia were enrolled in the study consisting of 20 adults and 9 children. While nineteen adult subjects were recruited from the community group, one individual with occasional blood transfusion was identified from the control (genotypic data are displayed in the previous report [9]). Demographic data and clinical severity parameters of the adult and

Table 1
Demographic data, clinical severity parameters, and genetic profile of pediatric and adult Hb E/β (NT -28 A > G) -thalassemia cases.

	Pediatric (*N* = 9)	Adult (*N* = 20)
Age (year) (median/range)	6 (0–13)[a]	30 (18–64)
Gender: male (number/%)	4 (44.4)	8 (40.0)
Age at presentation[b] (year) (median/range)	–	19 (6–62)
Age at first transfusion received[c] (year) (median/range)	–	27 (6–62)
Asymptomatic[d] (number/%)	9 (100.0)	13 (65.0)
Never received any blood transfusion for thalassemia (number/%)	9 (100.0)	14 (70.0)
Requirement for blood transfusion (number/%)		
– Regular	0 (0.0)	0 (0.0)
– Occasional	0 (0.0)	2 (10.0)
– Rare	0 (0.0)	4 (20.0)
Impalpable spleen (number/%)	9 (100.0)	13 (65.0)
Splenectomy (number/%)	0 (0.0)	0 (0.0)
Growth and development >25th percentile (number/%)	6 (85.7)[e]	18 (90.0)
Hb level before receiving transfusion (g/dL)	–	6.0, 7.5[f]
Hb level of subjects who never received any transfusion (g/dL) (mean ± SD)	10.52 ± 0.90	10.97 ± 1.24
Clinical severity (number/%)		
Mild	9 (100.0)	20 (100.0)
Moderate	0 (0.0)	0 (0.0)
Severe	0 (0.0)	0 (0.0)
Severity score (median/range)	0 (0–0.5)	0 (0–3)
Hb typing (number/%)		
EA	2 (22.2)	2 (10.0)
EFA	7 (77.8)	18 (90.0)
Coinheritance of α-thalassemia (number/%)		
α^0-thalassemia mutation		
• $--^{SEA}$	3 (33.3)	0 (0.0)
• $--^{THAI}$	0 (0.0)	0 (0.0)
α^+-thalassemia mutation		
• $-\alpha^{3.7}$	2 (22.2)	3 (15.0)
• $-\alpha^{4.2}$	0 (0.0)	0 (0.0)
Hb Constant Spring	0 (0.0)	3 (15.0)
α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$)	0 (0.0)	0 (0.0)
Single nucleotide polymorphism (allele frequency)		
BCL11A (rs766432) (C allele)	0.44	0.12
HBS1L-MYB (rs9399137) (C allele)	0.44	0.50
*Xmn*I (rs7482144) (A allele)	0.44	0.35

[a] The youngest is eight months old.
[b] Excludes asymptomatic subjects.
[c] Excludes cases who had never undergone any blood transfusion for thalassemia.
[d] Two asymptomatic subjects had blood transfusions for reason other than thalassemia.
[e] Two subjects cannot be evaluated by growth curve.
[f] Raw hemoglobin data of the two subjects who received occasional transfusion.

pediatric groups are presented in Table 1. All subjects were classified as having mild severity. Six adults (20.7 %) required blood transfusion: two patients with occasional and four cases with rare transfusion frequency (Table 2). Two subjects were given transfusion during their pregnancies, and never required it again. One individual was given blood at the age of six with unknown reason, and no further transfusion was needed. Besides blood transfusion and folate supplementation, no other treatment modalities were given for these six patients. There were two asymptomatic individuals experienced blood transfusion due to traffic accidents which required orthopedic surgery (individuals not included in transfusion-needed subjects). Hb analysis revealed a higher prevalence of the EA compared to the EFA pattern among pediatric subjects (Figs. 1 and 2). Coinherited deletional α^0-thalassemia ($--^{SEA}$), α^+-thalassemia ($-\alpha^{3.7}$) and Hb Constant Spring mutations, as genetic modifiers, were identified in 11 subjects (37.9 %). There were no anti-3.7 α-globin gene triplication detected. For allele frequency of the three SNPs, The C allele of *BCL11A* (rs766432) (prevalence of 0.22), C allele of *HBS1L-MYB* (rs9399137) (prevalence of 0.48), and A allele of $^{G}\gamma$*-globin* (rs7482144) (prevalence of 0.37) were considered minor. No homozygote for the minor frequency allele was detected in 29 subjects. Genetic profile of the adult and pediatric groups is presented in Table 1. Hematologic parameters and profile of Hb fractions are demonstrated in Table 3.

Clinical severity, laboratory features, and genetic profile between groups with EA and EFA pattern from Hb analysis are presented in Table 4. Even though the EA group exhibited a higher Hb A fraction compared to the EFA group, the higher value did not impact the hematological profile as well as clinical severity of the subjects. Hb analysis from the EA group resembled Hb E heterozygote but with higher Hb E levels. A significantly higher prevalence of the α^0-thalassemia allele ($--^{SEA}$) was observed in the EA group ($p = 0.042$). Severity scores and hematological parameters of Hb E/β (NT -28 A > G) -thalassemia with different forms of α-thalassemia are demonstrated in Table 5. Despite higher absolute Hb levels observed in subjects with α-thalassemia coinheritance, the difference was not statistically significant compared to individuals without α-thalassemia ($p = 0.279$). Individuals with coexistence of α-thalassemia exhibited substantially lower MCV ($p < 0.0001$) and Hb F ($p = 0.029$), but higher Hb A fraction ($p = 0.0096$) compared to subjects without α-thalassemia. For the multiple linear regression analyses, rs7482144-*Xmn*I was the only genetic modifier associated with clinical severity scores (Table 6).

4. Discussion

Our study is the first to provide sufficient clinical information on 29 adult and pediatric Hb E/β (NT -28 A > G) -thalassemia subjects to support prenatal genetic counseling for parents at risk of carrying, or who already have, fetuses with this condition. All 29 individuals were classified as having mild clinical severity. Only one subject with occasional transfusion was identified from hospital-recruited cohort. The majority were asymptomatic and had never received any blood transfusion for thalassemia. However, there were few patients requiring occasional transfusion who might have pre-transfusion Hb levels as low as 6.0 g/dL. The clinical severity score of Hb E/β (NT -28 A > G) -thalassemia subjects ranged from zero to three. Additionally, our study showed that concomitance of α-thalassemia did not significantly affect

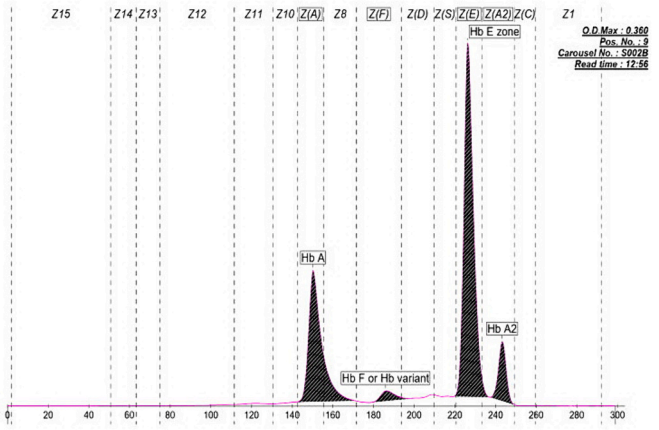

Fig. 1. Representative of samples with EA pattern from Hb analysis.

the clinical severity of Hb E/β (NT -28 A > G) -thalassemia as demonstrated by nearly-equivalent numbers of clinical severity score ($p = 0.27$). Nevertheless, laboratory severity seemed to be alleviated by α-thalassemia coexistence as indicated by higher total Hb values (without statistical significance) and Hb A levels compared between the EA and EFA Hb analysis pattern and between groups with and without α-thalassemia coinheritance. The positive impact of α-thalassemia may be subtle and obscured by the influence of the β^+-globin gene (NT -28 A > G) which is one of the major disease modifying factors of Hb E/β-thalassemia [9]. The subtle influence of other genetic modifiers in Hb E/β (NT -28 A > G) -thalassemia was also evidenced by multiple linear regression analysis (Table 6). Even though rs7482144-*Xmn*I was able to show a statistical significance for clinical severity reduction, the impact was rather small (0.847 score reduction, $p = 0.037$). The influence of the six genetic modifying factors in Hb E/β (NT -28 A > G) -thalassemia on clinical severity is weak as demonstrated by the F test in ANOVA ($F = 1.329$, $p = 0.286$). Of note, the high prevalence of α-thalassemia coexistence in this study may not be unexpected compared to the frequency of α-thalassemia in the region [2]. The coinheritance rate of α-thalassemia indicates the authenticity of community-acquired data which is different from hospital-based studies of Hb E/β-thalassemia that have been reported [13,14].

As observed in other Hb E/β^+-thalassemia cases, a lower Hb F fraction compared to Hb E/β^0-thalassemia subjects was one of its characteristics. Many individuals with Hb E/β (NT -28 A > G) -thalassemia had no elevation of Hb F levels (EA pattern), causing their electrophoregram/chromatogram to resemble those of Hb E heterozygote with higher-than-usual fraction of Hb E. This should be cautious and aware of misdiagnosis as Hb E heterozygote while interpreting Hb analyses. The majority of Hb E/β (NT -28 A > G) -thalassemia subjects had the EFA pattern of Hb analysis with low Hb F fraction. This could be explained by the small amount of normal β-globin chain that can be produced in β^+-thalassemia which reduces the Hb F fraction in Hb E/β^+-thalassemia. In general, the incorporation of Hb tetramer relies on the formation of dimers consisting of α- and β- (or β-liked [i.e. δ- and γ-]) globin chains. Dimer formation depends on the electrical charge of the non-α-monomer

Table 2
Clinical information of subjects who required blood transfusion.

Subject number	1	2	3	4	5	6
Gender	female	female	male	female	female	male
Age at presentation (year)	12	62	6	14	19	41
Age at first transfusion received (year)	28	62	6	26	19	41
Requirement for blood transfusion	rare	rare	rare	rare	occasional	occasional
Hb level (g/dL)	7.9	7.6	12.0	7.5	6.0	7.5
Severity score	0	2	1.5	2	2	3
Reason for transfusion	pregnancy	anemic symptom	unknown	pregnancy	anemic symptom	anemic symptom

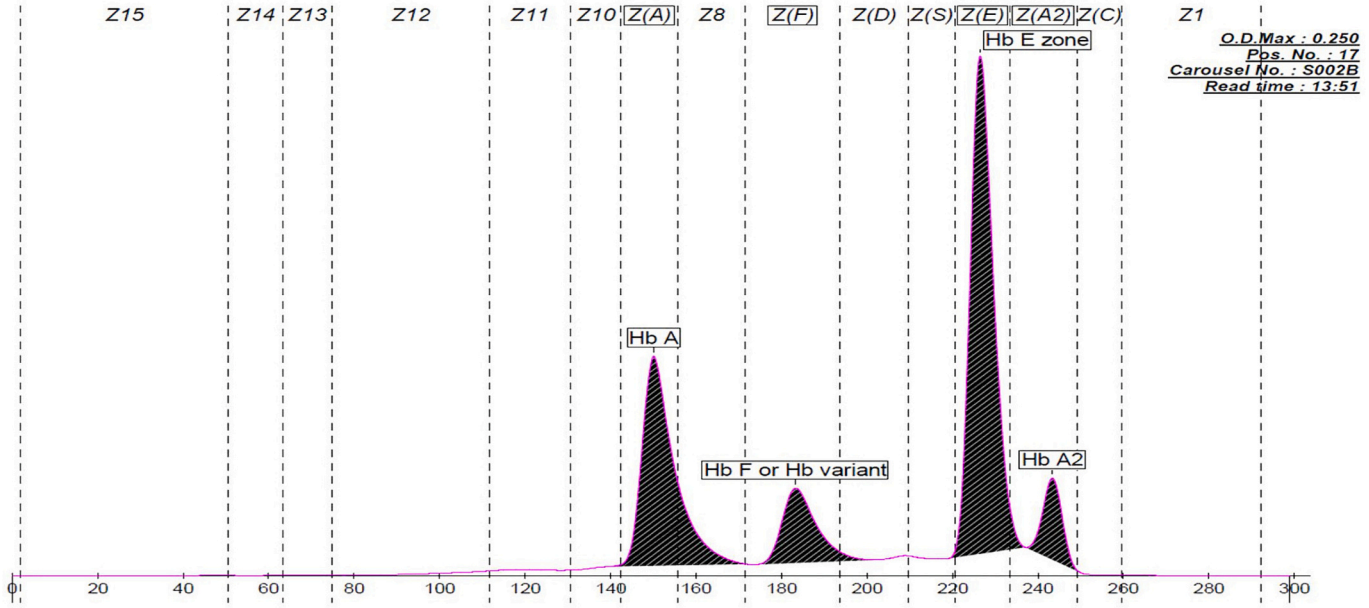

Fig. 2. Representative of samples with EFA pattern from Hb analysis.

Table 3
Hematological parameters and Hb analysis profile of Hb E/β (NT -28 A > G) -thalassemia cases with rare or no transfusion history.

	Pediatric (N = 9)	Adult (*N* = 18)
Hb level (g/dL)[a]	10.52 ± 0.90 (9.40–11.70)	10.48 ± 1.71 (7.50–13.10)
Hematocrit (%)[a]	32.27 ± 3.34 (27.70–37.50)	32.78 ± 5.41 (23.10–40.00)
Mean corpuscular volume (fL)[a]	52.89 ± 4.42 (43.30–58.60)	59.16 ± 3.64 (53.20–69.90)
Hb fraction (%) (median/range)		
Hb A	26.5 (15.1–31.5)	24.6 (17.1–29.9)
Hb A2	6.8 (3.8–9.2)	6.5 (4.0–8.0)
Hb E	52.1 (33.1–59.4)	51.5 (36.4–59.7)
Hb F	15.8 (1.4–48.0)	18.0 (2.6–42.5)

[a] Values are presented as mean ± SD (min-max).

which binds to the positively charged α-subunit by electrostatic attraction. Due to near-equivalent positive and negative charges of α- and β-subunit, αβ dimers form in preference to others. Therefore, with the presence of a small amount of normal β-globin chains to form Hb A in Hb E/β (NT -28 A > G) -thalassemia, other Hb fractions would be decreased especially Hb F. When situations with reduced α-globin chain production from coinheritance of α-thalassemia alleles occur, the effect of charge becomes exaggerated [15]. The preference of the reduced α-globin chains to bind more to the β-, δ-, and mutated β- (β^E) globin chains make it less in binding to γ-globin chains which produces the EA pattern of Hb analysis in some cases [16–18]. According to our findings, three fourth of the subjects with EA pattern had α-thalassemia coinheritance. Median Hb F fraction in Hb E/β (NT -28 A > G) -thalassemia subjects with and without α-thalassemia coinheritance were 15.0 % (1.4–18.4) and 19.3 % (2.6–48.0), respectively ($p = 0.029$).

While all pediatric Hb E/β (NT -28 A > G) -thalassemia subjects were asymptomatic, six adult individuals required transfusion, with age at first transfusion ranging from 6 to 62 years. However, for patient number three (Table 2) who experienced transfusion at six years of age for unknown reason, it may be reasonable to reclassify the subject as asymptomatic individual attributed to his current Hb value (12.0 g/dL). With one subject excluded, all five patients started their first transfusion beyond the age of 19 which corresponded to nine asymptomatic individuals from pediatric group. The late transfusion requirement implies an alleviating effect of β (NT -28 A > G) -allele and a potential influence of advancing age on clinical severity of Hb E/β-thalassemia. The fact that they had survived without any transfusion up to those points underscores the concept of an unstable Hb E/β-thalassemia phenotype [19,20]. It remains to be determined whether their bone marrow could not produce sufficient red blood cells as they got older, their exhausted vital organs could not tolerate longstanding anemia, or other unknown factors contributed to destabilization of phenotype with advancing patient age.

For the limitation of the study, community-recruited Hb E/β-thalassemia cases were a lot more difficult to be identified and enrolled compared with hospital/laboratory-based study. The small sample-size limited our ability to demonstrate statistically significant differences between groups especially between the EA and EFA pattern of Hb analysis. As a weak point of the Mahidol clinical severity score, information on late thalassemia complications due to chronic anemia and iron overload was not collected in the study.

5. Conclusion

The majority of individuals with Hb E/β (NT -28 A > G) -thalassemia were asymptomatic and never required blood transfusion; severity scores did not exceed three. However, a small subset of subjects did require transfusion. Pattern of Hb analysis, characterized by a low Hb F fraction, can resemble that of Hb E heterozygote, necessitating caution in interpretation. Three quarters of individuals exhibiting the EA pattern demonstrated α-thalassemia coinheritance. The positive impact of genetic modifiers could not be statistically demonstrated except rs7482144-*Xmn*I. Potential influence of genetic modifying factors on clinical severity may be obscured by the strong relieving effect of β (NT -28 A > G) -allele in Hb E/β-thalassemia.

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Table 4
Comparison of hematological parameters of Hb E/β (NT -28 A > G) -thalassemia cases between groups with EA and EFA pattern from Hb analysis.

	EA ($N = 4$)	EFA ($N = 25$)	p-value
Requirement for blood transfusion (number/%)			0.627[a]
– Regular	0 (0.0)	0 (0.0)	
– Occasional	1 (25.0)	1 (4.0)	
– Rare	0 (0.0)	4 (16.0)	
Hb level before receiving transfusion (g/dL)	6.0[b]	7.5[b]	–
Clinical severity (number/%)			
Mild	4 (100.0)	25 (100.0)	1.000[a]
Moderate	0 (0.0)	0 (0.0)	1.000[a]
Severe	0 (0.0)	0 (0.0)	1.000[a]
Severity score (median/range)	0 (0–2)	0 (0–3)	0.907[c]
Hematological parameters[d]			
Hb level (g/dL)[e]	11.3 (10.9–11.4)	10.7 (7.5–13.1)	0.279[c]
Hematocrit (%)[e]	36.5 (35.3–37.5)	32.8 (23.1–40.0)	0.105[c]
Mean corpuscular volume (fL)[e]	55.5 (53.9–55.5)	58.3 (43.3–69.9)	0.142[c]
Hb fraction (%)[d]			
Hb A[e]	30.3 (29.7–31.5)	24.6 (15.1–31.5)	0.0121[c]
Hb A2[e]	8.0 (7.7–9.2)	6.5 (3.8–7.5)	0.0054[c]
Hb E[e]	59.4 (56.8–59.7)	50.7 (33.1–57.7)	0.0069[c]
Hb F[e]	2.6 (1.4–3.7)	18.0 (6.9–48)	0.0055[c]
Coinheritance of α-thalassemia (number/%)			
α^0-thalassemia mutation			
• $-^{SEA}$	2 (50.0)	1 (4.0)	0.042[a]
• $-^{THAI}$	0 (0.0)	0 (0.0)	1.000[a]
α^+-thalassemia mutation			
• $-\alpha^{3.7}$	1 (25.0)	4 (16.0)	0.553[a]
• $-\alpha^{4.2}$	0 (0.0)	0 (0.0)	1.000[a]
Hb Constant Spring	0 (0.0)	3 (12.0)	0.629[a]
Total α-thalassemia alleles	3 (75.0)	8 (32.0)	0.139[a]

[a] *P*-value determined by Fisher's exact test.
[b] Value of a single case who received occasional transfusion.
[c] P-value determined by Mann-Whitney *U* test.
[d] Excludes two subjects who received occasional transfusion.
[e] Values are presented as median (range).

CRediT authorship contribution statement

Piyatida Chumnumsiriwath: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Prissana Charoenporn:** Investigation. **Sawichayaporn Jermnim:** Investigation. **Pawanrat Suannum:** Investigation. **Monthira Samaisombat:** Investigation. **Akamon Tapprom:** Investigation. **Rawisut Deoisares:** Investigation. **Peerapon Wong:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Table 6
Multiple linear regression analysis of relationship between genetic modifiers and severity scores and Hb levels.

Clinical severity scores				
Genetic modifier	Coefficient	Standard error	*p*-value	95 % CI
α^0-thalassemia mutation ($--^{SEA}$)	-0.481	0.557	0.396	(-1.636, 0.673)
α^+-thalassemia mutation ($-\alpha^{3.7}$)	-0.168	0.432	0.701	(-1.064, 0.728)
Hb Constant Spring	-0.676	0.503	0.192	(-1.719, 0.366)
Single nucleotide polymorphism				
BCL11A (rs766432, C/A)	0.131	0.365	0.723	(-0.626, 0.888)
HBS1L-MYB (rs9399137, C/T)	0.481	0.858	0.580	(1.298, 2.261)
*Xmn*I (rs7482144, A/G)	-0.847	0.382	0.037	(-1.639, -0.055)
$F = 1.329$, $p = 0.286$, $R^2 = 0.266$				

Hb levels				
Genetic modifier	Coefficient	Standard error	p-value	95 % CI
α^0-thalassemia mutation ($--^{SEA}$)	0.977	1.202	0.425	(-1.515, 3.470)
α^+-thalassemia mutation ($-\alpha^{3.7}$)	0.607	0.933	0.522	(-1.327, 2.542)
Hb Constant Spring	1.739	1.086	0.123	(-0.512, 3.991)
Single nucleotide polymorphism				
BCL11A (rs766432, C/A)	-0.789	0.788	0.327	(-2.424, 0.845)
HBS1L-MYB (rs9399137, C/T)	-1.377	1.853	0.465	(-5.220, 2.465)
*Xmn*I (rs7482144, A/G)	1.464	0.825	0.090	(-0.246, 3.174)
$F = 0.997$, $p = 0.452$, $R^2 = 0.214$				

CI, confidence interval; Hb, hemoglobin.

Table 5
Clinical and hematological parameters of Hb E/β (NT -28 A > G) -thalassemia subjects with different forms of α-thalassemia.

	N	Severity score (median/range)	Hb (g/dL) (mean ± SD/min-max)	MCV (fL) (mean ± SD/min-max)	Hb A (%) (median/range)	Hb F (%) (median/range)	Hb A2 (%) (median/range)	Hb E (%) (median/range)
No α-thalassemia	18	0 (0–3)	10.11 ± 1.50[a] (7.50–12.60)	58.50 ± 3.90[a] (51.80–69.90)	24.2[a] (15.1–29.7)	19.3[a] (2.6–48.0)	6.5[a] (3.8–8.0)	49.5[a] (33.1–59.7)
α^0-thalassemia mutation ($--^{SEA}$)	3	0 (0–0)	10.70 ± 1.10 (9.40–11.40)	50.90 ± 6.60 (43.30–55.50)	31.5 (30.3–31.5)	3.7 (1.4–16.0)	7.7 (6.3–9.2)	56.8 (43.6–59.4)
α^+-thalassemia mutation ($-\alpha^{3.7}$)	5	0.5 (0–2)	11.10 ± 1.60[a] (9.60–13.10)	55.30 ± 4.90[a] (49.70–60.20)	25.5[a] (24.2–27.9)	16.5[a] (6.9–18.4)	6.8[a] (6.2–7.5)	52.3[a] (49.3–57.7)
Hb constant spring	3	0 (0–3)	11.50 ± 0.50 (11.00–12.00)	57.40 ± 5.00 (53.20–62.90)	25.8 (24.1–29.9)	13.8 (11.1–18.1)	6.5 (5.4–6.9)	52.4 (52.1–53.9)
p-value		0.2700[b]	0.3578[c]	0.0643[c]	0.0138[b]	0.0834[b]	0.3495[b]	0.4361[b]

[a] Excludes a subject who received occasional transfusion.
[b] P-value determined by Kruskal-Wallis test.
[c] P-value determined by F-test in ANOVA.

Declaration of competing interest

None.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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References

[1] K. Paiboonsukwong, Y. Jopang, P. Winichagoon, S. Fucharoen, Thalassemia in Thailand, Hemoglobin 46 (1) (2022) 53–57.
[2] P. Wong, P. Suannum, S. Jermnim, P. Charoenporn, M. Chan-In, A. Tapprom, R. Deoisares, Lessons learned from a prenatal diagnosis program for thalassemia in Thailand, Prenat. Diagn. 40 (8) (2020) 998–1004.
[3] P. Winichagoon, S. Fucharoen, V. Thonglairoam, V. Tanapotiwirut, P. Wasi, Beta-thalassemia in Thailand, Ann. N. Y. Acad. Sci. 612 (1990) 31–42.
[4] S. Fucharoen, P. Ketvichit, P. Pootrakul, N. Siritanaratkul, A. Piankijagum, P. Wasi, Clinical manifestation of beta-thalassemia/hemoglobin E disease, J. Pediatr. Hematol. Oncol. 22 (6) (2000) 552–557.
[5] B. Boonyawat, C. Monsereenusorn, C. Traivaree, Molecular analysis of beta-globin gene mutations among Thai beta-thalassemia children: results from a single center study, Appl. Clin. Genet. 7 (2014) 253–258.
[6] P. Panichchob, P. Iamdeelert, P. Wongsariya, P. Wongsariya, P. Wongwattanasanti, W. Tepakhan, W. Jomoui, Molecular spectrum of β-thalassemia mutations in central to eastern Thailand, Hemoglobin 45 (2) (2021) 97–102.
[7] V. Viprakasit, C. Limwongse, S. Sukpanichnant, P. Ruangvutilert, C. Kanjanakorn, W. Glomglao, M. Sirikong, W. Utto, V.S. Tanphaichitr, Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand, Clin. Chem. Lab. Med. 51 (8) (2013) 1605–1614.
[8] S. Yamsri, K. Singha, T. Prajantasen, W. Taweenan, G. Fucharoen, K. Sanchaisuriya, S. Fucharoen, A large cohort of β(+)-thalassemia in Thailand: molecular, hematological and diagnostic considerations, Blood Cells Mol. Dis. 54 (2) (2015) 164–169.
[9] P. Wong, T. Chitsobhak, S. Jittasathian, C. Sirichantharawat, N. Cherdchoo, W. Prangcharoen, P. Jongautchariyakul, K. Jampachaisri, A. Tapprom, R. Deoisares, P. Chumnumsiriwath, Essential genetic modifiers and their measurable impact in a community-recruited population analysis for non-severe hemoglobin E/β-thalassemia prenatal genetic counseling, Blood Cells Mol. Dis. 103 (2023) 102765.
[10] O. Sripichai, W. Makarasara, T. Munkongdee, C. Kumkhaek, I. Nuchprayoon, A. Chuansumrit, S. Chuncharunee, N. Chantrakoon, P. Boonmongkol, P. Winichagoon, S. Fucharoen, A scoring system for the classification of beta-thalassemia/Hb E disease severity, Am. J. Hematol. 83 (6) (2008) 482–484.
[11] R. Saetung, S. Ongchai, P. Charoenkwan, T. Sanguansermsri, Genotyping of beta thalassemia trait by high-resolution DNA melting analysis, Southeast Asian J. Trop. Med. Public Health 44 (6) (2013) 1055–1064.
[12] Y.T. Liu, J.M. Old, K. Miles, C.A. Fisher, D.J. Weatherall, J.B. Clegg, Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions, Br. J. Haematol. 108 (2) (2000) 295–299.
[13] L. Nuntakarn, S. Fucharoen, G. Fucharoen, K. Sanchaisuriya, A. Jetsrisuparb, S. Wiangnon, Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-beta-thalassemia in Northeast Thailand, Blood Cells Mol. Dis. 42 (1) (2009) 32–35.
[14] O. Sripichai, T. Munkongdee, C. Kumkhaek, S. Svasti, P. Winichagoon, S. Fucharoen, Coinheritance of the different copy numbers of alpha-globin gene modifies severity of beta-thalassemia/Hb E disease, Ann. Hematol. 87 (5) (2008) 375–379.
[15] M.H. Steinberg, J.G. Adams, Hemoglobin A_2: origin, evolution, and aftermath, Blood 78 (1991) 2165–2177.
[16] W. Prasing, S. Pornprasert, Measurement of HbA_2 by capillary electrophoresis for diagnosing β-thalassemia/HbE disease in patients with low HbF, Lab. Med. 45 (3) (2014 Summer) 226–230.
[17] K. Singha, H. Srivorakun, G. Fucharoen, S. Fucharoen, Co-inheritance of α(0) -thalassemia elevates Hb A2 level in homozygous Hb E: diagnostic implications, Int. J. Lab. Hematol. 39 (5) (2017) 508–512.
[18] P. Wong, A. Srichaiya, P. Suannum, P. Charoenporn, S. Jermnim, M. Chan-In, A. Tapprom, R. Deoisares, Frequency of hemoglobin E/β-thalassemia compound heterozygotes with low hemoglobin F phenotype among cases with a diagnosis of hemoglobin E homozygote, determined by high-performance liquid chromatography, in prenatal control program for β-thalassemia, Ann. Hematol. 96 (10) (2017) 1763–1765.
[19] A. Premawardhena, C.A. Fisher, N.F. Olivieri, S. de Silva, M. Arambepola, W. Perera, A. O'Donnell, T.E. Peto, V. Viprakasit, L. Merson, G. Muraca, D. J. Weatherall, Haemoglobin E beta thalassaemia in Sri Lanka, Lancet 366 (2005) 1467–1470.
[20] N.F. Olivieri, Z. Pakbaz, E. Vichinsky, HbE/β-thalassemia: basis of marked clinical diversity, Hematol. Oncol. Clin. North Am. 24 (2010) 1055–1070.