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Genetic Modifiers in β-Thalassemia Intermedia: A Study on 102 Iraqi Arab Patients

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To determine the molecular basis of β-thalassemia intermedia (TI) and the contribution of the three hemoglobin F (HbF) quantitative trait loci (QTLs) on chromosomes 11, 2, and 6 to the milder phenotype, a total of 102 Iraqi Arab patients with TI were studied. The β and α genotypes as well as *HBG2 g.* 158 C>T (rs7482144), *BCL11A* (rs1427407 and rs10189857), and *HBS1L-MYB* (rs28384513 and rs9399137) by multiplex polymerase chain reaction and reverse hybridization were studied. A total of 21 different β-thalassemia mutations arranged in 35 different genotypes were identified. The genotypes encompassed β^+/β^+ mutations in 33 cases, β^+/β^0 in 17 cases, β^0/β^0 in 47 cases, β^0/wild type in 3 and β^0/Hb E in 2 cases. The most common was IVS-II-1 (G>A)/IVS-II-1 (G>A), followed by IVS-I-6 (T>C)/IVS-I-6 (T>C) and IVS-I-110 (G>A)/IVS-I-110 (G>A), in 31.4%, 17.6%, and 6.9%, respectively. Alpha-thalassemia mutations were found in 15.2% of those homozygous for the β-mutations, while α gene triplication was identified in all three heterozygotes. Of the five QTLs tested, only rs7482144 and rs10189857 were significantly associated with β^0/β^0 when compared to β^+/β^+, with odds ratios of 6.4 (95% confidence interval [CI] 2.9–14.0) and 3.2 (95% CI 1.2–8.6), respectively. In conclusion, this study has demonstrated that among Iraqi patients with thal intermedia, the main contributors to the milder phenotype were β^+ alleles, *Xmn*I polymorphism, and *BCL11A* (rs10189857), while other QTLs on chromosomes 2 and 6, as well as alpha-thalassemia, were not significantly relevant.

Introduction

Beta-thalassemias (β-thal) are autosomal recessive inherited disorders with clinical phenotypes ranging from the severe transfusion-dependent β-thalassemia major (TM) to the asymptomatic β-thalassemia minor. Between these two extremes lies β-thalassemia intermedia (TI), a less severe condition than TM, but more severe than β-thal minor (Camaschella and Cappellini, 1995). A variety of genetic mechanisms are responsible for the latter phenotype, including inheritance of mild or silent β-thalassemia alleles, coinheritance of α-thalassemia, and inheritance of determinants that are associated with increased γ-chain production (Taher *et al.*, 2013). Several genes have been identified that are involved in modified γ-chain production. The resulting increase of hemoglobin F (HbF) levels ameliorates the phenotype by reducing the α:β imbalance and ineffective erythropoiesis. The three major quantitative trait loci (QTLs) are the *Xmn*I -158 C>T promoter polymorphism in the *HBG2* gene, the *BCL11A* gene located on chromosome 2, and the *HBS1L-MYB* intergenic region on chromosome 6 (Thein *et al.*, 2009; Taher *et al.*, 2013). The *BCL11A* gene encodes a zinc finger transcription factor that is a critical modulator of hemoglobin switching and γ-gene silencing, and appears to do so by binding to the locus controlling region as well as the intergenic region within the β-gene cluster and not to the γ-gene promoter (Wilber *et al.*, 2011; Xu *et al.*, 2013). The *MYB*, on the other hand, is a proto-oncogene that encodes for a *c-MYB* transcription factor playing an essential role in erythroid differentiation and has been shown to modulate HbF levels in healthy as well as those with hemoglobinopathies (Jiang *et al.*, 2006; Menzel *et al.*, 2014). These two QTLs appear to be directly regulated by another key transcription factor, the Kruppell -like factor 1 (KLF 1), and they cooperate with DNA methyltransferase 1 to achieve fetal to adult hemoglobin switch (Tallack and Perkins, 2013; Roosjen *et al.*, 2014). The relative contributions of major QTLs to HbF regulation seem to vary among different populations (Fanis *et al.*, 2014).

There are a limited number of studies on the molecular basis of β-TI from the Middle East [including Iraq] (Al-Allawi *et al.*, 2014) and on the contributions of single-nucleotide polymorphisms (SNPs) in the three major QTLs to its milder phenotype; thus, the current study was initiated to address, in

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particular, the latter issue through studying a cohort of registered TI patients at a large thalassemia center in Iraq's capital, Baghdad, a city that is widely believed to be representative to a great extent of its Arab population.

Materials and Methods

Patients

In total, 102 Iraqi Arab patients with β-TI registered at the Ibn Albaladi hereditary anemia center in Baghdad were enrolled. The patients' diagnoses were based on the following criteria: first transfusion at or beyond the age of 2 years and/or relative independence on blood transfusion (Qatanani *et al.*, 2000). The distinction between TI and minor was based on the presence of mild to severe anemia with at least one of the following: (1) transfusion at some time during life; (2) splenomegaly or splenectomy; and (3) hemoglobin electrophoresis incompatible with thalassemia minor (Qatanani *et al.*, 2000). Patients were clinically and hematologically reevaluated. The study was approved by the appropriate ethics committee, and informed consents were obtained from all enrolled patients.

Genotyping

DNA was extracted using a QIAamp® DNA extraction blood minikit (Qiagen). All genotyping studies comprising the α- and β-globin genes, as well as the QTL genes, were carried out using ViennaLab StripAssays® (ViennaLab Diagnostics GmbH). These assays are based on multiplex polymerase chain reaction and subsequent reverse hybridization. DNA of patients, whose β-genotype was not fully characterized by the StripAssay, was subjected to sequencing of the whole β-globin gene using the Sanger sequencing service offered by Microsynth, Austria.

The β-Thal Modifier StripAssay® was used for genotyping the following QTLs: *HBG2* promoter SNP (g. -158C > T, rs7482144), two SNPs in the *BCL11A* gene (rs1427407 G > T, rs10189857 A > G), and two SNPs in the *HBS1L-MYB* intergenic region (rs28384513 A > C, rs9399137 T > C). The choice of these SNPs was based on previous studies linking them to HbF levels (Menzel *et al.*, 2007; Lettre *et al.*, 2008; Galarneau *et al.*, 2010).

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package. A variable was defined for each of the QTL SNPs and for α-thalassemia mutations as 0, 1, or 2 depending on the number of minor alleles or α-thal determinants detected. Logistic regression and Kruskal–Wallis test were used when appropriate. A p-value < 0.05 was considered significant.

Results

Patient characteristics

The enrolled patients, aged between 3 and 58 years (median 13 years), included 62 males and 40 females. The age at diagnosis ranged from 0.5 to 30 years (median 4 years). Eleven patients (10.8%) were splenectomized at the time of enrolment. Six patients were never transfused (5.9%); the remaining patients were first transfused between the age of 2 and 53 years (median 5 years). Median hemoglobin (Hb) before the next transfusion was 8.1 g/dL (range 4.4–11.0 g/dL), while median HbF was 93% (range 7.1–98.4%) [in those where it was available].

Molecular studies

β-Globin genotyping. The most common genotype encountered was homozygous IVS-II-1 G > A in 32 patients (31.4%), followed by homozygous IVS-I-6 T > C in 18 (17.6%), homozygous IVSI-110 G > A in 7 (6.9%), IVS-I-6 T > C/IVS-I-110 G > A in 5 (4.9%), and IVS-1-6 T > C/IVS-II-1 G > A in 4 patients (3.9%). The most common mutations were IVS-II-1 G > A (41.2%), IVS-1-6 T > C (24.0%), IVS-I-110 G > A (11.3%), codon 8-AA (3.9%), IVS-I -25 bp del (2.5%), and IVS-I-1 G > A (2%). Other mutations were less frequent or sporadic and are listed with their relative frequencies in Table 1. Overall, a total of 21 different mutations arranged in 35 different genotype combinations were detected (Table 2). The genotypes encompassed β^+/β^+ mutations in 33 cases, β^+/β^0 in 17 cases, β^0/β^0 in 47 cases, and β^0/wild type in 3 and β^0/Hb E in 2 cases.

α-Globin genotyping. α-thal mutations were found in 15 out of 99 patients with homozygous or compound heterozygous β-thal. The $\alpha\alpha\alpha^{anti\text{-}3.7}$ gene triplication was detected in all three patients with heterozygous β-thal. The following α-genotypes were found: $-\alpha^{3.7}/\alpha\alpha$ in 10 cases, $-\alpha^{3.7}/-\alpha^{3.7}$ and $-\alpha^{4.2}/\alpha\alpha$ in two cases each, and $\alpha^{PA2}\alpha/\alpha\alpha$ in one case.

Genotyping of SNPs in the major HbF QTLs. The frequencies of the minor alleles in patients with β^+/β^+ and β^0/β^0 are summarized in Table 3. Logistic regression analysis on these five SNPs and α-thal mutations revealed that *Xmn*I (rs7482144) and *BCL11A* (rs10189857) were, respectively, as follows: 6.4 times (95% confidence interval [CI] 2.9–14) and 3.2 times (95% CI 1.2–8.6) more frequent in the β^0/β^0 group than in the β^+/β^+ group. No significant differences were observed with other SNPs or concomitant α-thalassemia (Table 3). HbF percentages were available in 62 of the enrolled patients, and Table 4 shows the distribution of HbF (%) in relevance to the number of minor alleles in the five SNPs investigated and it shows that variation was only significant in association with *Xmn*I (rs7482144) polymorphism.

Discussion

The current study identified the β-thalassemia mutation spectrum among Iraqi Arab TI patients which comprised Mediterranean, Asian-Indian, Turkish, Egyptian, Kurdish, and Saudi Arabian mutations. All these mutations have been reported by earlier reports on β-thalassemia from Iraq, except for -101 (C > T) and codon 26 (G > A) [Hb E] mutations (Al-Allawi *et al.*, 2006, 2013, 2014; Jalal *et al.*, 2010). The promoter sequence mutation -101 (C > T) is considered the most common among silent β-thalassemia mutations in the Mediterranean populations and usually results in a clinical phenotype of nontransfusion-dependent thalassemia if it interacts with a severe β-thalassemia mutation (Maragoudaki *et al.*, 1999). In the current study, the patient in question was compound heterozygous for -101 (C > T) and codon 8 (-AA) with a mild phenotype presenting for the first time at the age of 28 years. Hemoglobin E, on the other hand, is one of the most common hemoglobinopathies in the Indian subcontinent

Table 1. The Allele Frequencies of β-Thalassemia Mutations in the Current and Earlier Studies from Iraq and Some Other Middle Eastern Countries

Mutations	*Iraqi Arabs (Baghdad)*	*Iraqi Kurds (Erbil)*	*Iraqi Kurds (Duhok)*	*Iran*	*Turkey*	*Lebanon*
IVS-II-1 (G > A)	41.2	27.7	20.6	43.6	24.4	8.9
IVS-I-6 (T > C)	24.0	33.1	32.4	7.4	28.0	40.4
IVS-I-110 (G > A)	11.3	2.5	2.0	4.3	6.1	7.5
Codon 8 (-AA)	3.9	7.2	7.8	—	22.0	2.7
IVS-1-25 bp del	2.5	—	—	—	—	0.7
IVS-I-1 (G > A)	2.0	3.6	3.9	2.1	1.2	4.8
IVS-I-128 (T > G)	1.5	6.0	2.0	2.1	—	—
IVS-II-848 (C > A)	1.5	—	—	—	—	—
Codon 44 (-C)	1.5	—	2.0	1.1	—	—
Codon41/42 (-TCTT)	1.5	—	—	—	—	—
-28 (A > C)	1.0	—	2.0	—	—	—
IVS-I-5 (G > C)	1.0	0.6	—	1.1	1.2	—
Codons 8/9 (+G)	1.0	4.8	—	2.1	—	0.7
IVS-I-130 (G > C)	1.0	—	1.0	—	—	—
Codon 26 (G > A) [Hb E]	1.0	—	—	—	—	—
-101 (C > T)	0.5	3.0	—	—	2.4	—
Codon 39 (C > T)	0.5	1.2	2.0	1.1	2.4	—
-30 (T > A)	0.5	—	1.0	—	2.4	—
Codon 36/37 (-T)	0.5	—	—	2.1	—	—
Codon 5 (-CT)	0.5	0.6	5.9	2.1	—	—
Codons 22/24 (-7 bp del)	0.5	1.2	—	4.3	—	—
Codons 82/83 (-G)	—	—	9.8	3.2	—	—
-87 (C > G)	—	3.0	—	—	—	1.4
Codon 29 (C > T)	—	—	—	—	—	12.3
Codon 30 (G > A)	—	—	—	—	—	12.3
δβ-thalassemia	—	—	—	3.2	7.3	1.4
Other mutations	—	1.2	2.9	10.5	2.4	6.8
Wild	1.5	4.2	2.9	9.6	—	—
No. of chromosomes	204	166	102	94	82	146
Reference	Current	Shamoon *et al.* (2015)	Al-Allawi *et al.* (2014)	Neishabury *et al.* (2008)	Altay and Gürgey (1990)	Qatanani *et al.* (2000)

and SE Asia, and the mutation, in addition to being a structural variant, also creates a cryptic splice site leading to a behavior similar to mild β-thal (Olivieri *et al.*, 2008). Hb E/β-thal is usually associated with a TI phenotype as it is the case in the two patients in the current study who were compound heterozygous for Hb E with codon 44 and IVS-II-1, respectively.

The three most common mutations identified in our enrolled β-TI patients were IVS-II-1, IVS-I-6, and IVS-I-110. An earlier study on the spectrum of β-thal mutations in Baghdad among obligate carriers (majority being parents of patients with thal major) revealed that IVS-1-110 and IVS-II-1 mutations were the most frequent, while IVS-I-6 constituted < 4% of mutations (Al-Allawi *et al.*, 2013). The higher contribution of IVS-I-6 in the current study is expected since the latter is a mild β^+ mutation and thus it is more likely to be associated with a TI phenotype in the homozygous and compound heterozygous state. A similar situation has also been reported among Cypriot TI patients (Verma *et al.*, 2007). When looking at our results in the context of surrounding countries, reports from Iran documented IVS-II-1 as the most frequent mutation among their TI patients (Banan *et al.*, 2013), while the mild IVS-1-6 was the most common among Iraqi Kurds, Lebanese, and Italian TI patients (Camaschella *et al.*, 1995; Qatanani *et al.*, 2000; Al-Allawi *et al.*, 2014; Shamoon *et al.*, 2015), and IVS-1-6 and IVS-1-110 were the most common among Cypriot TI patients (Verma *et al.*, 2007). The mutation spectrum of our TI patients seemed to be lying between the reported spectrum of TI in Iran to the East and that reported in Mediterranean countries to the West (Table 1). This finding is consistent with the geographic location of Iraq and its role throughout its long history as a link between the East and the West.

In the majority of studies, the most important contributor to TI was the inheritance of mild β-thalassemia alleles (Camaschella *et al.*, 1995; Qatanani *et al.*, 2000; Verma *et al.*, 2007; Al-Allawi *et al.*, 2014; Shamoon *et al.*, 2015). Our study showed that in 51% of TI patients one or both β-thal alleles were β^+ (β^+/β^0, β^+/β^+). It further showed that 46.1% of TI patients were homozygous or compound heterozygous for the severe β^0 alleles (β^0/β^0). Several genetic modulators have been implicated in ameliorating the phenotype in patients with such severe mutations. Previous studies investigated the modifying role of various SNPs in the three major QTLs by comparing patients with TM and TI. In the present study, we have chosen an alternative approach for investigating the effect of the selected SNPs by comparing TI patients with β^0/β^0 and β^+/β^+ genotypes. In our setting, the *Xmn*I (rs7482144) polymorphism turned out to be the most significant genetic modifier followed by the *BCL11A* rs10189857 SNP. The rs1427407 in *BCL11A*, the two SNPs in *HBS1L-MYB*, and the α-thal status were not found to play a significant role in phenotypic presentation of our cohort (Table 2).

Table 2. Summary of the Genotyping Results Among 102 Iraqi Thalassemia Intermedia Patients

Genotypes	No.	α-genotypes other than αα/αα	HBG2 g.158 C>T	BCL11A		HBS1L-Myb	
			rs7482144	*rs1427407*	*rs10189857*	*rs28384513*	*rs9399137*
β^+/β^+							
IVS-I-6/IVS-I-6	18	$\alpha^{PA2}\alpha/\alpha\alpha$ (1)	18 CC	10GG/6GT/2TT	9AA/4AG/5GG	9AA/6AC/3CC	10TT/7TC/1CC
IVS-I-110/IVS-I-110	7	$-\alpha^{3.7}/\alpha\alpha$ (1) $-\alpha^{3.7}/-\alpha^{3.7}$(1)	1CC/1CT/5TT	3GG/3GT/1TT	5AA/2AG/	5AA/2AC	5TT/1TC/1CC
IVS-I-6/IVS-I-110	5	$-\alpha^{3.7}/\alpha\alpha$ (2)	2CC/3CT	2GG/3GT	4AG/1GG	5AA	5TT
IVS-I-128/IVS-I-128	1		CC	GT	AA	AA	TC
IVS-II-848/IVS-II-848	1		CC	GG	AA	AA	TT
IVS-I-6/IVS-I-5	1		CT	GT	AG	AC	TT
Semitotal	33		23CC/5CT/5TT	16GG/14GT/3TT	16AA/11AG/6GG	21AA/9AC/3CC	22TT/9CT/2CC
β^+/β^0							
IVS-II-1/IVS-I-6	4	$-\alpha^{3.7}/\alpha\alpha$ (1)	1CC/3CT	2GG/1GT/1TT	2AA/2AG	2AA/1AC/1CC	2TT/2CC
IVS-I-110/IVS-II-1	3		1CC/2TT	2GT/1TT	1AA/2AG	2AA/1CC	1TT/1TC/1CC
IVS1.1/-28	1	$-\alpha^{3.7}/\alpha\alpha$	CC	GG	AG	AA	TT
IVS2.1/-28	1		CT	GG	GG	CC	TC
IVS2.1/IVS1.128	1		CT	GG	GG	AC	TT
IVS-I-6/Codon 8/9	1		CC	GT	AG	AA	TC
IVS-I-6/Codon 8	1		CT	GT	AA	CC	TC
IVS-I-6/IVS-I-1	1		CC	GT	AG	AA	TT
IVS-I-110/IVS-1-25b	1	$-\alpha^{3.7}/\alpha\alpha$	TT	GT	AA	AC	TT
-30/IVS-II-1	1		CT	GG	AA	AA	TC
-101/Codon 8	1		CC	GG	AG	AA	TT
IVS-II-848/IVS-II-1	1		CT	GG	AG	AA	TT
Semitotal	17		6CC/8CT/3TT	8GG/7GT/2TT	6AA/9 AG/2 GG	10AA/3AC/4CC	9TT/5TC/3CC
β^0/β^0							
IVS-II-1/IVS-II-1	32	$-\alpha^{3.7}/\alpha\alpha$(3) $-\alpha^{4.2}/\alpha\alpha$ (1)	2CC/4CT/26TT	15GG/14GT/3TT	7AA/15AG/10GG	22AA/6AC/4CC	23TT/7TC/2CC
IVS-I-1/IVS-I-1	1		TT	GG	GG	AA	TC
Codon 8/codon 8	2		2TT	2GG	AA/AG	2AA	2TT
IVS-1-25b/IVS-1-25b	2		2TT	2GG	1AG/1GG	1AC/1CC	2TC
Codon 8/IVS-II-1	2	$-\alpha^{4.2}/\alpha\alpha$ (1)	2TT	2GG	1AA/1AG	2AA	2TT
Codon 44/Codon 44	1		CC	GG	AG	CC	TC
IVS-I-130/IVS-I-130	1		CC	GT	AG	AA	TT
Codon 5/IVS-II-1	1		CT	GT	GG	AA	TT
Codon 41/42/Codon 41/42	1		CC	TT	AA	AA	TC
Codon 39/IVS-II-1	1	$-\alpha^{3.7}/\alpha\alpha$	CT	GG	AG	AA	TT
Codon 41/42/IVS-II-1	1		CT	GT	AG	AA	TT
Codon 36/37/IVS-II-1	1		CT	GT	AA	CC	TC
Codon 8/9/Codon 22	1	$-\alpha^{3.7}/-\alpha^{3.7}$	CC	TT	AA	AC	TT
Semitotal	47		6CC/8CT/33TT	24GG/18GT/5TT	12AA/22 AG/13GG	32AA/8AC/7CC	32TT/13TC/2CC
Beta/WT							
IVS-II-1/WT	2	$\alpha\alpha\alpha/\alpha\alpha$ (2)	2CT	2GT	2AG	2AC	TT/TC
IVS-I-5/WT	1	$\alpha\alpha\alpha/\alpha\alpha$	CC	TT	AA	AC	TT
Semitotal	3		2CT/CC	2GT/TT	2AG/AA	3AC	2TT/TC
Others							
IVS-II-1/Codon 26 (Hb E)	1		TT	GG	AG	AC	TC
Cd44/HB E	1		CT	GG	AG	AC	TC

Table 3. The Frequencies of Quantitative Trait Loci Single-Nucleotide Polymorphisms and α-Thalassemia Mutations Among Iraqi Thalassemia Intermedia Patients with β^+/β^+ and β^0/β^0 Genotypes

			Number (%)				
QTL			β^+/β^+	β^0/β^0	*OR*	*95% CI*	p-*Value*
HBG2 g. -158C>T	rs7482144	CC	23 (69.7)	6 (12.8)	6.4	2.9–14.0	<0.0005
		CT	5 (15.1)	8 (17.0)			
		TT	5 (15.2)	33 (70.2)			
BCL11A	rs1427407	GG	16 (48.5)	24 (51.1)	2.5	0.8–7.3	0.098
		GT	14 (42.4)	18 (38.3)			
		TT	3 (9.1)	5 (10.6)			
	rs10189857	AA	16 (48.5)	12 (25.5)	3.2	1.2–8.6	0.021
		AG	11 (33.3)	22 (46.8)			
		GG	6 (18.2)	13 (27.7)			
HBS1L-Myb	rs28384513	AA	21 (63.6)	32 (68.1)	1.0	0.3–3.2	0.998
		AC	9 (27.3)	8 (17.0)			
		CC	3 (9.1)	7 (14.9)			
	rs9399137	TT	22 (66.6)	32 (68.1)	1.5	0.3–6.1	0.607
		TC	9 (27.3)	13 (27.7)			
		CC	2 (6.1)	2 (4.2)			
α-thal mutations			5 (15.2)	7 (14.9)	1.4	0.4–4.7	0.607

OR, 95% CI, and significance were determined by logistic regression.
CI, confidence interval; OR, odds ratio; QTL, quantitative trait loci.

Our data corroborate the findings of many previous studies that among SNPs in the three major QTLs, the *Xmn*I polymorphism has the strongest effect on modifying disease severity of β-thalassemia (Nguyen *et al.*, 2010; Baden *et al.*, 2011; Danjou *et al.*, 2012; Banan *et al.*, 2013). The contributions of SNPs in the other two QTLs have been subject to controversy. While studies on Sardinian patients with β^0 thalassemias revealed a significant contribution of several SNPs in *BCL11A* and *HBL1S-MYB* to the TI phenotype (Galanello *et al.*, 2009; Danjou *et al.*, 2012), Nguyen *et al.* (2010) disputed the role of these two QTLs in their French TI patients, particularly in the presence of the *Xmn*I polymorphism. The latter authors suggested that the *Xmn*I effect on HbF production, which is potentiated by highly ineffective erythropoiesis of TI, could mask or inactivate the biological expression of the *BCL11A* and *HBS1L-MYB* genes. This explanation may also apply to our results.

One limitation of the current study is that it is a cross-sectional study and because of the fact that a good number of our patients were on regular transfusions at the time of enrolment, it was not possible to get HbF levels except in around 60% of patients. The latter would definitely limit the ability to link the SNPs studied to HbF levels. This limitation is not unique to the current study, but is shared by several previously published reports tackling the same issue (Weatherall, 2012); however, the observation of the current study linking some QTL SNPs with a milder phenotype of TI is significant and warrants further studies in Iraqi TI patients with known HbF levels.

In conclusion, the current report, which is the first on Iraqi Arab TI patients, revealed a relatively different mutation spectrum compared to an earlier study on Iraqi Kurds. Moreover, it showed that the main contributors to the less severe TI phenotype were the inheritance of mild β-thal determinants, *Xmn*I polymorphism and, to a lesser extent, the rs10189857 SNP in the *BCL11A* gene. Further studies, including patients with TM as well as genome-wide association analysis, may be more informative and may uncover other QTLs in this population.

Table 4. Mean (SD) of HbF Percentage and Its Association with Number of Minor Alleles in Each of the Five Studied Quantitative Trait Loci Single-Nucleotide Polymorphisms in the 62 Patients Who Had HbF% Available

QTL SNP	*HbF% mean (SD)*			
No. of minor alleles	*0*	*1*	*2*	p-*Value*
HBG2 g. -158 C>T				
rs7482144	36.86 (30.54)	74.8 (19.06)	92.31 (13.12)	<0.0005
BCL11A				
rs1427407	69.9 (34.21)	72.36 (30.05)	78.92 (28.46)	0.95
rs10189857	69.71 (32.1)	69.81 (31.92)	76.66 (32.99)	0.77
HBS1L-Myb				
rs28384513	75.55 (28.51)	62.58 (37.49)	70.91 (34.22)	0.94
rs9399137	75.55 (28.72)	68.2 (35.24)	72.2 (31.12)	0.98

HbF, hemoglobin F; SNP, single-nucleotide polymorphism.

Author Disclosure Statement

There are no conflicts of interest to report.

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