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ORIGINAL RESEARCH REPORT

Modifying effect of *XmnI*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β-thalassemia and hemoglobin E/β-thalassemia patients in Indonesia

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Thalassemia;
XmnI

Abstract
Objective/background: Thalassemia is a monogenic hematologic disease that has the highest prevalence globally. In addition, there is complexity of the genetic background associated with a variety of phenotypes presented among patients. Genetic heterogeneity related to fetal hemoglobin (HbF) production has been reported as an influencing phenotypic factor of β-thalassemia (β-thal). Therefore, this study aimed to find the effect of these genetic modifiers, especially in the *XmnI* locus, rs11886868, rs766432 (*BCL11A*), and rs9399137 (*HBS1L-MYB*), among β-thal and HbE/β-thal patients in Indonesia, according to laboratory and clinical

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outcomes, including HbF levels and clinical scores. This study was also designed to compare these modifying effects among β-thal and HbE/β-thal patients in Indonesia.
Methods: A total of 189 patients with genotyping of β-thal and HbE/β-thal were included in this study. The erythrocytes index and Hb electrophoresis measurements were calculated using appropriate methods. The severity of β-thal and HbE/β-thal was classified based on the Mahidol score. Polymorphism of the *XmnI* locus, rs11886868, rs766432 (*BCL11A*), and rs9399137 (*HBS1L-MYB*) was determined using polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) and amplification refractory mutation system (ARMS) methods.
Results: The distributions of minor allele in the *XmnI* locus, rs11886868, rs766432, and rs9399137 were 14%, 22%, 19% and 18% respectively. The variation allele in the *XmnI* locus, rs11886868, and rs766432 showed a significant value for modifying HbF and clinical score in HbE/β-thal patients, but rs9399137 did not demonstrate such features. In β-thal patients, however, no correlation was found for any single-nucleotide polymorphisms and clinical appearance.
Conclusion: The *XmnI* locus, rs11886868, and rs766432 have a modifying effect on HbF and clinical score in HbE/β-thal patients in Indonesia, but not in β-thal patients.

Introduction

The inherited disorders of hemoglobin (Hb), a disease with the highest prevalence and incidence rate worldwide, are now becoming a serious health problem given the hundreds of thousands of children who are born every year with these disorders [1]. Eighty to ninety million people, or ~1.5% of the global population, are now carrying beta-thalassemia alleles in their genetic make-up. Up to 60,000 children show clinical symptoms and require hospital care [2]. For patients with thalassemia, attention should not only be directed toward the severity of the clinical appearance, but also toward the complexity of the genetic background associated with the disease. So far, >800 types of mutations and structural variants in the *beta* gene have been well characterized using the existing genomic protocols [3]. Beta-thalassemia (β-thal) itself has over 200 point mutations that lead to various clinical states because of the varying arrangements of compound heterozygous alleles. Clinical features of this condition reflect on a wide range of transfusion requirements from ''do not require transfusions" to ''regular transfusions" dependent [4].

HbE/β-thal, a compound heterozygous mutation of the beta globin gene, is identified in ~30—50% of patients with thalassemia who require regular blood transfusions. In some regions, for example, the northern part of Thailand and Cambodia, this number has gone up to 70% [5]. Cryptic mutations associated with codon 26 (GAG > AAG) result in varying levels of HbE production. The clinical variability of HbE/β-thal patients also correlates with the type of mutation present in β-globin genes. However, variations in the β-globin gene mutations in many instances are not enough to explain the genotype—phenotype correlation in these patients [6]. The phenotypic diversity of these conditions are related to the influencing genetic factors, such as the presence of mild/silent β-thal alleles, and co-inheritance with α-thalassemia (α-β-thalassemia). Milder clinical symptoms are allegedly caused by a better balance ratio between the α-chain and the non-α-chain [7]. In particular, increasing fetal hemoglobin (HbF) level in many studies was shown to affect the clinical outcome of patients with sickle cell disease (SCD), β-thal, or HbE/β-thal [8,9].

Some genome-wide association studies have reported that there are at least three major loci that play a major role in increasing HbF levels. These include −158 C > T in the promoter gene Gamma 2 (locus *XmnI*), intergenic regions *HBS1L-MYB* in the 6q23.3 chromosomal region, and the *BCL11A* gene on chromosome 2p16.1 [10—12]. The three loci simultaneously have an influence on up to 20—50% of HbF variation in patients with β-thal or in healthy adults [6,13]. The effect of these loci has been studied among European, African, American, and other Asian populations. However, according to published literature, only a few reports related to these loci in the Indonesia population are available. Therefore, this study aimed to find the effect of these genetic modifiers, especially in the *XmnI* locus-rs11886868, rs766432 (*BCL11A*), and rs9399137 (*HBS1L-MYB*)-among β-thal and HbE/β-thal patients in Indonesia, according to laboratory and clinical outcomes, including HbF levels and clinical scores.

Materials and methods

Patients studied were part of a preliminary study on genetic epidemiology associated with β-thal in Central Java, Indonesia [14]. A total of 189 patients with genotyping of β-thal and HbE/β-thal were included in this study. An informed consent form was presented to the patients or their guardians and their consent was obtained. The ethical clearance certificate and consent form regarding this research was approved by the Medical Ethics Committee, Faculty of Medicine, Jenderal Soedirman University, Central Java, Indonesia. Patient registration was done in Banyumas General Hospital (Purwokerto), in collaboration with the Thalassemia Foundation of Indonesia. The severity of β-thal and HbE/β-thal was classified based on the Mahidol score. The clinical features considered included the first time thalassemic symptoms appeared, the first time

thalassemia was diagnosed, the first time of transfusion, Hb pretransfusion, periodicity of transfusion, and enlargement of the spleen. The details on Mahidol score can be obtained elsewhere [15]. The phenotypic score ranged between 0 (for asymptomatic value) and 10 (for the worst appearance). For calculating the erythrocytes index and for performing the Hb electrophoresis test, we used XT 2000 (Sysmex, Lincolnshire, IL, USA), BioRad VARIANT Hemoglobin Analyzer (Hercules, CA, USA), and Sebia CAPILLARYS 2 Flex (Évry, Essonne, France). Pretransfusion Hb sample was collected from patients for 3 years. Polymorphism of the *XmnI* locus, rs11886868, rs766432 (*BCL11A*), and rs9399137 (*HBS1L-MYB*) was determined using polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) and amplification refractory mutation system (ARMS) methods. Details of primers and enzymes used in this experiment are presented in Table 1. DNA electrophoresis was carried out on a 2.5% acrylamide gel. The DNA illumination band was presented as follows: in the ARMS examination of rs766432, a T allele was depicted as having 135 bp, whereas a C allele was depicted as having 116 bp. In the ARMS examination of rs9399137, a T allele was depicted as having 243 bp, and the C allele was depicted as having 178 bp. In the RFLP examination of rs11886868 with the *Mbo*II enzyme, the T and C alleles were indicated as having 540 bp and 478 bp, respectively. The *XmnI* locus alleles were identified as having 214 bp and 137 bp for the (+) allele, whereas the 51-bp, undigested PCR product was identified as the (−) allele.

A Hardy—Weinberg equilibrium (HWE) distribution was established on each allele and the genetic associations of single-nucleotide polymorphisms (SNPs) and clinical appearance of HbF were analyzed through a linear regression model using SNPStats, which is available online from Barcelona University, Barcelona, Spain [16]. An independent *t* test was used to determine whether there were any differences between the HbF level distribution according to the presence or absence of minor allele frequency in the studied loci. A p value <.05 was considered statistically significant.

Ethical approval and informed consent

All procedures performed in this study were performed in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Certificate of Approval was obtained from the Ethical Committee of Faculty of Medicine, Jenderal Soedirman University, and Department of Education and Training, Banyumas Hospital, Central Java, Indonesia.

Informed consent was obtained from all individual participants included in this study. The informed consent form was approved by the Ethical Committee of the Faculty of Medicine, Jenderal Soedirman University.

Results

Hematological index

Hematological values based on the type of allele found are presented in Tables 2 and 3. The data obtained (Tables 2 and 3) indicate that the lowest pretransfusion Hb level presented in the β^0/β^0 alleles with the average of 7.4 g/dL [standard deviation (SD) 0.92]. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were also low in the β^0/β^0 alleles. The hemoglobin A_2 (HbA_2) levels in β-thal mutations were in the range of 3.22% and 5.24%, whereas in the HbA/β-thal population, the determination of HbA_2 appeared to increase with the average level of 40% (SD 11.49). This result was confirmed by the presence of HbE, which has the same retention time in the high-performance liquid chromatography machine. The percentage of HbF in either β-thal or HbE/β-thal patients increased from the normal value. The β-thal patients showed HbF levels that varied from 59.7% to 91%, especially in the β^0/β^0 genotypes with the highest value of HbF was 81%; by contrast, HbE/β-thal patients had an average HbF level of ∼29%. It was also found that HbE/β-thal patients have a better correlation with the HbF level and the degree of the clinical score appearance than β-thal patients (r = .49, p = .01), as depicted in Figure 1.

DNA transillumination

A typical band of DNA electrophoresis is shown in Figure 2. Amplicons from a primer designed for the PCR method were then digested with the appropriate enzymes. Positive

Table 1 Primers and enzymes used for polymerase chain reaction—restriction fragment length polymorphism and amplification refractory mutation system application.

Locus	Primers	Amplicons (bp)	Enzyme	Alleles interpretation
XmnI	FG5: 5′-GAGCTACAGACAAGAAGGTG-3′ RG4: 5′-TTTTATTCTTCATCCCTAGC-3′	351	*XmnI*	(+): 214, 137 (−): 351
rs11886868	F868: 5′-TTTGGTGCTACCCTGAAAGAC-3′ R868: 5′-ACTCAACAGTAGCAGAATGAAAGAG-3′	540	*MboII*	C: 478, 62 T: 540
rs766432	432(A)F: 5′-TTGTTTCGCTTTAGCTTTATTAAGGTACAA-3′ 432(A)R: 5′-GACGTGTTCTGTATCTTGATTTTGGT-3′	135	—	A: 135
	432(C)F: 5′-CCAAACAGTTTAAAGGTTACAGACAGACT-3′ 432(C)R: 5′-AAAATGAATGACTTTTGTTGTATGTAGAG-3′	116		C: 116
rs9399137	137(C)F: 5′-AATGTAATTAACTGAACATATGGTTAGTC-3′ 137(C)R: 5′-TTTATTGTTACAAGGTTAATTCACTGCC-3′	178	—	C: 178
	137(T)F: 5′-GAAATACCATCACTGAGAAAAGCATAAG-3′ 137(T)R: 5′-CAGCAGGGTTGCTTGTGAAAAAACTT TA-3′	234		T: 234

Table 2 Erythrocyte index and fetal hemoglobin level in various alleles of β-thalassemia patients.

Parameter	β^0/β^0 (=7)	β^0/β^+ (severe) (n = 30)	β^+ (severe)/β^+ (severe) (n = 31)	β^+/β^+ (severe) (n = 4)	HbE/β^+ (severe) (n = 86)	HbE/β^0 (n = 30)	p (F test)
Hb pretransfusion (g/dL)	7.40 ± 0.92	8.28 ± 0.53	8.36 ± 0.56	8.39 ± 0.42	8.28 ± 0.61	8.34 ± 0.49	.035
Mean corpuscular volume (fL)	73.23 ± 3.98	74.41 ± 4.45	78.51 ± 2.61	77.08 ± 3.96	79.05 ± 5.44	76.86 ± 4.00	.373
Mean corpuscular hemoglobin (pg)	24.22 ± 1.65	25.94 ± 1.56	24.59 ± 2.10	25.56 ± 1.30	26.32 ± 0.96	24.60 ± 2.20	.326
Hemoglobin A_2 (+HbE) (%)[a]	3.85 ± 1.01	3.85 ± 2.48	5.24 ± 2.91	3.22 ± 2.32	40.33 ± 11.49	40.77 ± 10.68	.005
Fetal hemoglobin (%)	91.81 ± 2.10	69.08 ± 30.68	78.27 ± 24.49	59.70 ± 27	29.00 ± 11.41	29.45 ± 11.04	.002

[a] Hemoglobin A_2 and Hemoglobin E (HbE) is on the same value (high-performance liquid chromatography Variant II).

Table 3 Alleles and genotypes percentage of *XmnI*, rs9399137, rs11886868, and rs766432.

Gene (chromosome)	Single-nucleotide polymorphism locus	Genotype			Allele			Hardy–Weinberg equilibrium (χ^2)[a]
		Type	Frequency	Proportion	Type	Frequency	Proportion	
HBG2 (11.p15.5)	*XmnI*	+/+	7	0.04	+	53	0.14	0.063
		−/+	39	0.21	−	325	0.86	
		−/−	143	0.76				
HBS1L-MYB (6.q23.3)	rs9399137	C/C	10	0.05	C	69	0.18	0.085
		T/C	49	0.26	T	309	0.82	
		T/T	130	0.69				
BCL11A (2.p16.2)	rs11886868	T/T	12	0.06	T	82	0.22	0.20
		C/T	58	0.31	C	296	0.78	
		C/C	119	0.63				
BCL11A (2.p16.2)	rs766432	C/C	11	0.06	C	72	0.19	0.058
		A/C	50	0.26	A	306	0.81	
		A/A	128	0.68				

[a] $\chi^2 > 0.05$ = alleles in the equilibrium.

digestion indicated the presence of polymorphisms at these sites. On the ARMS examination, a primer mismatch created for the PCR amplification process showed the base sequence polymorphism in accordance with the specific primer. A positive band corresponding to the specific primer showed the type of polymorphism in question.

SNPs distribution and phenotypic relationship

Genotype distribution, allele proportions, and HWE are determined using SNPStats software and depicted in Table 3. Positive alleles (+) of *XmnI* constituted 14% of the whole chromosomes examined, with a homozygosity (+/+) percentage of 4%. The major allele of the rs11886868 locus was C, accounting for 78% of the total chromosomes. By contrast, rs766432 and rs9399137 only had 19% and 18% of minor allele C. Population alleles of each locus showed HWE over 0.05.

Minor alleles at the polymorphism loci did not show a significant relationship to changes in the HbF concentration and the degree of clinical appearance of β-thal patients (Table 4). Nevertheless, the mean levels of HbF and the clinical scores of minor alleles showed a better value than the wild-type allele. In the HbE/β-thal group, the minor allele plays a role in increasing HbF concentrations mainly in the *XmnI* locus, rs11886868, and rs766432 (Table 4), whereas rs9399137 did not modify either the HbF level or phenotype appearance.

Regression analysis carried out to understand the impact of SNPs in shaping HbF levels in the HbE/β-thal patients showed that rs766432 polymorphism displayed a better coefficient (4.7%) in increasing the HbF level compared with

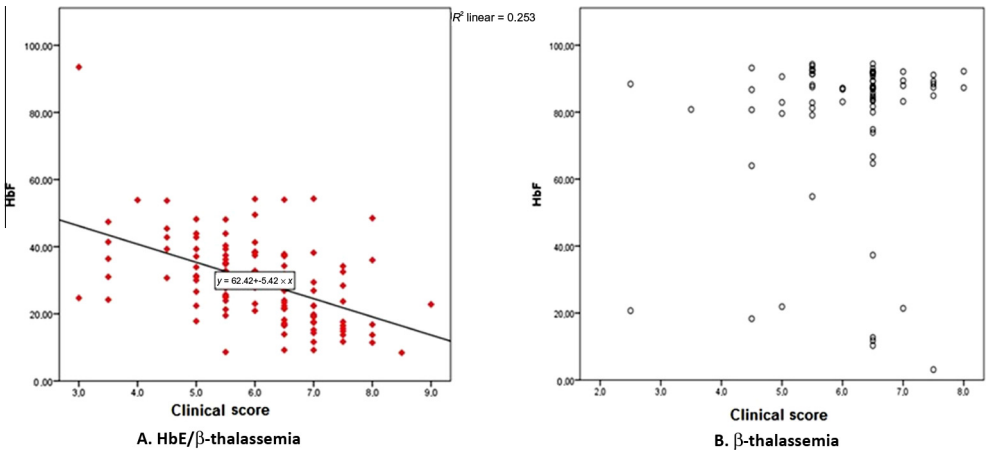

Figure 1 Relationship between fetal hemoglobin (HbF) level and clinical score in patients with β-thalassemia (β-thal) and hemoglobin E/β-thal (HbE/β-thal). HbE/β-thal patients provide a better correlation with HbF and clinical score association (Pearson r = .49 and p = .001), compared with β-thal patients (Pearson r = .01 and p = .98).

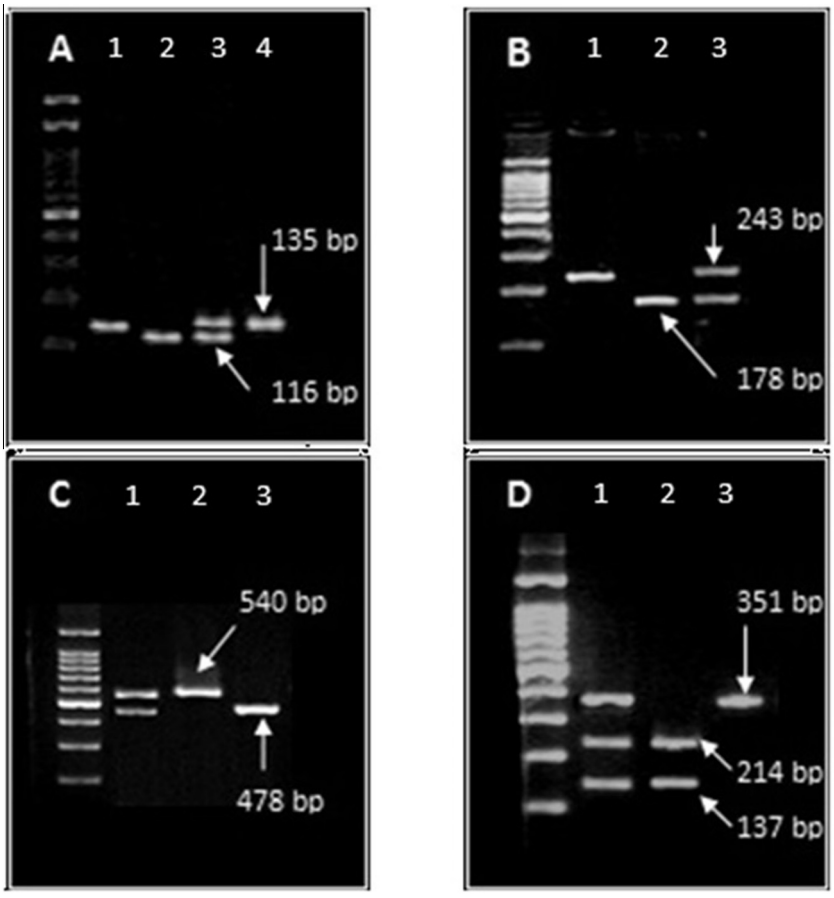

Figure 2 Transillumination DNA examination of *XmnI*, rs766432, rs11886868, and rs9399137. (A). Amplification refractory mutation system (ARMS) examination of rs766432: Bands 1 and 4 are homozygous AA, Band 2 is homozygous CC, and Band 3 is heterozygous AC. (B). ARMS examination of rs9399137: Band 1 is homozygous TT, Band 2 is homozygous CC, and Band 3 is heterozygous TC. (C). Restriction fragment length polymorphism (RFLP) examination of rs11886868 with the *Mbo*II enzyme. Band 1 is heterozygous TC, Band 2 is homozygous TT, and Band 3 is homozygous CC. (D). RFLP examination of *XmnI* locus with the *Xmn*I enzyme digestion. Band 3 shows the undigested polymerase chain reaction product reflecting the homozygous −/− genotype. Band 2 is homozygous +/+ and Band 1 is heterozygous −/+.

Table 4 Relationship between minor allele, fetal hemoglobin, and clinical presentation of β-thalassemia and hemoglobin E/β-thalassemia.

Gene (chromosome)	Locus	Genotype	Frequency	Mean		*t* test (*p*)	
				HbF (%)	Clinical score	Fetal hemoglobin	Clinical score
β-thal patients							
HBG2	*XmnI*	−/−	55	75.12	6.33	0.67	0.24
(11.p15.5)		−/+ + +/+	17	77.94	6.00		
HBS1L-MYB	rs9399137	TT	53	75.94	5.39	0.78	0.10
(6.q23.3)		TC + CC	19	77.75	6.33		
BCL11A	rs11886868	CC	43	78.15	6.23	0.71	0.15
(2.p16.2)		CT + TT	29	75.98	5.86		
BCL11A	rs766432	AA	55	75.56	5.47	0.70	0.17
(2.p16.2)		AC + CC	17	77.81	6.27		
HbE/β-thal patients							
HBG2	*XmnI*	−/−	88	31.32	6.15	0.01	0.02
(11.p15.5)		−/+ + +/+	29	38.42	5.58		
HBS1L-MYB	rs9399137	TT	82	32.22	6.23	0.17	0.44
(6.q23.3)		TC + CC	35	35.10	5.50		
BCL11A	rs11886868	CC	82	38.75	5.80	0.02	0.04
(2.p16.2)		CT + TT	35	30.66	6.31		
BCL11A	rs766432	AA	73	31.80	6.20	0.01	0.02
(2.p16.2)		AC + CC	44	35.21	5.69		

other polymorphisms. The determinant coefficient of the *XmnI* allele was 3.2%, whereas that of rs11886868 was 2.8%. Meanwhile, the C allele at rs9399137 showed no significant association ($p > .05$; Table 5).

Discussion

Hematological features

In patients with thalassemia, especially those with thalassemia intermedia and major, experts argue that the clinical situation is not always directly proportional to the specific type of mutation. Various factors affect the patient's clinical presentation. However, the International Thalassaemia Federation agreed that the clinical disturbance is greatly influenced by the degree of red blood cell indexes, primarily the Hb status [17]. The Hb status in this study, however, showed significant improvement during the study period, in both β-thal and HbE/β-thal patients. In the beginning, before commencing routine transfusion, the mean Hb levels were in the range of 4—6 g/dL (preliminary data, unpublished). In the later stages, however, this value increased up to 8 g/dL. This significant improvement is attributed to our multiple efforts to improve patient's quality of life, one of which is to maintain Hb levels above 9 g/dL with regular transfusions. This process significantly reduces hepatosplenomegaly due to extramedullary hematopoiesis, bone deformity, and enlargement of the heart [18].

Individuals with homozygous β-thal have similar Hb pretransfusion, MCV, and MCH values between the classification of the type of mutation (73 fL and 74 fL for a mutation containing the β^0 allele, and 77 fL and 78 fL for the β^+ mutation; Table 2). In general, patients have lower Hb and red blood cell indices than normal individuals. The Hb level in HbE/β^0-thalassemia patients showed slightly lower levels than the β^+ patients (severe). An extensive review conducted by the Gene Reviews team noted that regardless of the mutation, Hb, MCV, and MCH values in patients with thalassemia major are at very low levels. They noted that Hb level, in general, is <7 g/dL, MCV is between 50 fL and 70 fL, and MCH level is between 12 pg and 20 pg [18]. In line with these findings, a previous Indonesian study noted that Hb levels in patients with thalassemia in Semarang varied from 4.3 f/dL to 12.5 g/dL with a mean value of 7.8 g/dL [19], whereas in Jakarta and Bandung these values were 4.4—8.6 g/dL [20] and 7.3 g/dL [21], respectively.

In a previous study, the average Hb value of HbE/β-thal patients without the co-inheritance involving the deletion of α gene was 7.1 g/dL with an MCV below 60 fL [5]. Similar

Table 5 Regression value of *XmnI*, rs9399137, rs11886868, and rs766432 for the fetal hemoglobin level.

Gene (chromosome)	Locus	Allele	Allele frequency	Correlation (*r*)	Determinant coefficient (r^2)	(*p*)
HBG2 (11.p15.5)	*XmnI*	+	0.14	.18	.032	.028
HBS1L-MYB (6.q23.3)	rs9399137	C	0.18	.06	.004	.395
BCL11A (2.p16.2)	rs11886868	C	0.88	.17	.028	.042
BCL11A (2.p16.2)	rs766432	C	0.19	.19	.047	.039

data were presented by researchers in Sri Lanka, who illustrated that the Hb level in HbE/β-thal patients with no splenectomy or splenectomy is not much different, with an average value between 7.6 g/dL and 7.9 g/dL [22]. Data on Hb, MCV, and MCH in this study are slightly higher than the existing data presented in previous publications. This can be explained as follows: all patients in Banyumas, regardless of the type of mutation, have been receiving a regular blood transfusion to achieve Hb levels of over 10 g/dL. In addition, the patients were asked to return to the hospital within 3–4 weeks for multiple rounds of transfusion until this goal is achieved. It is to be noted that patients with mutations in β^+ may still have better pretransfusion Hb level than those with β^+ mutations.

Patients with the β^0/β^0 alleles showed the highest level of HbF (91.81% ± 2.10%), whereas those with β^+/β^+ alleles had HbF levels of 59.70% ± 27% [23]. These data indicate that that β-thal patients have a wide range of HbF concentration, which ranges from 50% to 98% under various circumstances (i.e., based on the type of mutation). Another study reported that homozygous or compound heterozygous mutations in β-thal patients produce HbF in the range of 70–90% [18]. Our study patients presented HbF levels in the range of 80–98% (β^0/β^0), whereas the other allele group (β^+/β^+) showed a highly variable level ranging from 50% to 98%. This high value represents the inability of the cell to produce the β-globin chain.

In the HbE/β-thal group, the HbF degree increased in both β^+ (29.00% ± 11.41%) and β^0 alleles (29.45% ± 11.04%). The increased level of HbF in HbE/β-thal patients is a response to, or a compensation for, the reduced levels of HbA and HbE to varying degrees due to cryptic mutations. There are at least two basic mechanisms that play a role in this regard: maintaining a high number of gamma-globin chain and increasing the rate of erythropoiesis [24]. In a subsequent study, large numbers of gamma globin chain were noted due to suppression or variations in *BCL11A* protein-coding genes and other transcription factors, including *EKLF* and *GATA1* [25]. With regard to the other mechanism, overexpression of erythropoiesis increases the concentration of erythrocyte F cells in peripheral blood circulation [26].

In our study, the range of HbA_2 in β-thal patients was 3.22–5.23%. According to a systematic review, this level was in the normal range (2–5%) [18]. The HbE/β-thal patients, however, had HbA_2 levels of ∼40%. This high concentration is actually the resultant value of HbE and HbA_2, both of which have the same electrophoresis zone in the high-performance liquid chromatography method [27,28]. These increased levels (i.e., ∼40%) are in accordance with previous studies that found levels of 37–71% [29] and 30–70%, respectively [5]. From these values, it can be concluded that HbA_2 levels in HbE/β-thal patients vary between 3% and 6%. Another report indicated that that the HbA_2 level in HbE/β-thal patients is in the range of 4–4.5% [30].

Relationship between SNPs distribution and clinical appearance

The *XmnI* locus in our patients included 14% minor allele, which is within the scope of other Asian distribution studies, which reported the range of minor allele in this locus to be 8–36%. A study in Pakistan reported that a minor allele in the *XmnI* locus has a frequency of 23–36% [31], whereas this was reported to be 16% [32] and 8% [33] in Indian and Malay populations, respectively. In accordance with the data from the HapMap project (updated 2012), the rs11886868 genotype has a wide distribution that varies in populations throughout the world. Major CC alleles are widespread in the Asian population, including China and Japan (80–95%), whereas the TT alleles are most widely documented in the populations of Europe and Latin American plains [34]. The distribution of rs11886868 genotype in this study has a similar frequency with the populations of China and Thailand, as reported by Pakdee and colleagues [35]. The rs766432 allele distribution in this study followed the pattern of alleles seen in almost all of the world's populations. Various populations, including whites in Europe, the population of white Americans, and Asian populations (e.g., China, Japan, and India), indicate that the major allele of A has a frequency of 51–88%. The rs9399137 genotype had a similar distribution in various ethnic groups. European populations presented genotypes of CC, CT, and TT as follows: 4.5%, 31.2%, and 64.3%, respectively. The Asian population, however, shows a diverse variation: 2.4–12.8% for CC, 38–42.9% for CT, and 48–54% for TT [34].

Quantitative trait locus either on the *XmnI* locus, rs9399137, rs11886868, or rs766432 in β-thal patients showed no significant relationship, in either the HbF level or clinical degree. There are two possibilities to explain this condition. Almost all allele mutations in β-thal patients were a combination of β^0 and β^+ (severe). By contrast, the HbF and clinical appearance of these patients were pooled in the severe-type condition. In HbE/β-thal patients, there were three SNPs, namely, *XmnI*, rs11886868, and rs766432, which were significantly associated with either HbF levels or the degree of clinical patients. Nevertheless, the rs9399137 genotype did not show a similar relationship.

XmnI polymorphism has a coefficient determinant of 3.2% ($p = .028$; Table 5). This number is in the moderate value range compared with a previous study, in which SNP rs7482144 (*XmnI* locus) explained 2.2% of the variation in HbF production for SCD cases [36]. A higher variation was found in the study of Chinese populations (9%) [37] and in a European study (10.2%) [38].

The rs766432 and rs11886868 loci are the SNPs in *BCL11A* genes that are known to be associated with increasing HbF levels. The gene is a kind of regulator responsible for the transition of HbF into HbA [39,40]. Studies on populations in Europe and Central Asia showed that rs11886868 had a strong relationship with the increase in HbF, in both normal individuals and thalassemia patients [12,13,41]. In a previous study, a group of researchers considered that rs766432 had a better relationship with the percentage of HbF in various cases of hemoglobinopathies in Thai, Chinese, and African American populations [42]. The SNP rs766432 was also subsequently found to play a role in other Thai populations [43]. Thus, our finding is in line with previous studies confirming that SNP rs766432 has a better correlation with increasing HbF levels among thalassemia patients. SNP rs9399137, however, neither significantly correlated with HbF concentrations nor with the degree of clinical presentations. These findings are thus not consistent with the data

previously reported, such as those on European [11], Brazilian, or African-American populations [13,36], which reported the effect of variation of rs9399137 on HbF concentration. However, when considering other genetic epidemiology studies, our data are in accordance with those identified in several research centers, including data from studies in Hong Kong [44], Cameroon [45], and the Middle East [46]. These different results between cross studies demonstrate the fact that the influence of SNPs can be different among different regions, reflecting a link between ethnicity and other genetic susceptibility factors.

The combination of the four alleles studied indicated that the −CAT allele is a common allele in this population. In another section, the +CCC alleles provide a good explanation of the change in HbF levels compared with the other allele population. The combination of these alleles has a determinant value of 10.7% against HbF changes in HbE/β-thal patients. Similar findings were reported by Banan et al. [47] who reported that the +CC allele for *XmnI*, rs766432, and rs9399137 showed a good response to changes in HbF in patients with thalassemia intermedia receiving hydroxyurea supplementation. In addition, the combination of alleles +CCC contributed to increase HbF levels (about 17.5%) among SCD patients in African populations [48]. The findings of genetic epidemiology in this study complement previous data indicating that variations of SNP loci in the *XmnI* site, *BCL11A*, and *HBS1L-MYB* are specific to a particular geographical area, although this is not a causal relationship.

Conclusion

We have herein reported that SNPs of the *XmnI* site, rs766432, and rs11886868 have a relationship with HbF levels and the clinical appearance among HbE/β-thal patients in an Indonesian cohort, whereas rs9399137 did not modify either HbF or clinical presentation. This study also reported that the SNPs had no correlation with either the HbF level or the clinical presentation of β-thal patients.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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