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Original Article

α^0-thalassemia in affected fetuses with hemoglobin E-β^0-thalassemia disease in a high-risk population in Thailand

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Abstract: Objectives: A co-inheritance of α^0-thalassemia can ameliorate the clinical severity of the hemoglobin (Hb) E-β-thalassemia disease. This information should be provided at prenatal diagnosis. Identification of α^0-thalassemia in an affected fetus is therefore valuable. We have explored this genetic interaction in a large cohort of affected fetuses with hemoglobin (Hb) E-β-thalassemia in northeast Thailand. Methods: A study was done retrospectively on 1,592 couples at risk of having fetuses with Hb E-β^0-thalassemia, encountered from January 2011 to December 2019. A total of 415 left-over DNA specimens of the affected fetuses with Hb E-β^0-thalassemia disease were further investigated. Examination of α^0-thalassemia was done using gap-PCR or a multiplex PCR assay for simultaneous detection of Hb E and α^0-thalassemia mutations. Results: Of the 415 affected fetuses, the two most common β^0-thalassemia genes found were the codons 41/42 (-TTCT) (199/415; 48.0%) and codon 17 (A-T) (115/415; 27.7%). α^0-thalassemia was found unexpectedly in 21 (5.1%) fetuses. Hematologic phenotypes of the parents indicated that it was impossible to differentiate a pure β^0-thalassemia carrier from a double β^0-thalassemia/α^0-thalassemia heterozygote unless DNA analysis is performed. In contrast, a reduced level of Hb E in the Hb E carrier (<25%) is a valuable marker for predicting double heterozygosity for Hb E/α^0-thalassemia. This could be further confirmed using a multiplex PCR assay. Conclusions: There is a high prevalence of co-inheritance of α^0-thalassemia in fetuses with Hb E-β^0-thalassemia disease. In a high-risk population such as Thailand, we recommend screening for α^0-thalassemia in all affected fetuses with Hb E-β^0-thalassemia disease and providing complete genetic information to the parents to make appropriate decisions at prenatal diagnosis and genetic counseling.

Keywords: α-thalassemia, β-thalassemia, Hb E-β-thalassemia, prenatal diagnosis, genetic interaction

Introduction

Hemoglobin (Hb) E-β^0-thalassemia is the most common form of thalassemia found in northeast Thailand [1]. The disease exhibits variable clinical phenotype, ranging from mild non-transfusion dependent thalassemia (NTDT) to severe transfusion-dependent thalassemia (TDT) [2, 3]. This marked phenotypic diversity is associated with a great variety of genotypes, including different β-thalassemia alleles, co-inheritance of α-thalassemia, and the presence of genetic determinants associated with increased production of γ-globin chains and consequent ability to produce functional fetal hemoglobin (Hb F) in adult life [4-7]. Among these genetic modifiers, co-inheritance of α^0-thalassemia is a significant factor that can dramatically reduce the clinical severity of the cases in our population [8, 9]. In Thailand, according to the national prevention and control program of thalassemia, Hb E-β^0-thalassemia is one of the targeted severe thalassemia diseases [10]. Prenatal diagnosis is routinely offered to a couple at risk of having a fetus with the disease [1]. Therefore, identification of α^0-thalassemia in an affected fetus is useful. Due to the variability of the phenotype associated with the disease, it is essential to examine the possible co-inheritance of genetic modifiers in the fetus, especially α^0-thalassemia, before a decision is made appropriately. The micromap-

ping survey in our area in northeast Thailand has identified the prevalence of 5.8% for α^0-thalassemia, 0.9% for β-thalassemia, and 41.7% for Hb E [11]. A genetic co-inheritance of these thalassemia defects in the population is expected. Our previous studies in adult subjects showed that the prevalence of α^0-thalassemia in β-thalassemia carrier, Hb E carriers, and homozygous Hb E were 4.4%, 10.4%, and 4.6%, respectively [12-14]. In this study, we examine the prevalence of co-inheritance of α^0-thalassemia in a large retrospective cohort of affected fetuses with Hb E-β^0-thalassemia encountered at our prenatal diagnosis service nine consecutive years during January 2011 to December 2019.

Material and methods

Subjects

Ethical approval of the study protocol was obtained from the Institutional Review Board (IRB) of Khon Kaen University, Khon Kaen, Thailand (HE622173). A retrospective review was made on 1,592 couples at risk of having fetuses with Hb E-β^0-thalassemia referred to our Thalassemia Service Unit, Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Khon Kaen University, Khon Kaen, Thailand, for prenatal diagnosis during January 2011 to December 2019. Left-over DNA specimens were selectively recruited from 415 affected fetuses with Hb E-β^0-thalassemia disease. These DNA specimens were prepared routinely from chorionic villi, amniotic fluid, or fetal blood specimens obtained at prenatal diagnosis [1].

DNA analysis

At our routine setting, identification of β-thalassemia and Hb E mutations are carried out using allele-specific PCR and related techniques [1]. Screening for α^0-thalassemia mutation (SEA & THAI deletions) was done in the 415 affected fetuses using gap-PCR as previously described [15, 16]. Alternatively, α^0-thalassemia was detected simultaneously with the Hb E gene in a modified multiplex PCR manner, as shown in **Figure 1** [17]. The multiplex PCR was carried out in a reaction volume of 50 μL containing 1 μg of fetal DNA in a PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 0.001% gelatin), 200 mM dNTPs, 1.005 M Betaine, 2% Dimethyl sulfoxide, 1.5 pmol each of primer; γ4 (5'-GGCCTAAAACCACAGAGT-3'), γ5 (5'-CCAGAAGCGAGTGTGTGGAA-3'), S3 (5'-TCCCATAGACTCACCCTGAA-3') and G24 (5'-CGTGGATGAAGTTGGTGGTA-3'), 2.4 pmol each of primer; αG64 (5'-CGATCTGGGCTCTGTGTTCT-3') and αG65 (5'-TGGAGTGCAGTGTTGTAGTC-3'), and 2 units *Taq* DNA polymerase (Biotools, B&M Labs, Madrid, Spain). After initial heating at 95°C for 5 min, a PCR process (95°C for 1 min, 63°C for 1 min, and 72°C for 90 sec) was carried out on a SimpliAmp Thermal Cycler (Applied Biosystems, Waltham, MA, USA) for 30 cycles. The PCR amplicon was analyzed on 2% agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide.

Statistical analysis

Frequency of α^0-thalassemia was presented as number and percentage. Hematological values and Hb profiles among at-risk couples were described by mean and standard derivation. All descriptive statistics were performed using Minitab version 16 (Minitab, Inc., USA).

Results

Thalassemic diseases at-risk for the fetuses

From January 2011 to December 2019, a total of 3,699 at-risk couples were referred to our center for prenatal diagnosis of thalassemia. Of these, 1,592 couples were at risk of having fetuses with Hb E-β^0-thalassemia disease based on initial Hb and DNA analyses. Some of them also carried additional risks for homozygous α^0-thalassemia and homozygous β-thalassemia, as shown in **Table 1**. As shown in the Table, Hb analysis revealed, in most couples (1,544/1,592; 97.0%) with Hb A_2A (Hb A_2>3.5% for β-thalassemia trait) in one of the couples and EA (Hb E>25% for Hb E trait) in another, indicating a 25% risk of Hb E-β-thalassemia in the fetuses (Group 1). Based on the national screening protocol, prenatal testing of these at-risk couples in group 1 focuses mainly on β-thalassemia and Hb E, and analysis of α^0-thalassemia is not performed. In the remaining couples, 45 (45/1592; 2.8%) had additional risks for homozygous α^0-thalassemia since one of the couples carried β-thalassemia heterozygote, Hb E homozygote, or Hb E heterozygote (with Hb E<25%, possibly

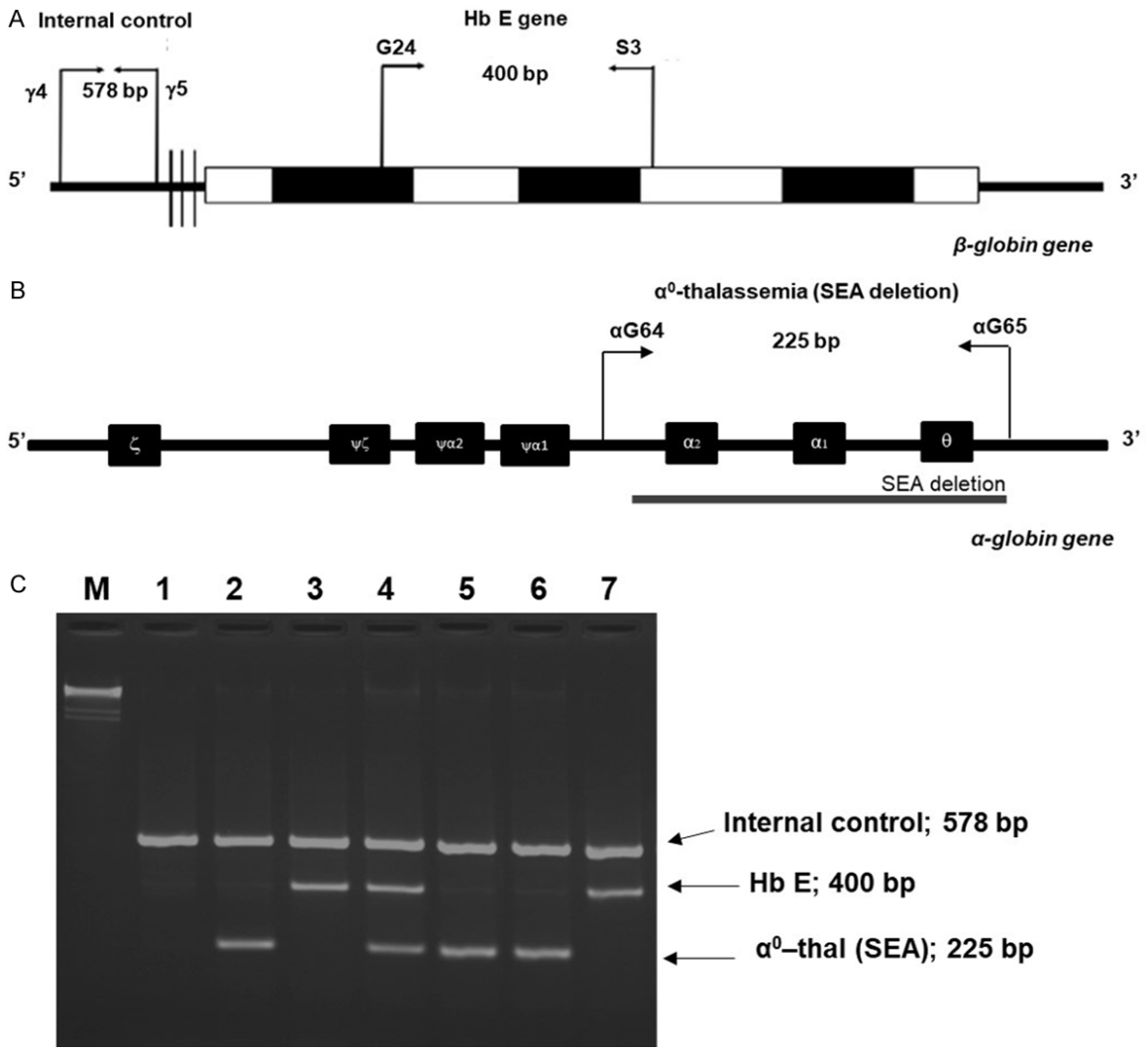

Figure 1. Multiplex PCR for simultaneous identification of Hb E and α⁰-thalassemia (SEA deletion) genes. The locations and orientations of the primers used in the PCR assay are depicted. A: The Hb E allele-specific primer (G24) is used with a common primer (S3) to produce a 400 bp Hb E-specific fragment. Two common primers, γ4 and γ5 for ^{G}γ-globin gene promoter, are used to generate a 578 bp internal control fragment. B: The primers αG64 and αG65 are used to produce a 225-bp specific fragment of α⁰-thalassemia (SEA deletion). C: A representative 2% agarose gel electrophoresis. M is λ/Hind III DNA markers. Lane 1 is normal, lanes 2, 5, and 6 are positive for the α⁰-thalassemia (SEA deletion), lanes 3 and 7 are positive for Hb E, and lane 4 is positive for both Hb E and α⁰-thalassemia (SEA deletion).

Table 1. Results of Hb analysis of 1,592 at-risk couples, classified into 6 different groups and the diseases at-risk for the fetuses

Group	Results of Hb analysis		Diseases at-risk	No.	%
	Husband/Wife	Husband/Wife			
1	A_2A; Hb A_2>3.5%	EA; Hb E>25%	a	1,544	96.9
2	A_2A; Hb A_2>3.5%	EA; Hb E≤25%	a, b	29	1.8
3	A_2A; Hb A_2>3.5%	EE	a, b	9	0.6
4	EF	EA; Hb E≤25%	a, b	4	0.3
5	EF	EE	a, b	3	0.2
6	A_2A; Hb A_2>3.5%	EF, EE/EF	a, b, c	3	0.2
	Total			1,592	100

A: Hb E-β⁰-thalassemia disease; b: Homozygous α⁰-thalassemia, and c: Homozygous β⁰-thalassemia.

Table 2. Number of affected fetuses with Hb E-β⁰-thalassemia according to β-thalassemia mutations *in trans* of Hb E mutation and number of fetuses with co-inheritance of α⁰-thalassemia

β-thalassemia mutations	No	(%)	Number of fetuses with α⁰-thalassemia (SEA) (%)
CD 41/42 (-TTCT)	199	(48.0)	11 (2.7)
CD 17 (A-T)	115	(27.7)	7 (1.7)
IVSI-1 (G-T)	26	(6.3)	2 (0.5)
IVSI-5 (G-C)	19	(4.6)	
CD 71/72 (+A)	18	(4.3)	
3.4-kb deletion	10	(2.4)	
IVSII-654 (C-T)	8	(1.9)	
CD 35 (C-A)	5	(1.2)	
CD 41 (-C)	3	(0.7)	
CD 26 (C-T)	2	(0.5)	
CD 27 (+C)	2	(0.5)	1 (0.2)
105 bp del	2	(0.5)	
FIL del	1	(0.2)	
CD 43 (G-T)	1	(0.2)	
CD 30 (G-C)	1	(0.2)	
CD 15 (-T)	1	(0.2)	
CD 33/34 (+C)	1	(0.2)	
CD 95 (+A)	1	(0.2)	
Total	415	(100)	21 (5.1)

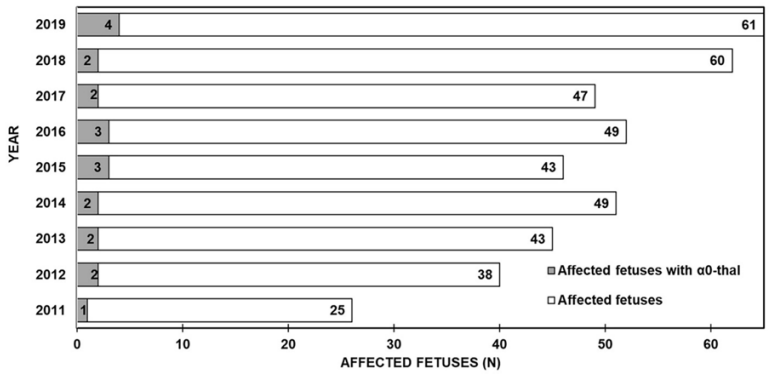

Figure 2. Numbers of affected fetuses with Hb E-β⁰-thalassemia disease encountered within nine years from 2011 to 2019. The white bars indicate the total number of Hb E-β⁰-thalassemia affected fetuses and the gray boxes indicate the number of affected fetuses that co-inherited α⁰-thalassemia.

co-inheritance of α⁰-thalassemia requiring further PCR analysis of α⁰-thalassemia). Therefore, these 45 couples were at risk of having fetuses with both Hb E-β⁰-thalassemia and homozygous α⁰-thalassemia. Thus, the fetuses of these 45 couples were tested at prenatal diagnosis for β⁰-thalassemia, Hb E, and α⁰-thalassemia mutations. In contrast, the last three couples (3/1,592; 0.2%) were at risk for Hb E-β-thalassemia, homozygous α⁰-thalassemia and homozygous β-thalassemia. In this last group, one partner of the couples was a carrier of β-thalassemia, and their partner carried Hb E-β-thalassemia or homozygous Hb E disorders (**Table 1**).

β-thalassemia mutations and α⁰-thalassemia identified in affected fetuses

Retrospective reviews at prenatal diagnosis of these 1,592 at-risk couples showed that 415 (26.1%) fetuses were affected by Hb E-β⁰-thalassemia disease. A total of 18 different β-thalassemia mutations were identified. As expected, the two most common mutations were codons 41/42 (-TTCT) and codon17 (A-T), accounting for 48.0% and 27.7%, respectively. Other β-thalassemia mutations were encountered at much lower frequencies, as shown in **Table 2**. DNA samples of these 415 affected fetuses were further investigated for co-inheritance of α⁰-thalassemia (SEA & THAI deletions). With this analysis, we found 21 of 415 (5.1%) fetuses with Hb E-β⁰-thalassemia disease to carry α⁰-thalassemia (SEA deletion). **Figure 2** summarizes a total number of affected fetuses with Hb E-β⁰-thalassemia disease and those with α⁰-thalassemia (SEA deletion) found each year during 2011-2019. Hematologic features and the result of DNA analysis of the parents of these 21 affected fetuses are presented in **Table 3**. As shown in the table, α⁰-thalassemia was identified in the parents with heterozygous β-thalassemia (n=13), heterozygous Hb E (n=7), homozygous Hb E (n=2), and Hb E-β-thalassemia (n=1). Except for a reduced level of Hb E in Hb E

Table 3. Hematologic values and Hb profiles of 21 at-risk couples who had affected fetuses with Hb E-β^0-thalassemia and heterozygous α^0-thalassemia (SEA), including results of α^0-thalassemia analysis of the couples

Group	Number of couples (%)	Hb analysis		α^0-thal gene (SEA)	Rbc ($x10^{12}$/L)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	RDW (%)	Hb A_2/E (%)	Hb F (%)
1	10 (47.6)	Husband/Wife	A_2A; Hb A_2>3.5%	Pos	5.4±1.0	11.6±1.8	35.7±5.1	66.1±4.9	21.1±1.6	15.4±2.1	5.8±0.8	1.3±0.5
		Husband/Wife	EA; Hb E>25%	Neg	5.3±0.6	12.8±2.3	38.8±6.5	73.1±8.0	24.4±3.3	14.1±2.8	28.4±1.1	1.2±0.7
2	3 (14.3)	Husband/Wife	A_2A; Hb A_2>3.5%	Pos	5.8±0.7	11.2±2.7	35.1±7.2	59.6±5.5	19.0±24	18.2±2.0	5.7±0.6	0.5±0.4
		Husband/Wife	EA; Hb E≤25%	Pos	4.9±0.1	10.5±0.4	31.5±1.1	64.8±3.7	21.5±0.7	14.6±0.7	21.7±0.7	0.5±0.3
3	3 (14.3)	Husband/Wife	A_2A; Hb A_2>3.5%	Neg	6.3±0.7	12.2±1.4	40.0±3.5	63.9±3.1	19.5±1.0	13.9±0.1	5.8±0.5	1.7±0.9
		Husband/Wife	EA; Hb E≤25%	Pos	5.8±0.7	12.1±1.5	38.3±5.0	71.3±5.8	21.0±0.1	16.0±2.5	19.7±0.8	1.2±0.8
4	1 (4.8)	Husband	EE	Neg	6.8	13.4	42.5	62.8	19.8	15.3	98.8	1.2
		Wife	A_2A; Hb A_2>3.5%	Pos	5.3	11.0	34.9	65.9	20.8	16.9	5.7	0
5	1 (4.8)	Husband	A_2A; Hb A_2>3.5%	Neg	4.6	10.9	30.4	66.5	23.9	13.8	5.2	0.5
		Wife	EE	Pos	5.8	13.6	40.1	69.0	23.3	20.1	98.5	1.5
6	1 (4.8)	Husband	EE	na	5.6	11.8	34.8	62.0	21.0	15.8	96.5	3.5
		Wife	A_2A; Hb A_2>3.5%	na	5.3	11.4	36	67.0	21.3	14.8	5.2	1.1
7	1 (4.8)	Husband	EE	Pos	6.3	13.2	37.9	60.1	20.9	18.6	90.2	1.9
		Wife	EF	Pos	4.9	8.2	23.8	48.8	16.7	21.9	66.6	33.4
8	1 (4.8)	Husband	EF	Neg	5.4	10.2	33.4	61.3	18.7	27.1	63.2	36.8
		Wife	EA; Hb E≤25%	Pos	5.2	10.7	34	65.7	20.7	16.4	19.2	0.7

Values are presented as mean ± standard deviation or as raw data where appropriated. Pos: Positive; Neg: Negative; na: not available.

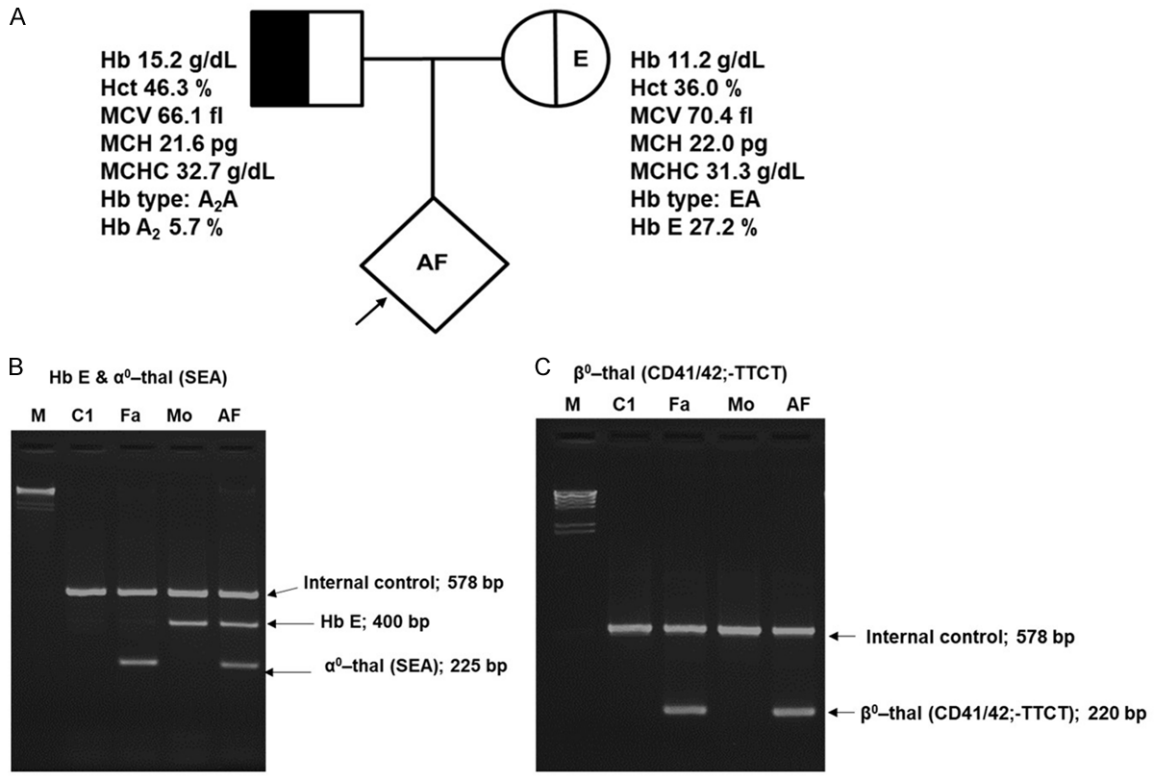

Figure 3. Prenatal diagnosis of Hb E-β⁰-thalassemia in a representative family. A: Pedigree analysis with hematological and Hb analysis results of the couples at risk. The arrow indicates the fetus whose amniotic fluid specimen (AF) was obtained at 16 weeks of gestation. A representative 2% agarose gel electrophoresis of the PCR products are shown. M is λ/*Hind* III DNA markers. B: The multiplex PCR assay for Hb E and α⁰-thalassemia (SEA deletion) showed that the father (Fa), the mother (Mo), and the fetus (AF) were respectively positive for α⁰-thalassemia, Hb E, and both Hb E and α⁰-thalassemia. C1 is a negative control. C: Allele-specific PCR assay for β⁰-thalassemia (CD 41/42; -TTCT). The result indicated that the father (Fa) and the fetus (AF) but not the mother (Mo) were positive for β⁰-thalassemia (CD 41/42; -TTCT). Therefore, the fetus was affected by Hb E-β⁰-thalassemia disease with a co-inherited α⁰-thalassemia (SEA deletion).

heterozygote with α⁰-thalassemia (Hb E<25%), we did not observe a difference in the hematologic features of these double heterozygotic subjects and those of heterozygotic subjects without α⁰-thalassemia in our archives. Therefore, screening of such thalassemia interactions in the parents based on hematological grounds may be difficult.

Simultaneous detection of α⁰-thalassemia and Hb E

Simultaneous identification of α⁰-thalassemia in the fetus with Hb E-β-thalassemia at prenatal diagnosis is the best alternative. This is shown representatively in **Figure 3**. Hematologic findings indicated that the father was a β⁰-thalassemia carrier (CD 41/42; -TTCT) associated with reduced MCV (66.1 fL) & MCH (21.6 pg), and elevated Hb A_2 (5.7%). The mother was diagnosed as a Hb E carrier with 27.2% Hb E. The couple had a 25% risk of having a fetus with Hb E-β⁰-thalassemia disease. As shown in the figure, a multiplex PCR assay identified unexpectedly that the fetus was affected by Hb E-β⁰-thalassemia and α⁰-thalassemia, and the father was, in fact, a double β⁰-thalassemia/α⁰-thalassemia heterozygote.

Discussion

Hb E-β⁰-thalassemia is a significant public health problem in Thailand and many Southeast Asian countries [1, 14]. Generally, the patient requires regular blood transfusion for effective treatment, although variable phenotypic expression varying from mild to severe phenotype has been noted [3, 19]. Several genetic factors have been identified as genetic modifiers of this disease. A study of Hb E-β⁰-thalassemia patients with non-transfusion-dependent thalassemia (NTDT) phenotype in north-

east Thailand also showed many genetic factors, such as co-inheritance of α-thalassemia, ^{G}γ-*Xmn*I polymorphism, and other genetic determinants associated with increased production of γ-globin chains play important role in the severity of the disease [3, 9, 20, 21]. Among these genetic modifiers, co-inheritance of α^0-thalassemia was found to be a major genetic modifier for Hb E-β^0-thalassemia disease. Our recent study in northeast Thailand demonstrated that Hb E-β^0-thalassemia patients with co-inherited α^0-thalassemia showed NTDT phenotype with a mean Hb level of 10.5±2.9 g/dL [21]. This is also the case in northern Thai patients where it was found that Hb E-β^0-thalassemia patients with co-inherited α^0-thalassemia showed a mild clinical phenotype with the mean Hb level of 7.9±1.5 g/dL, and growth was close to normal [22]. Therefore, identifying the co-inheritance of α^0-thalassemia in the fetus with Hb E-β^0-thalassemia disease is important additional information for phenotypic prediction of the expecting fetus and genetic counseling the parents. It is understandable that the affected fetus with this genetic thalassemia interaction would have a mild clinical phenotype. In this study, the prevalence of 5.1% (**Table 2**) co-inheritance of α^0-thalassemia in fetuses with Hb E-β^0-thalassemia disease indicates that this should not be overlooked at prenatal diagnosis in Thai population. A prevalence of 5.8% α^0-thalassemia has been documented in the northeast Thai population [11]. Our previous study revealed that the prevalence of co-inherited α^0-thalassemia in β-thalassemia heterozygote in our population is around 4.4% [12]. As shown in **Table 3**, among 21 couples who had fetuses with Hb E-β^0-thalassemia disease with α^0-thalassemia, only one of the couples carried α^0-thalassemia. Accordingly, these couples do not carry the risk for fetal homozygous α^0-thalassemia disease (Hb Bart's hydrops fetalis) in their fetuses, information related to α^0-thalassemia gets ignored, and no α^0-thalassemia screening is carried out at prenatal diagnosis and counseling. Thus, two approaches may be carried out prospectively, i.e., massive screening of α^0-thalassemia in all couples at risk for fetal Hb E-β^0-thalassemia or selective identification of α^0-thalassemia in all fetuses with Hb E-β^0-thalassemia disease.

It is conceivable, as shown by **Table 3** that based on hematological grounds, selective screening of α^0-thalassemia in the couples with heterozygous β-thalassemia may be difficult. The hematologic features of the β-thalassemia heterozygote with or without α^0-thalassemia are similar, especially since they both have hypochromic microcytosis and elevated Hb A_2 i.e., still within a diagnostic range for a β-thalassemia heterozygote [12, 23]. In contrast, for Hb E heterozygote, the reduced level of Hb E (plus Hb A_2) (<25%) is a useful marker for recognition of a double heterozygosity for Hb E/α^0-thalassemia [13]. In addition, as shown in **Table 3**, as compared to a plain Hb E trait (Hb E>25%), a double heterozygous Hb E/α^0-thalassemia (Hb E<25%) had relatively lower MCV & MCH values [24]. According to the screening protocol, an individual with Hb E heterozygote and Hb E>25% is considered a plain Hb E carrier and no α^0-thalassemia screening is essential [1, 10]. For homozygous Hb E, it is helpful to do Hb analysis using capillary electrophoresis rather than Hb-HPLC to determine the level of Hb A_2 since capillary electrophoresis, but not HPLC, can report the level of Hb A_2 in the presence of Hb E. Selection of suspected cases with homozygous Hb E and α^0-thalassemia is possible. We have demonstrated that co-inheritance of α^0-thalassemia elevates the Hb A_2 level in homozygous Hb E (>4.5%) [25]. These characteristics are helpful in selective screening of α^0-thalassemia in couples with β-thalassemia heterozygote or Hb E-related disorders. However, this selective screening in couples can add to workload, and be labor-intensive, and expensive due to a high number of pregnancies each year.

Alternatively, screening of α^0-thalassemia can be effectively performed in all fetuses with Hb E-β-thalassemia using a multiplex PCR assay to detect Hb E and α^0-thalassemia as shown in **Figure 3**. In this approach, the fetus is screened for α^0-thalassemia simultaneously with Hb E. Co-inheritance of α^0-thalassemia with the Hb E-β-thalassemia disease in the fetus is readily identified, no matter on the status of α^0-thalassemia of the parents. Complete thalassemia genetic interaction in the fetus can then be provided to the parents at genetic counseling before making an appropriate decision. It is also noteworthy that another approach is to perform a prenatal diagnosis using a fetal blood specimen and Hb fractionation using

capillary electrophoresis. This Hb analysis system can demonstrate and quantitate Hb Bart's (γ_4) because of a co-inheritance of α⁰-thalassemia. Hb Bart's is a homotetramer of excess γ-globin chain, presented in a fetus and newborn with α-thalassemia. It is a useful marker in cord blood for screening of α-thalassemia in newborns [26-28]. We have demonstrated previously that heterozygous α⁰-thalassemia fetuses produced Hb Bart's around 5.0% when examined using capillary electrophoresis [29]. The fetus with Hb E-β⁰-thalassemia and α⁰-thalassemia would demonstrate this amount of Hb Bart's in addition to Hb F and Hb E without Hb A.

In conclusion, this study in Thailand confirmed that as many as 5.1% of affected fetuses with Hb E-β⁰-thalassemia disease carried α⁰-thalassemia (SEA deletion). These affected fetuses should have a mild clinical phenotype, and their parents should receive this complete genetic information at genetic counseling before making an appropriate decision. Therefore, we recommend screening of α⁰-thalassemia in all fetuses with Hb E-β⁰-thalassemia disease at routine prenatal diagnosis in the region to obtain the correct genotype of the affected fetuses, which is necessary for genetic counseling.

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Disclosure of conflict of interest

None.

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References

[1] Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-ung N, Ratanasiri T and Fucharoen S. Prevention of severe thalassemia in northeast Thailand: 16 years of experience at a single university center. Prenatal Diagnosis 2010; 30: 540-546.

[2] Winichagoon P, Fucharoen S, Chen P and Wasi P. Genetic factors affecting clinical severity in beta-thalassemia syndromes. J Pediatr Hematol Oncol 2000; 22: 573-580.

[3] Prayalaw P, Teawtrakul N, Jetsrisuparb A, Pongudom S, Fucharoen G and Fucharoen S. Phenotype and genotype in a cohort of 312 adult patients with nontransfusion-dependent thalassemia in Northeast Thailand. Acta Haematol 2016; 135: 15-20.

[4] Lettre G, Sankaran VG, Bezerra MA, Araujo AS, Uda M, Sanna S, Cao A, Schlessinger D, Costa FF, Hirschhorn JN and Orkin SH. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci U S A 2008; 105: 11869-11874.

[5] Garner C, Tatu T, Reittie JE, Littlewood T, Darley J, Cervino S, Farrall M, Kelly P, Spector TD and Thein SL. Genetic influences on F cells and other hematologic variables: a twin heritability study. Blood 2000; 95: 342-346.

[6] So CC, Song YQ, Tsang ST, Tang LF, Chan AY, Ma ES and Chan LC. The HBS1L-MYB intergenic region on chromosome 6q23 is a quantitative trait locus controlling fetal haemoglobin level in carriers of beta-thalassaemia. J Med Genet 2008; 45: 745-751.

[7] Sedgewick AE, Timofeev N, Sebastiani P, So JCC, Ma ESK, Chan LC, Fucharoen G, Fucharoen S, Barbosa CG, Vardarajan BN, Farrer LA, Baldwin CT, Steinberg MH and Chui DHK. BCL11A is a major HbF quantitative trait locus in three different populations with beta-hemoglobinopathies. Blood Cells Mol Dis 2008; 41: 255-258.

[8] Sripichai O, Munkongdee T, Kumkhaek C, Svasti S, Winichagoon P and Fucharoen S. Co-inheritance of the different copy numbers of alpha-globin gene modifies severity of beta-thalassemia/Hb E disease. Ann Hematol 2008; 87: 375-379.

[9] Nuntakarn L, Fucharoen S, Fucharoen G, Sanchaisuriya K, Jetsrisuparb A and Wiangnon S. Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-beta-thalassemia in Northeast Thailand. Blood Cells Mol Dis 2009; 42: 32-35.

[10] Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S and Fucharoen S. A simplified screening strategy for thalassaemia and hae-

moglobin E in rural communities in south-east Asia. Bull World Health Organ 2004; 82: 364-372.
[11] Tritipsombut J, Sanchaisuriya K, Phollarp P, Bouakhasith D, Sanchaisuriya P, Fucharoen G, Fucharoen S and Schelp FP. Micromapping of thalassemia and hemoglobinopathies in diferent regions of northeast Thailand and Vientiane, Laos People's Democratic Republic. Hemoglobin 2012; 36: 47-56.
[12] Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N and Fucharoen S. Genotype and phenotype characterizations in a large cohort of beta-thalassemia heterozygote with different forms of alpha-thalassemia in Northeast Thailand. Blood Cells Mol Dis 2011; 47: 120-124.
[13] Sanchaisuriya K, Fucharoen G, Sae-ung N, Jetsrisuparb A and Fucharoen S. Molecular and hematologic features of hemoglobin E heterozygotes with different forms of alpha-thalassemia in Thailand. Ann Hematol 2003; 82: 612-616.
[14] Fucharoen G, Trithipsombat J, Sirithawee S, Yamsri S, Changtrakul Y, Sanchaisuriya K and Fucharoen S. Molecular and hematological profiles of hemoglobin EE disease with different forms of alpha-thalassemia. Ann Hematol 2006; 85: 450-454.
[15] Sae-ung N, Fucharoen G, Sanchaisuriya K and Fucharoen S. Alpha(0)-thalassemia and related disorders in northeast Thailand: a molecular and hematological characterization. Acta Haematol 2007; 117: 78-82.
[16] Chaibunruang A, Prommetta S, Yamsri S, Fucharoen G, Sae-Ung N, Sanchaisuriya K and Fucharoen S. Molecular and hematological studies in a large cohort of alpha(0)-thalassemia in northeast Thailand: data from a single referral center. Blood Cells Mol Dis 2013; 51: 89-93.
[17] Siriratmanawong N, Fucharoen G, Sanchaisuriya K, Ratanasiri T and Fucharoen S. Simultaneous PCR detection of beta-thalassemia and alpha-thalassemia 1 (SEA type) in prenatal diagnosis of complex thalassemia syndrome. Clin Biochem 2001; 34: 377-380.
[18] Fucharoen S and Winichagoon P. Hemoglobinopathies in Southeast Asia. Hemoglobin 1987; 11: 65-88.
[19] Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A and Wasi P. Clinical manifestation of beta-thalassemia/hemoglobin E disease. J Pediatr Hematol Oncol 2000; 22: 552-557.
[20] Phanrahan P, Yamsri S, Teawtrakul N, Fucharoen G, Sanchaisuriya K and Fucharoen S. Molecular analysis of non-transfusion dependent thalassemia associated with hemoglobin E-beta-thalassemia disease without alpha-thalassemia. Mediterr J Hematol Infect Dis 2019; 11: e2019038.
[21] Yamsri S, Pakdee N, Fucharoen G, Sanchaisuriya K and Fucharoen S. Molecular understanding of non-transfusion-dependent thalassemia associated with hemoglobin E-beta-thalassemia in Northeast Thailand. Acta Haematol 2016; 136: 233-239.
[22] Charoenkwan P, Teerachaimahit P and Sanguansermsri T. The correlation of alpha-globin gene mutations and the XmnI polymorphism with clinical severity of Hb E/beta-thalassemia. Hemoglobin 2014; 38: 335-338.
[23] Singha K, Taweenan W, Fucharoen G and Fucharoen S. Erythrocyte indices in a large cohort of beta-thalassemia carrier: implication for population screening in an area with high prevalence and heterogeneity of thalassemia. Int J Lab Hematol 2019; 41: 513-518.
[24] Karnpean R, Fucharoen G, Fucharoen S and Ratanasiri T. Fetal red blood cell parameters in thalassemia and hemoglobinopathies. Fetal Diagn Ther 2013; 34: 166-171.
[25] Singha K, Srivorakun H, Fucharoen G and Fucharoen S. Co-inheritance of alpha(0)-thalassemia elevates Hb A2 level in homozygous Hb E: diagnostic implications. Int J Lab Hematol 2017; 39: 508-512.
[26] Tritipsombut J, Sanchaisuriya K, Fucharoen S, Fucharoen G, Siriratmanawong N, Pinmuangngam C and Sanchaisuriya P. Hemoglobin profiles and hematologic features of thalassemic newborns: application to screening of alpha-thalassemia 1 and hemoglobin E. Arch Pathol Lab Med 2008; 132: 1739-1745.
[27] Srivorakun H, Fucharoen G, Changtrakul Y, Komwilaisak P and Fucharoen S. Thalassemia and hemoglobinopathies in Southeast Asian newborns: diagnostic assessment using capillary electrophoresis system. Clin Biochem 2011; 44: 406-411.
[28] Chaibunruang A, Sornkayasit K, Chewasateanchai M, Sanugul P, Fucharoen G and Fucharoen S. Prevalence of thalassemia among newborns: a re-visited after 20 Years of a prevention and control program in northeast Thailand. Mediterr J Hematol Infect Dis 2018; 10: e2018054.
[29] Srivorakun H, Fucharoen G, Sae-Ung N, Sanchaisuriya K, Ratanasiri T and Fucharoen S. Analysis of fetal blood using capillary electrophoresis system: a simple method for prenatal diagnosis of severe thalassemia diseases. Eur J Haematol 2009; 83: 57-65.