

Modifying_effect_of_Xmnl.pdf

by

Submission date: 04-May-2021 02:47PM (UTC+0700)

Submission ID: 1577607087

File name: Modifying_effect_of_Xmnl.pdf (602.41K)

Word count: 6970

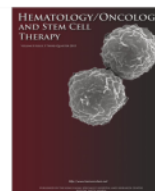
Character count: 36240



ELSEVIER

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/hemonc

ORIGINAL RESEARCH REPORT

Modifying effect of *Xmn1*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β -thalassemia and hemoglobin E/ β -thalassemia patients in Indonesia

Lantip Rujito^{a,*}, Muhammad Basalamah^b, Wahyu Siswandari^c,
Joko Setyono^d, Gondo Wulandari^e, Sri Mulatsih^f, Abdul Salam M. Sofro^g,
Ahmad Hamim Sadewa^h, Sutaryo Sutaryo^f

^a Department of Molecular Biology, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia

^b Department of Pediatrics, Banyumas General Hospital, Banyumas, Central Java, Indonesia

^c Department of Clinical Pathology, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia

^d Department of Biochemistry, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia

^e Indonesian Red Cross, Banyumas Unit, Purwokerto, Central Java, Indonesia

^f Department of Pediatrics, Sardjito Central General Hospital, Sekip, Yogyakarta, Indonesia

^g Department of Biochemistry, Faculty of Medicine, Yayasan Rumah Sakit Islam (YARSI) University, Cempaka Putih, Jakarta, Indonesia

^h Department of Biochemistry, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Received 26 October 2015; accepted 26 February 2016

KEYWORDS

BCL11A;
HBS1L-MYB;
Modifier effect;
Thalassemia;
Xmn1

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 *Xmn1* locus, rs11886868, rs766432 (*BCL11A*), and rs9399137 (*HBS1L-MYB*), among β -thal and HbE/ β -thal patients in Indonesia, according to laboratory and clinical

* Corresponding author at: Department of Molecular Biology, Faculty of Medicine, Jenderal Soedirman University, Jalan Medika Gumbreg Number 1, Purwokerto 53146, Central Java, Indonesia.

E-mail address: l.rujito@unsoed.ac.id (L. Rujito).

<http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

1658-3876/© 2016 King Faisal Specialist Hospital & Research Centre. Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Please cite this article in press as: Rujito L et al., Modifying effect of *Xmn1*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β -thalassemia and hemoglobin ..., *Hematol Oncol Stem Cell Ther* (2016), <http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

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.

© 2016 King Faisal Specialist Hospital & Research Centre. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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 $-15 > 1$ 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 (*B_g11A*), 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 *MbolI* enzyme, the T and C alleles were indicated 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, Anyumas 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

Hematology 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 A₂ (HbA₂) 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 HbA₂ 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 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
<i>XmnI</i>	FG5: 5'-GAGCTACAGACAAGAAGGTG-3' RG4: 5'-TTTTATTCTTCATCCCTAGC-3'	351	<i>XmnI</i>	(+): 214, 137 (–): 351
rs11886868	F868: 5'-TTTGGTGCTACCTGAAAGAC-3' R868: 5'-ACTCAACAGTAGCAGAAATGAAAGAG-3'	540	<i>MbolI</i>	C: 478, 62 T: 540
rs766432	432(A)F: 5'-TTGTTTCGCTTTAGCTTTATTAAGGTACAA-3' 432(A)R: 5'-GACGTGTTCTGTATCTTGATTTTGGT-3' 432(C)F: 5'-CCAAACAGTTTAAAGGTTACAGACAGACT-3' 432(C)R: 5'-AAAATGAATGACTTTTGTGTATGTAGAG-3'	135 116	—	A: 135 C: 116
rs9399137	137(C)F: 5'-AATGTAATTAAGTGAACATATGGTTAGTC-3' 137(C)R: 5'-TTTATTGTTACAAGGTTAATTCACTGCC-3' 137(T)F: 5'-GAAATACCATCACTGAGAAAAGCATAAG-3' 137(T)R: 5'-CAGCAGGGTTGCTTGTGAAAAAAGCTT TA-3'	178 234	—	C: 178 T: 234

Please cite this article in press as: Rujito L et al., Modifying effect of *XmnI*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β -thalassemia and hemoglobin ..., *Hematol Oncol Stem Cell Ther* (2016), <http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

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

Parameter	β^0/β^0 (n=7)	β^0/β^+ (severe) (n=30)	β^+/β^+ (severe) (n=31)	β^+/β^+ (severe) (n=4)	HbE/ β^+ (severe) (n=86)	HbE/ β^0 (n=30)	p (F test)
Hb	7.40 $\pm$ 0.92	8.28 $\pm$ 0.53	8.36 $\pm$ 0.56	8.39 $\pm$ 0.42	8.28 $\pm$ 0.61	8.34 $\pm$ 0.49	.035
Transfusion (g/dL)							
Mean	73.23 $\pm$ 3.98	74.41 $\pm$ 4.45	78.51 $\pm$ 2.61	77.08 $\pm$ 3.96	79.05 $\pm$ 5.44	76.86 $\pm$ 4.00	.373
corpuscular volume (fL)							
Mean	24.22 $\pm$ 1.65	25.94 $\pm$ 1.56	24.59 $\pm$ 2.10	25.56 $\pm$ 1.30	26.32 $\pm$ 0.96	24.60 $\pm$ 2.20	.326
corpuscular hemoglobin (pg)							
Hemoglobin A ₂ (+HbE) (%) ^a	3.85 $\pm$ 1.01	3.85 $\pm$ 2.48	5.24 $\pm$ 2.91	3.22 $\pm$ 2.32	40.33 $\pm$ 11.49	40.77 $\pm$ 10.68	.005
Fetal hemoglobin (%)	91.81 $\pm$ 2.10	69.08 $\pm$ 30.68	78.27 $\pm$ 24.49	59.70 $\pm$ 27	29.00 $\pm$ 11.41	29.45 $\pm$ 11.04	.002

^a Hemoglobin A₂ 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	
<i>HBG2</i> (11.p15.5)	<i>XmnI</i>	+/+	7	0.04	+	53	0.14	0.063
		–/+	39	0.21	–	325	0.86	
		–/–	143	0.76				
<i>HBS1L-MYB</i> (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				
<i>BCL11A</i> (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				
<i>BCL11A</i> (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

Please cite this article in press as: Rujito L et al., Modifying effect of *XmnI*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β -thalassemia and hemoglobin ... Hematol Oncol Stem Cell Ther (2016), <http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

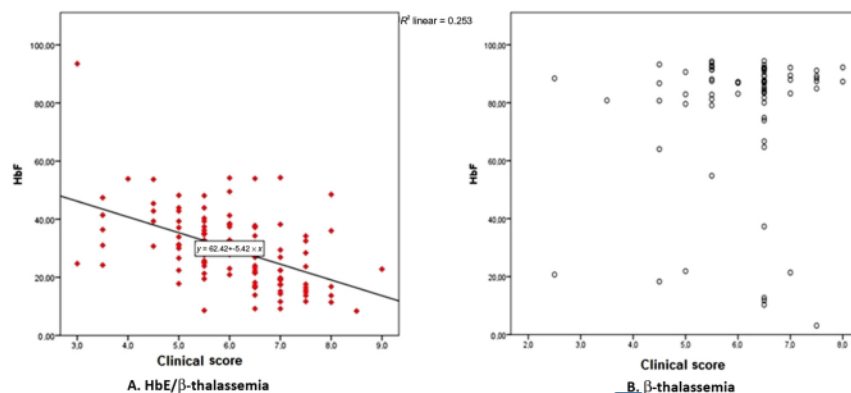


Figure 1 Relationship between fetal hemoglobin (HbF) level and clinical score in patients with β-thalassemia (β-thal) and hemoglobin E/β-thal (HbE/β-thal). HbE/β-thal patients provide a better correlation with HbF and clinical score association (Pearson $r = .49$ and $p = .001$), compared with β-thal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

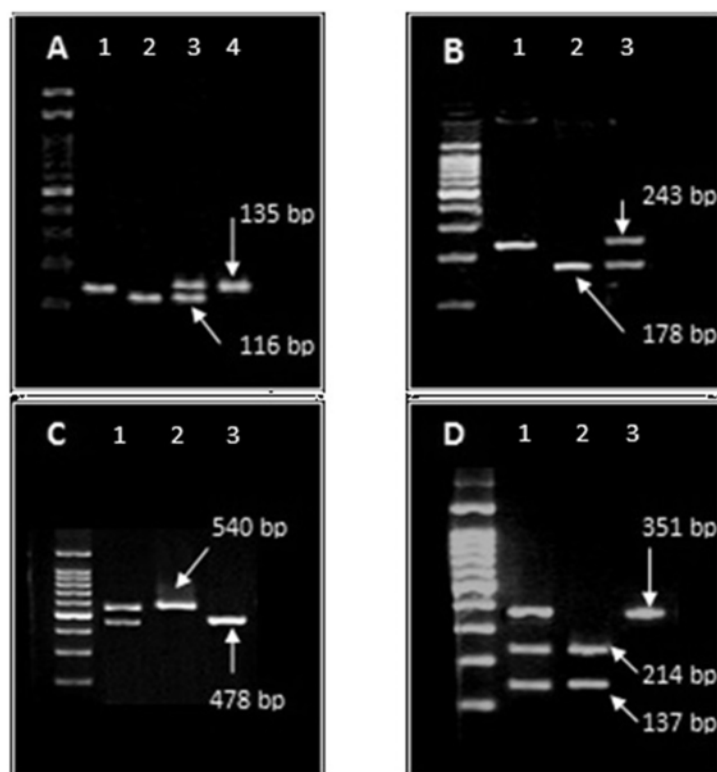


Figure 2 Transillumination DNA examination of *XmnI*, rs766432, rs11886868, and rs9399137. (A). Amplification refractory mutation system (ARMS) examination of rs766432: Bands 1 and 4 are homozygous AA, Band 2 is homozygous CC, and Band 3 is heterozygous AC. (B). ARMS examination of rs9399137: Band 1 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 *MbolI* 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 *XmnI* enzyme digestion. Band 3 shows the undigested polymerase chain reaction product reflecting the homozygous $-/-$ genotype. Band 2 is homozygous $+/+$ and Band 1 is heterozygous $-/+$.

Please cite this article in press as: Rujito L et al., Modifying effect of *XmnI*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β-thalassemia and hemoglobin ..., Hematol Oncol Stem Cell Ther (2016), <http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

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
<i>β-thal patients</i>							
HBG2	<i>XmnI</i>	−/−	55	75.12	6.33	0.67	0.24
(11.p15.5)		19 + +/+	17	77.94	6.00		
<i>HBS1L-MYB</i>	rs9399137	TT	53	75.94	5.39	0.78	0.10
(6.q23.3)		TC + CC	19	77.75	6.33		
<i>BCL11A</i>	rs11886868	CC	43	78.15	6.23	0.71	0.15
(2.p16.2)		CT + TT	29	75.98	5.86		
<i>BCL11A</i>	rs766432	AA	55	75.56	5.47	0.70	0.17
(2.p16.2)		AC + CC	17	77.81	6.27		
<i>HbE/β-thal patients</i>							
HBG2	<i>XmnI</i>	−/−	88	31.32	6.15	0.01	0.02
(11.p15.5)		19 + +/+	29	38.42	5.58		
<i>HBS1L-MYB</i>	rs9399137	TT	82	32.22	6.23	0.17	0.44
(6.q23.3)		TC + CC	35	35.10	5.50		
<i>BCL11A</i>	rs11886868	CC	82	38.75	5.80	0.02	0.04
(2.p16.2)		CT + TT	35	30.66	6.31		
<i>BCL11A</i>	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 Thalassemia 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 pre-transfusion, 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 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)	<i>XmnI</i>	+	0.14	.18	.032	.028
<i>HBS1L-MYB</i> (6.q23.3)	rs9399137	C	0.18	.06	.004	.395
<i>BCL11A</i> (2.p16.2)	rs11886868	C	0.88	.17	.028	.042
<i>BCL11A</i> (2.p16.2)	rs766432	C	0.19	.19	.047	.039

Please cite this article in press as: Rujito L et al., Modifying effect of *XmnI*, *BCL11A*, and *HBS1L-MYB* on clinical appearances: A study on β -thalassemia and hemoglobin ... Hematol Oncol Stem Cell Ther (2016), <http://dx.doi.org/10.1016/j.hemonc.2016.02.003>

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^0 mutations.

Patients with the β^0/β^0 alleles showed the highest level of HbF ($91.81\% \pm 2.10\%$), whereas those with β^+/ β^+ alleles had HbF levels of $59.70\% \pm 27\%$ [23]. These data indicate 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^+ ($29.00\% \pm 11.41\%$) and β^0 alleles ($29.45\% \pm 11.04\%$). 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 [36] [26].

In our study, the range of HbA₂ 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 HbA₂ levels of ~40%. This high concentration is actually the resultant value of HbE and HbA₂, 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 HbA₂ levels in HbE/ β -thal patients vary between 3% and 6%. Another report indicated that the HbA₂ 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.

20

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This study was a part of Decentralization Research Grant (Grant No. 1024/UN.23.10/PN/2014) from the Ministry of Research, Technology, and Higher Education of Indonesia to L.R.

References

- [1] Weatherall DJ. Thalassemia as a global health problem: recent progress toward its control in the developing countries. *Ann N Y Acad Sci* 2010;1202:17–23.
- [2] Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis* 2010;5:1–15.
- [3] Giardine B, Borg J, Viennas E, Pavlidis C, Moradkhani K, Joly P, et al. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. *Nucl Acids Res* 2014;42: D1063–D9.
- [4] Cao A, Moi P, Galanello R. Recent advances in beta-thalassemias. *Pediatr Rep* 2011;3:e17.
- [5] Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. *Cold Spring Harb Perspect Med* 2012;2(8), a011734.
- [6] Galanello R, Perseu L, Satta S, Demartis FR, Campus S. Phenotype-genotype correlation in β -thalassemia. *Thalassemia Rep* 2011;1:e6.
- [7] Akhtar MS, Qaw F, Borgio JF, Albuali W, Suliman A, Nasserullah Z, et al. Spectrum of α -thalassemia mutations in transfusion-dependent β -thalassemia patients from the Eastern Province of Saudi Arabia. *Hemoglobin* 2013;37:65–73.
- [8] Haj Khelil A, Morinière M, Laradi S, Khelif A, Perrin P, Ben Chibani J, et al. *XmnI* polymorphism associated with concomitant activation of γ and δ globin gene transcription on a beta0-thalassemia chromosome. *Blood Cells Mol Dis* 2011;46:133–8.
- [9] Mtatiro SN, Makani J, Mmbando B, Thein SL, Menzel S, Cox SE. Genetic variants at HbF-modifier loci moderate anemia and leukocytosis in sickle cell disease in Tanzania. *Am J Hematol* 2015;90:E1–4.
- [10] Danjou F, Anni F, Perseu L, Satta S, Dessi C, Lai ME, et al. Genetic modifiers of β -thalassemia and clinical severity as assessed by age at first transfusion. *Haematologica* 2012;97:989–93.
- [11] Thein SL. Genetic modifiers of the β -haemoglobinopathies. *Br J Haematol* 2008;141:357–66.
- [12] Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, et al. An erythroid enhancer of *BCL11A* subject to genetic variation determines fetal hemoglobin level. *Science (New York, NY)* 2013;342:253–7.
- [13] Galarneau G, Palmer CD, Sankaran VG, Orkin SH, Hirschhorn JN, Lettre G. Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation. *Nat Genet* 2010;42:1049–51.
- [14] Rujito L, Basalamah M, Mulatsih S, Sofro ASM. Molecular scanning of β -thalassemia in the southern region of Central Java, Indonesia: a step towards a local prevention program. *Hemoglobin* 2015;39:330–3.
- [15] Sripichai O, Makarasara W, Munkongdee T, Kumkhaek C, Nuchprayoon I, Chuansumrit A, et al. A scoring system for the classification of beta-thalassemia/Hb E disease severity. *Am J Hematol* 2008;83:482–4.
- [16] Solé X, Guinó E, Valls J, Iñiesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 2006;22:1928–9.
- [17] Thalassemia International Federation. Guidelines for the management of transfusion dependent thalassemia (TDT). 3rd ed. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. Nicosia, Cyprus: Thalassemia International Federation Publisher; 2014.
- [18] Cao A, Galanello R, Origa R. Beta-thalassemia. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, et al., editors. *GeneReviews*: 1993–2016. Seattle, WA: University of Washington; 2015. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1116/> [Accessed 9 March 2016].
- [19] Bulun S. Factors associated with quality of life in children with beta thalassemia major. Semarang, Central Java, Indonesia: Universitas Diponegoro; 2009.
- [20] Andriastuti M, Sari TT, Wahidiyat PA, Putriasih SA. Blood transfusion need on post-splenectomy thalassemia major. *Sari Pediatri* 2011;13:244–9 [in Bahasa].
- [21] Ermaya YS, Hilmanto D, Reniarti L. Relationship between hemoglobin pre-transfusion, iron chelating agent and growth among thalassemia major patients. *Madjalah Kedokt Indones* 2007;57:380–4 [in Bahasa].

- [22] Pratunmo K, Jetsrisuparb A, Fucharoen S, Tripatara A. Hepcidin expression from monocyte of splenectomized and non-splenectomized patients with HbE- β -thalassemia. *Hematology* 2014;19:175–80.
- [23] Weatherall DJ, Clegg JB. The thalassaemia syndromes. 4th ed. Oxford, UK: Blackwell Science; 2008.
- [24] Rees DC, Porter JB, Clegg JB, Weatherall DJ. Why are hemoglobin F levels increased in HbE/ β -thalassemia? *Blood* 1999;94:3199–204.
- [25] Satta S, Perseu L, Maccioni L, Giagu N, Galanello R. Delayed fetal hemoglobin switching in subjects with KLF1 gene mutation. *Blood Cells Mol Dis* 2012;48:22–4.
- [26] Rahim F, Allahmoradi H, Salari F, Shahjahani M, Fard AD, Hosseini SA, et al. Evaluation of signaling pathways involved in γ -globin gene induction using fetal hemoglobin inducer drugs. *Int J Hematol Oncol Stem Cell Res* 2013;7:41–6.
- [27] Ou CN, Rognerud CL. Diagnosis of hemoglobinopathies: electrophoresis vs HPLC. *Clin Chim Acta* 2001;313:187–94.
- [28] Pant L, Kalita D, Singh S, Kudesia M, Mendiratta S, Mittal M, et al. Detection of abnormal hemoglobin variants by HPLC method: common problems with suggested solutions. *Int Sch Res Notices* 2014;2014:10.
- [29] Tyagi S, Pati HP, Choudhry VP, Saxena R. Clinico-haematological profile of HbE syndrome in adults and children. *Hematology* 2004;9:57–60.
- [30] Mais DD, Gulbranson RD, Keren DF. The range of hemoglobin A2 in hemoglobin E heterozygotes as determined by capillary electrophoresis. *Am J Clin Pathol* 2009;132:34–8.
- [31] Ali N, Ayyub M, Khan SA, Ahmed S, Abbas K, Malik HS, et al. Frequency of $\gamma\gamma$ -globin promoter –158 (C>T) *XmnI* polymorphism in patients with homozygous/compound heterozygous beta thalassaemia. *Hematol Oncol Stem Cell Ther* 2015;8:10–5.
- [32] Bhagat S, Patra PK, Thakur AS. Association between *XmnI* polymorphism and HbF level in sickle cell disease patients from Chhattisgarh. *Int J Biomed Sci* 2012;8:36–9.
- [33] Wong YC, George E, Tan KL, Yap SF, Chan LL, Jama T. Molecular characterisation and frequency of $\gamma\gamma$ Xmn I polymorphism in Chinese and Malay β -thalassaemia patients in Malaysia. *Malays J Pathol* 2006;28:17–21.
- [34] Database of Single Nucleotide Polymorphisms (dbSNP). National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {rs766432, rs11886868, rs9399137}). Available from: <http://www.ncbi.nlm.nih.gov/SNP/2015>. [Accessed 9 March 2016].
- [35] Pakdee N, Yamsri S, Fucharoen G, Sanchaisuriya K, Pissard S, Fucharoen S. Variability of hemoglobin F expression in hemoglobin EE disease: hematological and molecular analysis. *Blood Cells Mol Dis* 2014;53:11–5.
- [36] Lettre G, Sankaran VG, Bezerra MA, Araujo AS, Uda M, Sanna S, et al. 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 USA* 2008;105:11869–74.
- [37] Sney GT, Panhuysen CM, So JC, Ma EK, Ha SY, Li CK, et al. Variation and heritability of Hb F and F-cells among β -thalassemia heterozygotes in Hong Kong. *Am J Hematol* 2008;83:458–64.
- [38] Thein SL, Menzel S, Peng X, Best S, Jiang J, Close J, et al. Intergenic variants of *HBS1L-MYB* are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc Natl Acad Sci USA* 2007;104:11346–51.
- [39] Sankaran VG, Xu J, Orkin SH. Advances in the understanding of haemoglobin switching. *Br J Haematol* 2010;149:181–94.
- [40] Xu J, Bauer DE, Kerenyi MA, Vo TD, Hou S, Hsu Y-J, et al. Corepressor-dependent silencing of fetal hemoglobin expression by *BCL11A*. *Proc Natl Acad Sci USA* 2013;110:6518–23.
- [41] Karimi M, Haghpanah S, Farhadi A, Yavarian M. Genotype–phenotype relationship of patients with β -thalassemia taking hydroxyurea: a 13-year experience in Iran. *Int J Hematol* 2012;95:51–6.
- [42] Sedgewick AE, Timofeev N, Sebastiani P, So JC, Ma ES, Chan LC, et al. *BCL11A* is a major HbF quantitative trait locus in three different populations with beta-hemoglobinopathies. *Blood Cells Mol Dis* 2008;41:255–8.
- [43] Solovieff N, Milton JN, Hartley SW, Sherva R, Sebastiani P, Perkins DA, et al. Fetal hemoglobin in sickle cell anemia: genome-wide association studies suggest a regulatory region in the 5' olfactory receptor gene cluster. *Blood* 2010;115:1815–22.
- [44] So CC, Song YQ, Tsang ST, Tang LF, Chan AY, Ma ES, et al. 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;45:745–51.
- [45] Cardoso GL, Diniz IG, Martins da Silva ANL, Cunha DA, da Silva Junior JS, Carvalho Uchôa CT, et al. DNA polymorphisms at *BCL11A*, *HBS1L-MYB* and *Xmn1-HBG2* site loci associated with fetal hemoglobin levels in sickle cell anemia patients from Northern Brazil. *Blood Cells Mol Dis* 2014;53:176–9.
- [46] Alsultan A, Solovieff N, Aleem A, AlGahtani FH, Al-Shehri A, Al-Osman M, et al. Fetal hemoglobin in sickle cell anemia: Saudi patients from the Southwestern province have similar HBB haplotypes but higher HbF levels than African Americans. *Am J Hematol* 2011;86:612–4.
- [47] Banan M, Bayat H, Azarkeivan A, Mohammadparast S, Kamali K, Rashi S, et al. The *XmnI* and *BCL11A* single nucleotide polymorphisms may help predict hydroxyurea response in Iranian beta-thalassemia patients. *Hemoglobin* 2012;36:371–80.
- [48] Wonkam A, Bitoungui VJ, Vorster AA, Ramesar R, Cooper RS, Tayo B, et al. Association of variants at *BCL11A* and *HBS1L-MYB* with hemoglobin F and hospitalization rates among sickle cell patients in Cameroon. *PLoS ONE* 2014;9:e92506.

Modifying_effect_of_Xmnl.pdf

ORIGINALITY REPORT

19%

SIMILARITY INDEX

14%

INTERNET SOURCES

13%

PUBLICATIONS

10%

STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Malta Student Paper	3%
2	core.ac.uk Internet Source	2%
3	hsrc.himmelfarb.gwu.edu Internet Source	1%
4	www.tandfonline.com Internet Source	1%
5	docplayer.net Internet Source	1%
6	Attaphon Chamchoi, Sirada Srihirun, Kittiphong Paiboonsukwong, Thanaporn Sriwantana et al. "Hemoglobin - bound platelets correlate with the increased platelet activity in hemoglobin E/ β - thalassemia", International Journal of Laboratory Hematology, 2020 Publication	1%
7	www.researchgate.net Internet Source	1%

8

Ravindra Kumar, Rajiv Yadav, Sweta Mishra, M. P. S. S. Singh, Anil Gwal, Praveen K. Bharti, Shanmugam Rajasubramaniam. "Krüppel-like factor 1 (KLF1) gene single nucleotide polymorphisms in sickle cell disease and its association with disease-related morbidities", *Annals of Hematology*, 2021

Publication

1 %

9

Z.-H. Yang, J.-X. Huang, Y.-J. Yao. "Autoscreening of Restriction Endonucleases for PCR-Restriction Fragment Length Polymorphism Identification of Fungal Species, with *Pleurotus* spp. as an Example", *Applied and Environmental Microbiology*, 2007

Publication

1 %

10

journals.plos.org

Internet Source

1 %

11

hsdsignaling.com

Internet Source

<1 %

12

Aumpika Kesornsit, Nutjaree Jeenduang, Dararat Horpet, Thunyaluk Plyduang, Manit Nuinoon. " Quantitative Trait Loci Influencing Hb F Levels in Southern Thai Hb E (: c.79G>A) Heterozygotes ", *Hemoglobin*, 2018

Publication

<1 %

13

www.ncbi.nlm.nih.gov

Internet Source

<1 %

14

Submitted to Universiti Sains Malaysia

Student Paper

<1 %

15

Omega Mellyana, Edi Dharmana, Hardhono Susanto, Nanan Sekarwana. "Effects of combined supplementation of vitamins C and E on the oxidative modification of low-density lipoprotein, soluble form of CD36, soluble vascular cell adhesion molecule-1, and nitrite/nitrate oxide levels in idiopathic nephrotic syndrome", Biomarkers and Genomic Medicine, 2015

Publication

<1 %

16

Florence Urio, Siana Nkya, Helen Rooks, Josephine A. Mgaya et al. "F cell numbers are associated with an X - linked genetic polymorphism and correlate with haematological parameters in patients with sickle cell disease", British Journal of Haematology, 2020

Publication

<1 %

17

Submitted to Universitas Jenderal Soedirman

Student Paper

<1 %

18

Submitted to Higher Education Commission Pakistan

Student Paper

<1 %

19	www.rug.nl Internet Source	<1 %
20	pdfs.semanticscholar.org Internet Source	<1 %
21	Mariko BANNO. "Association of Genetic Polymorphisms of Endothelin-Converting Enzyme-1 Gene with Hypertension in a Japanese Population and Rare Missense Mutation in Preproendothelin-1 in Japanese Hypertensives", Hypertension Research, 2007 Publication	<1 %
22	Shiromi Perera, Angela Allen, Ishari Silva, Menaka Hapugoda et al. "Genotype-phenotype association analysis identifies the role of α globin genes in modulating disease severity of β thalassaemia intermedia in Sri Lanka", Scientific Reports, 2019 Publication	<1 %
23	www.lib.kobe-u.ac.jp Internet Source	<1 %
24	damanhour.edu.eg Internet Source	<1 %
25	www.freepatentsonline.com Internet Source	<1 %
26	Janet L. Kwiatkowski. "Transfusional iron overload in children with sickle cell anemia on	<1 %

chronic transfusion therapy for secondary stroke prevention", American Journal of Hematology, 02/2012

Publication

27

L. Jouini, C. A. Sahli, N. Laaouini, F. Ouali et al. "Association between clinical expression and molecular heterogeneity in β -thalassemia Tunisian patients", Molecular Biology Reports, 2013

Publication

<1 %

28

ajbd.us

Internet Source

<1 %

29

journals.sagepub.com

Internet Source

<1 %

30

Alex E. Felice, Joseph Borg, Monica Pizzuto, Wilhelmina Cassar et al. " A Review of Interplay Between DNA Sequences 5' to the γ - and β -Globin Genes Among Hb F-Malta-I Heterozygotes/Homozygotes and β -Thalassemia Homozygotes/Compound Heterozygotes, and the Effects of Hydroxyurea on the Hb F/F-Erythrocyte; the Need for Large Multicenter Trials ", Hemoglobin, 2009

Publication

<1 %

31

Vip Viprakasit, Paul Tyan, Sarayuth Rodmai, Ali T Taher. "Identification and key management of non-transfusion-dependent thalassaemia

<1 %

patients: not a rare but potentially under-recognised condition", Orphanet Journal of Rare Diseases, 2014

Publication

32

ftp.pagepress.org

Internet Source

<1 %

33

onlinelibrary.wiley.com

Internet Source

<1 %

34

www.cure2children.org

Internet Source

<1 %

35

www.spandidos-publications.com

Internet Source

<1 %

36

Aditi Baruah, Mrinal Kumar Baruah. "Phenotypic Diversity and Clinico-Hematological Profile of Hb E-Beta Thalassemic Children", Indian Journal of Hematology and Blood Transfusion, 2019

Publication

<1 %

37

Evy Sulistyoningrum, Eka Rachmani, Hanif Baroroh, Lantip Rujito. "Annona muricata Leaves Extract Reduce Proliferative Indexes And Improve Histological Changes In Rat's Breast Cancer", Journal of Applied Pharmaceutical Science, 2017

Publication

<1 %

38

Tiwaporn Nualkaew, Pinyaphat Khamphikham, Phitchapa Pongpaksupasin,

<1 %

Woratree Kaewsakulthong et al. "UNC0638 induces high levels of fetal hemoglobin expression in β -thalassemia/HbE erythroid progenitor cells", Annals of Hematology, 2020

Publication

39

Ylenia Barbanera, Francesco Arcioni, Hovirag Lancioni, Roberta La Starza et al.

"Comprehensive analysis of mitochondrial and nuclear DNA variations in patients affected by hemoglobinopathies: A pilot study", PLOS ONE, 2020

Publication

<1 %

40

jdc.jefferson.edu

Internet Source

<1 %

41

repository.cshl.edu

Internet Source

<1 %

42

www.haematologica.org

Internet Source

<1 %

43

www.hindawi.com

Internet Source

<1 %

44

www.ithanet.eu

Internet Source

<1 %

45

"Gene and Cell Therapies for Beta-Globinopathies", Springer Science and Business Media LLC, 2017

Publication

<1 %

46

Barbara J. Bain. "Haemoglobinopathy
Diagnosis", Wiley, 2020

Publication

<1 %

47

Budi Aji, Herman Sumawan. "Hospital with
No-class Wards Policy: An Effort to Create the
Right to Access to Quality Health Care for the
Poor", Journal of Health Management, 2019

Publication

<1 %

48

Sujana Biswas, Arijit Nag, Kanjaksha Ghosh,
Rudra Ray, Kaushik Roy, Anish
Bandyopadhyay, Maitreyee Bhattacharyya.
"Genetic determinants related to
pharmacological induction of foetal
haemoglobin in transfusion-dependent HbE- β
thalassaemia", Annals of Hematology, 2018

Publication

<1 %

49

Ramin Ravangard, Zahra Mirzaei, Khosro
Keshavarz, Sezaneh Haghpanah, Mehran
Karimi. "Blood transfusion versus
hydroxyurea in beta-thalassemia in Iran: a
cost-effectiveness study", Hematology, 2017

Publication

<1 %

Exclude quotes

On

Exclude matches

Off

Exclude bibliography

On