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Genotyping of *BCL11A* and *HBS1L-MYB* Single Nucleotide Polymorphisms in β-thalassemia/HbE and Homozygous HbE Subjects with Low and High Levels of HbF

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Abstract

Whether multiple single nucleotide polymorphisms (SNPs) of *BCL11A* and *HBS1L-MYB* genes affect hemoglobin (Hb)F production and hematological parameter variation in β-thalassemia/HbE and homozygous HbE in Thai subjects with low and high HbF levels are still unclear. Three SNPs of *BCL11A* gene (rs1427407, rs10189857 and rs11886868) and 3 SNPs of *HBS1L-MYB* gene (rs4895441, rs9399137 and rs28384513) were analyzed in 45 β-thalassemia/HbE patients who had HbF levels lower and higher than 15 %, and in 50 homozygous HbE who had HbF levels lower and higher than 5 %. Their hematological parameters were measured using automated blood counter. The HbF level was analyzed using high performance liquid chromatography (HPLC). There were no statistical significant differences of allele and genotype frequencies of 3 SNPs in the *BCL11A* gene between the groups of β-thalassemia/HbE patients and homozygous HbE subjects with low and high HbF levels. Significant differences in the allele frequencies in *HBS1L-MYB* SNP rs4895441 (p = 0.041) and rs9399137 (p = 0.048) were observed in homozygous HbE subjects with HbF ≤ 5 % and > 5 %. Moreover, significant differences in MCV (p = 0.005) and trends towards significant differences in MCH (p = 0.057) and HbF levels (p = 0.051) were found in *HBS1L-MYB* SNP rs9399137 of homozygous HbE subjects. Therefore, the *HBS11L-MYB* SNPs especially rs9399137 had an effect on HbF production and the variation of hematological parameters in homozygous HbE subjects, but not in β-thalassemia/HbE patients.

Keywords: β-thalassemia/Hb disease, HbF, homozygous HbE, *BCL11A*, *HBS1L-MYB*

Introduction

The β-thalassemia is an autosomal genetic disorder resulting in decreased (β^+-thalassemia) or completely absent (β^0-thalassemia) production of the normal β-globin chain and is associated with increased levels of hemoglobin (Hb) A_2 and slightly increased of HbF [1]. Point mutations, small deletions or insertions in the nucleotide sequences are mainly responsible for the molecular defect of β-thalassemia around the world [2]. Homozygous β^0-thalassemia and β-thalassemia/HbE disease are the most common form of β-thalassemia syndrome in South-East Asia [3]. Moreover, one of the most common hemoglobinopaties due to point mutation in the β-globin gene is known as HbE [β26 (B8) Glu→Lys, GAG>AAG], which induces alternative splicing and thus results in a decreased β^E-globin

chain [3]. The HbE trait has no clinical significance while homozygous HbE individuals are asymptomatic with very mild anemia and microcytosis [4]. In contrast, β-thalassemia/HbE disease has a widely disparate range of clinical and hematological parameters, ranging from mild and asymptomatic anemia to transfusion dependent [5].

One of the major genetic factors that influence the clinical phenotype and anemia severity in β-thalassemia is the persistence of HbF production [6,7]. Several studies have presented evidence of single nucleotide polymorphisms (SNPs) which influence γ-globin gene expression, HbF production and erythropoiesis. Especially, the SNPs in 3 loci, including *HBS1L-MYB* intergenic region on chromosome 6q23, *BCL11A* on chromosome 2p16, and the β-globin cluster on chromosome 11 (*Xmn*1-^{G}γ polymorphism) have been identified to be associated with the elevation of HbF levels in β-thalassemia/HbE disease or HbE trait [8-11]. The variation in HbF level is likely to be a factor related to heterogeneity in clinical severity in β-thalassemia/HbE disease. In general, most β-thalassemia/HbE patients have a high level of HbF (≥ 15 %), whereas others have a low HbF level (< 15 %) [12,13]. In addition, homozygous HbE individual has variability of HbF level; ranging from 0 - 15 % [9,14]. However, the HbF level in homozygous HbE is usually less than 5 % [12]. The previous study presented that *BCL11A* and *HBS1L-MYB* SNPs were not correlated with HbF level and also suggested that *BCL11A* and *HBS1L-MYB* loci had a minor effect on HbF level when compared to the *Xmn*1-^{G}γ polymorphism in β-thalassemia intermedia patients [15]. Our previous study showed significant difference in the frequency of *Xmn*1-^{G}γ polymorphism between the 2 groups of β-thalassemia/HbE patients with HbF < 15 % and ≥ 15 %. Whereas, there was no significant difference of those between the 2 groups of homozygous HbE patients with HbF ≤ 5 % and > 5 %. Thus, the *Xmn*1-^{G}γ polymorphism may be one factor that influences the production of HbF in β-thalassemia/HbE patients but not in homozygous HbE subjects [16]. However, only the *Xmn*1-^{G}γ polymorphism could not improve clinical symptoms. Pakdee *et al*. reported that 4 SNPs of *Xmn*1-^{G}γ (rs7482144), *HBS1L-MYB* (re4895441), *HBS1L-MYB* (re9399137), and *BCL11A* (rs4671393) were associated with a high HbF level (> 5 %) in homozygous HbE Thai subjects [9]. Therefore, in the current study, we determined the influence of the *BCL11A* and *HBS1L-MYB* SNPs with HbF production and hematological parameter variation in β-thalassemia/HbE patients and homozygous HbE subjects with low and high HbF levels.

Materials and methods

Subjects and hematological analysis

Subjects and hematological data were from our previous study [16]. Hematological parameters were measured with an automated blood counter (Sysmex KX-21; Sysmex Corporation, Kobe, Japan). Quantification of HbA_2 (for detection of β-thalassemia), HbF and identification of hemoglobinopathies including HbE was performed using high performance liquid chromatography (HPLC, VARIANT II, β-thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). Blood samples were diagnosed as β-thalassemia trait when the level of HbA_2 was 4 - 9.9 %, HbE trait when the level of HbA_2/E was 10 - 29.9 %, β-thalassemia/HbE when levels of HbA_2/E and HbF were 30 - 60 % and ≥ 15 %, respectively and homozygous HbE when levels of HbA_2/E and HbF were > 65 % and < 5 %, respectively [12,17]. Samples containing HbE > 65 % and HbF levels varying from 5 to 15 % were further discriminated for β-thalassemia/HbE and homozygous HbE by molecular analysis systems. This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.

Molecular analysis for thalassemia diagnosis

DNA was extracted from EDTA blood samples using the NucleoSpin® kit (Macherey-Nagel, KG, Duren, Germany) according to manufacturer's instructions. DNA extracts were stored at –20 °C until used. The diagnosis for α-thalassemia-1 South-East Asian (SEA) and Thai type deletion was detected using real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis as previously described [18,19]. The β-thalassemia codons 71/72 (+A), 41/42 (-TCTT), 17 (A>T) and IVSI-nt1 (G>T)

mutations were analyzed by the multiplex amplification refractory mutation system (MARMS)-PCR according to the protocol described previously [20,21]. Moreover, the amplification refractory mutation system (ARMS)-PCR was performed in order to provide a rapid molecular diagnosis for heterozygous and homozygous HbE as previously described [12].

Molecular analysis for single nucleotide polymorphisms

Analysis of 6 SNPs including *HBS1L-MYB* rs4895441 (A-G) and rs28384513 (A-C) and *BCL11A* rs1427407 (G-T), rs10189857 (A-G) and rs11886868 (C-T) was performed using real-time PCR on the LightCycler® 480 (Roche Diagnostics, USA) and *HBS1L-MYB* (rs9399137) (T-C) was determined using direct sequencing on a ABI Prism 3130 XL (Applied Biosystems, Courtaboeuf, France) as previously described [22].

Statistical analysis

Statistical analyses were performed using the SPSS software package (Statistical Package for the Social Sciences 11.0, Chicago, IL, USA). The normal distribution of the quantitative data was tested in each group of β-thalassemia/HbE disease and homozygous HbE by using Kolmogorov-Smirnov test. The hematological parameters and HbF level were presented as mean and standard deviation (SD). The differences of the allele and genotype frequencies of SNPs between 2 groups of subjects were determined using the Fisher's exact test. Differences in mean hematological parameter and HbF level of 3 genotypes of each SNP were analyzed using the Kruskal-Wallis test. Significance was determined as $p < 0.05$. All reported *p*-values were 2-sided.

Results and discussion

SNPs distribution

Tables 1 and **2** list the major and minor alleles and genotype frequencies found for 6 analyzed SNPs of 45 β-thalassemia/HbE patients and 50 homozygous HbE subjects, respectively. Population alleles of each locus between the 2 groups of subjects were similar and they showed Hardy-Weinberg equilibrium (HWE) over 0.05.

Comparison of *HBS1L-MYB* SNP haplotypes between β-thalassemia/HbE patients and homozygous HbE subjects with low and high HbF levels

There are no significant differences in the frequencies of allele and genotype of all analyzed SNPs between the β-thalassemia/HbE patients with HbF < 15 % and ≥ 15 % (**Table 3**). Moreover, the frequencies of allele and genotype of 3 SNPs in *BCL11A* gene were not statistically significantly different between the groups of homozygous HbE subjects with HbF ≤ 5 % and > 5 % (**Table 4**). Among 3 SNPs in *HBS1L-MYB* gene, the significant differences of the allele frequencies in rs4895441 ($p = 0.041$) and rs9399137 ($p = 0.048$) were observed in homozygous HbE subjects with HbF ≤ 5 % and > 5 % while trends to significant differences in the genotype frequencies of these 2 SNPs, rs4895441 ($p = 0.050$) and rs9399137 ($p = 0.056$), were also found in these 2 subject groups (**Table 4**). In addition, there was significant difference in the genotype frequency in rs28384513 ($p = 0.043$) between the groups of homozygous HbE subjects with HbF ≤ 5 % and > 5 % (**Table 4**).

Association of *HBS1L-MYB* SNP haplotypes with hematological parameters of β-thalassemia/HbE patients and homozygous HbE subjects

The β-thalassemia/HbE patients had no significant differences in levels of all assessed hematological parameters (RBC, Hb, Hct, MCV, MCH, and MCHC) and HbF among the 3 genotypes of each SNP in *BCL11A* and *HBS1L-MYB* gene (**Table 5**). The homozygous HbE subjects also had no significant differences in levels of all assessed hematological parameters and HbF among the 3 genotypes of each SNP in the *BCL11A* gene (**Table 6**). However, significant differences in the levels of Hct were found in *HBS1L-MYB* SNP rs4895441 ($p = 0.037$). Moreover, the MCV among the 3 genotypes of

HBS1L-MYB SNPs rs4895441 (p = 0.007), rs9399137 (p = 0.005) and rs28384513 (p = 0.032) was significantly different (**Table 6**). Furthermore, the significant difference of MCH (p = 0.020) was found in only *HBS1L-MYB* SNP rs28384513 while trends to significant differences in MCH (p = 0.057) and HbF levels (p = 0.051) were found in *HBS1L-MYB* SNP rs9399137 (**Table 6**).

Table 1 The allele and genotype frequencies of each single nucleotide polymorphism (SNP) among 45 β-thalassemia/HbE patients.

SNPs		Allele	Frequency (%)	Genotype	Frequency (%)	HWE equilibrium (X^2 test)[a]
Bcl11A	rs1427407 (G-T)	G	78.9	GG	62.2	0.996
		T	21.1	GT	33.3	
				TT	4.4	
	rs10189857 (A-G)	A	21.1	AA	4.4	0.996
		G	78.9	AG	33.3	
				GG	62.2	
	rs11886868 (C-T)	C	94.4	CC	88.9	0.693
		T	5.6	CT	11.1	
				TT	-	
HBS1L-Myb	rs4895441 (A-G)	A	82.2	AA	68.9	0.556
		G	17.8	AG	26.7	
				GG	4.4	
	rs9399137 (T-C)	T	85.6	TT	73.3	0.941
		C	14.4	TC	24.4	
				CC	2.2	
	rs28384513 (A-C)	A	71.6	AA	47.7	0.250
		C	28.4	AC	47.7	
				CC	4.5	

[a]X^2 test to check the Hardy-Weinberg equilibrium (HWE). A p-value > 0.05 means that population meets the assumption of the HWE.

Table 2 The allele and genotype frequencies of each SNP among 50 homozygous HbE subjects.

SNPs		Allele	Frequency (%)	Genotype	Frequency (%)	HWE equilibrium (X^2 test)[a]
Bcl11A	rs1427407 (G-T)	G	86.0	GG	74.0	0.981
		T	14.0	GT	24.0	
				TT	2.0	
	rs10189857 (A-G)	A	16.0	AA	2.0	0.981
		G	84.0	AG	28.0	
				GG	70.0	
	rs11886868 (C-T)	C	90.0	CC	82.0	0.432
		T	10.0	CT	16.0	
				TT	2.0	
HBS1L-Myb	rs4895441 (A-G)	A	81.0	AA	64.0	0.460
		G	19.0	AG	34.0	
				GG	2.0	
	rs9399137 (T-C)	T	79.0	TT	60.0	0.304
		C	21.0	TC	38.0	
				CC	2.0	
	rs28384513 (A-C)	A	73.0	AA	54.0	0.799
		C	27.0	AC	38.0	
				CC	8.0	

[a]X^2 test to check the HWE. A P-value > 0.05 means that population meets the assumption of the HWE.

Table 3 The allele and genotype frequencies of each SNP in β-thalassemia/HbE patients with low and high HbF levels.

SNPs		Allele	Frequency (n, %)		p-value[a]	Genotype	Frequency (n, %)		p-value[a]
			HbF < 15 %	HbF ≥ 15 %			HbF < 15 %	HbF ≥ 15 %	
Bcl11A	rs1427407 (G-T)	G	26 (86.7%)	45 (75.0%)	0.276	GG	11 (73.3%)	17 (56.7%)	0.471
		T	4 (13.3%)	15 (25.0%)		GT	4 (26.7%)	11 (36.7%)	
						TT	-	2 (6.7%)	
	rs10189857 (A-G)	A	4 (13.3%)	15 (25.0%)	0.276	AA	-	2 (6.7%)	0.471
		G	26 (86.7%)	45 (75.0%)		AG	4 (26.7%)	11 (36.7%)	
						GG	11 (73.3%)	17 (56.7%)	
	rs11886868 (C-T)	C	28 (93.3%)	57 (95.0%)	1.000	CC	13 (86.7%)	27 (90.0%)	1.000
		T	2 (6.7%)	3 (5.0%)		CT	2 (13.3%)	3 (10.0%)	
						TT	-	-	

SNPs		Allele	Frequency (n, %)		*p*-value [a]	Genotype	Frequency (n, %)		*p*-value [a]
			HbF < 15 %	HbF ≥ 15 %			HbF < 15 %	HbF ≥ 15 %	
HBS1L-Myb	rs4895441	A	25 (83.3%)	49 (81.7%)	1.000	AA	10 (66.7%)	21 (70.0%)	0.643
	(A-G)	G	5 (16.7%)	11 (18.3%)		AG	5 (33.3%)	7 (23.3%)	
						GG	-	2 (6.67%)	
	rs9399137	T	26 (86.7%)	51 (85.0%)	1.000	TT	11 (73.3%)	22 (73.3%)	1.000
	(T-C)	C	4 (13.3%)	9 (15.0%)		TC	4 (26.7%)	7 (23.3%)	
						CC	-	1 (3.3%)	
	rs28384513	A	20 (71.4%)	43 (71.7%)	1.000	AA	6 (42.9%)	15 (50.0%)	0.663
	(A-C)	C	8 (28.6%)	17 (28.3%)		AC	8 (57.1%)	13 (43.3%)	
						CC	-	2 (6.7%)	

[a]Fisher's exact test

Table 4 The allele and genotype frequencies of each SNP in homozygous HbE subjects with low and high HbF levels.

SNPs		Allele	Frequency (n, %)		*p*-value [a]	Genotype	Frequency (n, %)		*p*-value [a]
			HbF ≤ 5%	HbF > 5%			HbF ≤ 5%	HbF > 5%	
Bcl11A	rs1427407	G	46 (85.2%)	40 (87.0%)	1.000	GG	19 (70.4%)	18 (78.2%)	0.413
	(G-T)	T	8 (14.8%)	6 (13.0%)		GT	8 (29.6%)	4 (17.4%)	
						TT	-	1 (4.4%)	
	rs10189857	A	9 (16.7%)	7 (15.2%)	1.000	AA	-	1 (4.4%)	0.439
	(A-G)	G	45 (83.3%)	39 (84.8%)		AG	9 (33.3%)	5 (21.7%)	
						GG	18 (66.7%)	17 (73.9%)	
	rs11886868	C	49 (90.7%)	41 (89.1%)	1.000	CC	22 (81.5%)	19 (82.6%)	0.571
	(C-T)	T	5 (9.3%)	5 (10.87%)		CT	5 (18.5%)	3 (13.0%)	
						TT	-	1 (4.4%)	
HBS1L-Myb	rs4895441	A	48 (88.9%)	33 (71.7%)	0.041	AA	21 (77.8%)	11 (47.8%)	0.050
	(A-G)	G	6 (11.1%)	13 (28.3%)		AG	6 (22.2%)	11 (47.8%)	
						GG	-	1 (4.4%)	
	rs9399137	T	47 (87.0%)	32 (69.6%)	0.048	TT	20 (74.1%)	10 (43.5%)	0.056
	(T-C)	C	7 (13.0%)	14 (30.4%)		TC	7 (25.9%)	12 (52.1%)	
						CC	-	1 (4.4%)	
	rs28384513	A	41 (75.9%)	32 (69.6%)	0.505	AA	14 (51.8%)	13 (56.5%)	0.043
	(A-C)	C	13 (24.1%)	14 (30.4%)		AC	13 (48.2%)	6 (26.1%)	
						CC	-	4 (17.4%)	

[a]Fisher's exact test

Table 5 Hematological parameters and HbF levels among 45 β-thalassemia/HbE patients in each genotype of SNPs.

	SNPs	Genotype	No.	Hematological parameters													
				RBC ($x10^6/mm^3$)		Hb (g/dL)		Hct (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)		HbF (%)	
				Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]
Bcl11A	rs1427407	GG	28	4.4±0.9	0.520	7.6±1.3	0.252	24.9±4.1	0.526	57.0±7.7	0.407	17.3±2	0.297	30.6±2.8	0.496	20.3±11.3	0.151
	(G-T)	GT	15	4.4±1.2		8.0±1.7		26.0±5.5		60.4±6.0		18.5±2		30.6±2.1		25.5±14.1	
		TT	2	3.7±0.2		6.5±0.0		22.5±0.7		61.0±5.7		17.6±1.1		28.9±0.9		34.2±5.1	
	rs10189857	AA	2	3.7±0.2	0.520	6.5±0.0	0.252	22.5±0.7	0.526	61.0±5.7	0.407	17.6±1.1	0.297	28.9±0.9	0.496	34.2±5.1	0.151
	(A-G)	AG	15	4.4±1.2		8.0±1.7		26.0±5.5		60.4±6.0		18.5±2		30.6±2.1		25.5±14.1	
		GG	28	4.4±0.9		7.6±1.3		24.9±4.1		57.0±7.7		17.3±2		30.6±2.8		20.3±11.3	
	rs11886868	CC	40	4.4±1.0	0.327	7.7±1.4	0.506	25.2±4.5	0.868	57.7±7.3	0.057	17.6±1.9	0.542	30.7±2.3	0.284	22.4±12.3	0.551
	(C-T)	CT	5	4.0±0.9		7.2±1.5		25.1±5.4		62.9±2.4		18.3±2.9		29.0±4.0		25.0±14.4	
		TT	-	-		-		-		-		-		-		-	
HBS1L-Myb	rs4895441	AA	31	4.3±0.9	0.630	7.4±1.3	0.087	24.4±4.2	0.118	57.8±7.5	0.262	17.5±2.0	0.181	30.4±2.6	0.878	22.9±12.5	0.374
	(A-G)	AG	12	4.5±1.1		8.0±1.5		26.1±5.1		58.6±6.4		18.0±1.9		30.8±2.4		20.4±12.8	
		GG	2	4.8±0.4		9.6±0.7		31.0±1.4		65.0±2.8		20.1±0.3		30.9±0.9		32.0±5.9	
	rs9399137	TT	33	4.4±1.0	0.662	7.5±1.3	0.156	24.8±4.3	0.301	57.8±7.4	0.585	17.4±2.0	0.217	30.3±2.6	0.519	22.8±12.7	0.476
	(T-C)	TC	11	4.4±1.0		8.0±1.5		25.8±5.1		59.5±6.6		18.5±1.8		31.1±2.3		21.2±11.9	
		CC	1	5.1		10.1		32.0		63.0		19.9		31.6		36.2	
	rs28384513	AA	21	4.2±0.8	0.511	7.3±1.2	0.137	24.2±3.9	0.193	58.7±7.8	0.513	17.5±2.0	0.866	30.0±2.9	0.229	23.4±13.1	0.984
	(A-C)	AC	21	4.6±1.1		8.1±1.5		26.7±4.9		58.8±6.6		17.9±2.1		30.6±2.0		22.5±12.6	
		CC	2	4.2±0.1		7.5±0.8		22.5±2.1		54.1±4.2		17.9±1.6		33.1±0.3		23.1±2.3	

[a]Kruskal-Wallis test

Table 6 Hematological parameter and HbF levels among 50 homozygous HbE subjects in each genotype of SNPs.

SNPs		Genotype	No.	Hematological parameters													
				RBC ($x10^6/mm^3$)		Hb (g/dL)		Hct (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)		HbF (%)	
				Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]
Bcl11A	rs1427407	GG	37	5.6±0.9	0.479	11.6±1.5	0.416	36.6±5.5	0.672	65.4±5.9	0.177	20.7±1.8	0.516	31.8±1.6	0.229	4.8±2.6	0.194
	(G-T)	GT	12	5.6±0.7		11.2±1.6		35.5±6		63.5±6.9		20.0±1.8		31.6±1.3		3.9±1.5	
		TT	1	4.9		9.8		40.0		82.3		20.2		24.5		9.0	
	rs10189857	AA	1	4.9	0.477	9.8	0.436	40.0	0.708	82.3	0.223	20.2	0.710	24.5	0.201	9.0	0.168
	(A-G)	AG	14	5.6±0.7		11.2±1.5		35.8±5.7		64.2±6.6		20.2±1.7		31.5±1.2		3.9±1.6	
		GG	35	5.6±0.9		11.6±1.6		36.5±5.6		65.3±6		20.7±1.8		31.8±1.7		4.9±2.6	
	rs11886868	CC	41	5.6±0.8	0.661	11.4±1.6	0.689	36.2±5.9	0.842	64.8±6.9	0.387	20.4±1.8	0.284	31.6±2.0	0.520	4.7±2.5	0.879
	(C-T)	CT	8	5.7±0.8		11.9±1.2		37.7±4.2		66.9±4.7		21.2±1.4		31.7±1.2		4.7±2.2	
		TT	1	5.1		11.2		36.6		71.9		22.0		30.6		5.3	
HBS1L-Myb	rs4895441	AA	32	5.6±0.8	0.974	11.2±1.7	0.197	35±6	0.037	63.2±6.1	0.007	20.2±1.8	0.118	32.1±1.7	0.074	4.1±2	0.077
	(A-G)	AG	17	5.7±0.8		11.9±1.2		38.8±3.7		68.9±5.8		21.1±1.6		30.6±1.8		5.5±2.9	
		GG	1	5.5		12.4		40.0		73.0		22.6		31.0		8.3	
	rs9399137	TT	30	5.6±0.9	0.961	11.2±1.7	0.288	35.1±6.1	0.090	62.9±6.2	0.005	20.1±1.8	0.057	32.1±1.7	0.162	4.1±1.9	0.051
	(T-C)	TC	19	5.6±0.8		11.8±1.3		38.3±4.0		68.7±5.5		21.1±1.6		30.8±1.8		5.5±2.8	
		CC	1	5.5		12.4		40.0		73.0		22.6		31.0		8.3	
	rs28384513	AA	17	5.6±0.8	0.541	11.4±1.6	0.642	36.5±5.7	0.685	65.3±6.7	0.032	20.4±1.7	0.020	31.4±2.2	0.651	5.1±2.8	0.123
	(A-C)	AC	19	5.6±0.9		11.4±1.6		35.8±5.8		63.8±6.3		20.3±1.7		31.9±1.4		3.8±1.7	
		CC	4	5.3±0.4		12.1±0.9		38.7±2.8		72.5±0.6		22.7±0.5		31.3±0.6		6.1±1.5	

[a]Kruskal-Wallis test

Discussion

The persistence of HbF level suggests that variability is not from only one of the β-globin gene clusters, but from a combination of several gene loci on different chromosomes. The expression of HbF is associated with the presence of 3 main quantitative trait loci (QTL) including the *Xmn*1-$^{G}\gamma$ polymorphism on chromosome 11 (11p15), *HBS1L-MYB* locus on chromosome 6 (6p23) and the *BCL11A* gene on chromosome 2 (2q16) [8,23]. Our previous study [16] showed that the *Xmn*1-$^{G}\gamma$ polymorphism is likely to be one factor that influences HbF production in β-thalassemia/HbE patients, but not in homozygous HbE subjects and the results suggested that the other polymorphisms might play a major role in ameliorating the severity of anemia and hematological parameter variation. Thus, in the current study, we analyzed the *BCL11A* and *HBS1L-MYB* SNPs with HbF production and hematological parameters variation in β-thalassemia/HbE patients and homozygous HbE individuals with low and high HbF levels. The results showed that the polymorphic SNPs in *BCL11A* and *HBS1L-MYB* gene did not affect the HbF production in β-thalassemia/HbE patients. This is consistent with the study of Nguyen *et al.* [15] who also found a strong correlation between HbF expression in the Mediterranean β-thalassemia patients with only *Xmn*1-$^{G}\gamma$ polymorphism but no other *BCL11A* and *HBS1L-MYB* polymorphisms [15]. This is in contrast with some previous studies, which showed that the *BCL11A* and *HBS11L-MYB* SNPs had a strong association with HbF variation in β-thalassemia and β-thalassemia/HbE disease [22,24]. Moreover, the SNP rs11886868 in the BCL11A gene was found to have a strong association with HbF levels and could help to ameliorate the β-thalassemia phenotype [25]. The increased HbF production had ameliorated the severity of anemia in homozygous β-thalassemia and β-thalassemia/HbE disease [7,13]. However, in the current study, the influence of *BCL11A* and *HBS11L-MYB* SNPs on HbF levels and hematological parameters in β-thalassemia/HbE disease was not observed. Furthermore, it seems likely that the *HBS11L-MYB* SNP (rs9399137) only but not *BCL11A* is a factor for HbF regulation and hematological parameter amelioration in homozygous HbE subjects. Consistent with the previous study, the C allele of *HBS11L-MYB* SNP (rs9399137) was strongly associated with increased HbF expression in HbE subjects in the Thai population [9]. The different results from many previous studies [22,24,25], especially those of *BCL11A*, could be explained by the different ethnical origins that play a significant role in the studied populations.

The study by Pakdee *et al*. suggested the co-inheritance of α-thalassemia could result in a lower level of HbF and the subjects with α-thalassemia should be excluded from the analysis of SNPs [9]. In the current study, a few subjects with α-thalassemia-1 SEA trait were found in each group, 3 (20.0 %) and 2 (6.7 %) β-thalassemia/HbE patients with HbF < 15 % and ≥ 15 %, respectively and 2 (7.4 %) and 1 (4.3 %) homozygous HbE subjects with HbF ≤ 5 % and > 5 %, respectively. These subjects were not excluded from the analysis because they did not significantly affect the analysis results. The co-inheritance of the α-thalassemia-2 trait in β-thalassemia/Hb E disease was associated with a mild clinical outcome [26]. The subjects who co-inherited α-thalassemia-2 may also present low HbF levels because of the α-globin chain reduction. The previous study showed that the homozygous HbE subjects with one α-globin gene defect had lower mean ± SD of HbF levels than those without α-globin gene defects (2.7 ± 1.5 % vs 4.2 ± 3.2 %). However, it looks like these levels may not have any significant difference. Furthermore, the hematological parameters between these 2 groups of subjects were comparable [9]. In addition, HbF levels of Moroccan adult subjects who had co-inheritance of the α-thalassemia-2 trait (3.7 kb deletion) and β-thalassemia varied from 0.6 to 6.0 % (mean ± SD; 2.8 ± 2.2 %) [27]. Thus, the molecular analysis for diagnosis of α-thalassemia-2 (3.7 and 4.2 kb deletions) was not performed in the present study.

Conclusions

The *HBS11L-MYB* SNPs especially rs9399137 is likely to be one factor that influences the production of HbF and ameliorates the abnormal hematological parameters in homozygous HbE subjects, but not in β-thalassemia/HbE patients. However, the other factors that influence the increase of HbF production and ameliorate the clinical symptoms presented in both β-thalassemia/HbE patients and the hematological abnormalities presented in homozygous HbE subjects, such as KLF1, a direct activator of BCL11A in globin gene switching should be analyzed further. We expect our findings will help to characterize the molecular mechanisms of γ-globin gene regulation and could eventually contribute to the development of new therapeutic approaches for β-thalassemia/HbE and homozygous HbE.

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