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The Authority of Gene Modifiers in β-Thalassemia Major and Its Relationship to the Pathophysiology of the Disease

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Abstract: β-Thalassemia, an inherited red blood cell disorder, presents a significant health problem worldwide and is caused by defects in the β-globin gene, resulting in the reduction or absence of β-globin chain synthesis. That leads to blood transfusion dependency with its terrible complications. Polymorphisms at position -158 of XmnI-HBG2 on chromosome 11 and BCL11A site on chromosome 2p16 might be linked with elevated hemoglobin F (HbF) appearance, which may, in turn, improve β-thalassemia sternness. This study aims to walk around the amending effects of XmnI and BCL11A loci on HbF levels in Egyptian β-thalassemia patients. Material and Methods. A prospective case-control study of 70 multi-transfused β-thalassemia major patients and 22 controls was performed in the Paediatric hematology unit of Assiut university hospital from June 2019 till April 2021. PCR-RFLP was used to detect single nucleotide polymorphisms at XmnI and BCL11A site loci. Results. XmnI Polymorphism was detected in 9 of 70 patients and associated with higher mean HbF levels (53.48%) than patients without polymorphism (mean Hb level was 42.23%)(P-Value=0.035). The frequency of CT heterozygous genotype was 8 (11.4%), TT homozygous genotype was (1.4%), while the wild genotype CC was detected in 61 (87.1%) of the cases. While BCL11A Polymorphism detected in 21 of 70 patients did not affect either Hb or HbF levels (P-Value =0.26). The TT genotype frequency was 49 (70%), and TC heterozygous genotype was detected in 21 (30 %) of patients. The CC genotype was absent. Conclusion: XmnI -158Gγ polymorphism, but not BCL11A polymorphism, has a modifying effect on both Hb and HbF levels in Egyptian β-thalassaemia major patients. Our research goal is to investigate the frequency of XmnI and BCL11A polymorphism in thalassemia major and investigate the HbF level difference according to the XmnI and BCL11A polymorphism of phenotype group in thalassemia major.

Keywords: β-Thalassemia major, XmnI polymorphism, BCL11A polymorphism.

重型 β-地中海贫血基因修饰剂的权威性及其与疾病病理生理学的关系

摘要：β 地中海贫血是一种遗传性红细胞疾病，在世界范围内呈现出严重的健康问题，由 β 珠蛋白基因缺陷引起，导致 β 珠蛋白链合成减少或缺失。这导致输血依赖及其可怕的并

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发症。11 号染色体上 XmnI 血红蛋白亚基伽玛 2 位点 158 和 2p16 号染色体上 B 细胞淋巴瘤/白血病 11A 位点的多态性可能与血红蛋白 F 出现升高有关，这反过来可能会改善 β 地中海贫血的严重性。本研究旨在探讨 XmnI 和 B 细胞淋巴瘤/白血病 11A 基因座对埃及 β 地中海贫血患者血红蛋白 F 水平的修正作用。材料与方法。2019 年 6 月至 2021 年 4 月，在阿西乌特大学医院儿科血液科对 70 名多次输血的 β 地中海贫血重度患者和 22 名对照进行了前瞻性病例对照研究。聚合酶链反应-限制性片段长度多态性 用于检测 XmnI 和 B 细胞淋巴瘤/白血病 11A 位点基因座。结果。 70 名患者中有 9 名检测到 XmnI 多态性，其平均血红蛋白水平（53.48%）高于无多态性患者（平均血红蛋白水平为 42.23%）（P 值=0.035）。CT 杂合基因型频率为 8（11.4%），TT 纯合基因型频率为（1.4%），而野生基因型 CC 在 61 例（87.1%）中检出。虽然在 70 名患者中的 21 名中检测到 B 细胞淋巴瘤/白血病 11A 多态性不影响 血红蛋白 或 血红蛋白 F 水平（P 值 =0.26）。TT 基因型频率为 49（70%），21 例（30%）患者检测到 TC 杂合基因型。CC 基因型不存在。结论：XmnI-158Gγ 多态性，而非 B 细胞淋巴瘤/白血病 11A 多态性，对埃及 β-地中海贫血重度患者的血红蛋白和血红蛋白 F 水平具有调节作用。我们的研究目标是调查重型地中海贫血中 XmnI 和 B 细胞淋巴瘤/白血病 11A 多态性的频率，并根据重型地中海贫血表型组的 XmnI 和 B 细胞淋巴瘤/白血病 11A 多态性研究 血红蛋白 水平差异。

关键词：β-地中海贫血，XmnI 多态性，B 细胞淋巴瘤/白血病 11A 多态性。

1. Introduction

Beta-thalassemia is a monogenic autosomal recessive disease. It is the most common chronic hemolytic disease in which β-globin chain synthesis is either decreased or absent [1]. In Egypt, β-thalassemia carriers are about 5.3% to ≥ 9% [2], with 1000/1.5 million live births having thalassemia disease in Egypt per year [3]. The pathophysiology of the β-thalassemias is mainly due to the excess α-globin chain, leading to the declaration of impenetrable haemachromes leading to ineffective erythropoiesis and shortened half-life of RBCs. Disease cruelty depends on the α-chain excess degree [4].

Co-inheritance of α-thalassemia or factors that increase γ-globin chain and hemoglobin F (HbF) production alleviate the strictness of β-thalassemia. So, γ-globin chains balance the excess α-globin by inducing HbF [4].

Several genetic mechanisms modifying induction were illustrated. Primary modifiers can influence the severity of thalassemia by changing the degree of globin chain disparity, and the secondary modifiers which not connected to globin imbalance, but they moderate the problems of β thalassemia, which are related to the anemia, or therapy such as iron absorbed from the intestine [5]. β-thalassemia severity is determined by the degree of β-chain deficiency [5]. Three major quantitative trait loci (QTL) have been initiated for variations in HbF in thalassemia patients and normal adults. These QTLs include XmnIC/T polymorphism at position -158 of HBG2, BCL11A on chromosome 2p16, and HBS1L-MYB intergenic region on chromosome 6q23 [6] In this study, we aimed to explore XmnI-$^{G}\gamma$ single nucleotide polymorphisms (SNPs) at position −158 of the HBG2 promoter area (rs7482144) and BCL11A polymorphism (rs11886868) and their perturbation effect on the level of with HbF level in Egyptian β-Thalassemia major patients.

2. Subjects and Methods

2.1. Subjects

This prospective case-control study was recruited from the Paediatric hematology unit of Assiut University hospital from January 2016 to April 2017 on 70 blood transfusion-dependent β-thalassemia major (β-TM) patients; 40 male and 30 female. Their ages ranged from two to 18 years old. Twenty-two healthy age-matched children were included as controls. Patients diagnosed as transfusion-dependent β-TM according to the criteria of Thalassemia International Federation [7]. All patients subjected to routine investigations included complete blood count on CELL-DYN 3700 (Abbott-Germany) and Hb electrophoresis on D10 Automated high-performance liquid chromatography (BIO-RAD Laboratories Inc., Hercules, CA, USA. The Scientific and Ethical Committees approved the study at the Faculty of

Medicine, Assiut University. According to the Declaration of Helsinki, informed consent was obtained from all participants' guardians.

2.2. Methods

Blood was collected aseptically into (K3 EDTA) containing tubes and stored at -20C° until used later for DNA extraction. The DNA analysis of two single nucleotide polymorphisms; XmnI-HBG2-158 (rs7482144) C/T and BCL11A (rs11886868) SNP were done by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). It was done in the Laboratory of Clinical Immunology, Clinical Pathology Department, Assiut University Hospital, Egypt.

DNA extracted from the blood samples by Gene JET Whole Blood Genomic DNA Purification Mini Kits (catalog no. K0781, Thermo Scientific, USA). The primer set used for DNA amplification [8] were as follow:XmnI SNP study Forward primer: 5'GAACTTAAGAGATAATGGCCTAA-3' Reverse primer: 5'-ATGACCCATGGCGTCTGGACTAG-3'BCL11A SNP study Forward primer: 5′-TTTGGTGCTACCCTGAAAGAC-3′Reverse primer 5′-ACTCAACAGTAGCAGAATGAAAGAG-3′ PCR mixture (Total amount 25µl) was prepared as follows: MyTaq™ Red Mix (2x) 12.5µl, Forward Primer (20 µmol) 1 µl, Reverse Primer (20 µmol) 1 µl, Deionised water (RNase/DNase free) 7.5µl, DNA 3 µl. Cycling conditions have been done on (Veriti 96 well-thermal cycler Applied Biosystems ®USA). Initial denaturation for 1 min at 95°C Then, 35 cycles (95ºC for 15 seconds, annealing at 62ºC for 15 seconds, and extension at 72ºC for 10 seconds) followed by a final extension step of 7 min at 72ºC.

For XmnI polymorphism detection, the PCR products were analyzed by restriction enzyme digestion using XmnI enzyme (Catalog no. FD1534, Thermo scientific, USA). After electrophoresis 2%agarose gel, the wild CC genotype showed non-cleavedfragment650 bp; the CT heterozygous genotype gives 3 bands at 650 bp, 418 bp, and 223 bp, and TT homozygous mutant genotype give2 bands at 418 bp and 223 bp (Fig. 1).

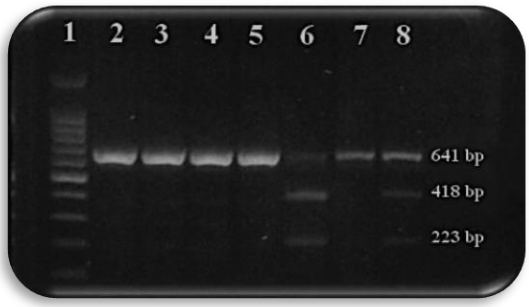


Fig. 1 XmnI-158 C/T genotyping pattern on 2% agarose gel after XmnI (PdmI) restriction enzyme digestion: Lane 1: 100 bp ladder; Lanes (2, 3, 4, 5, and 7) show CC wild homozygous genotype (band at 641 bp); lanes (6 & 8) show CT heterozygous genotype (3 bands at 641 bp, 418 bp, and 223 bp)

For BCL11A polymorphism detection, digestion with MboII restriction enzyme (Catalog no. EE0821, Thermo scientific, USA) revealed a 540 bp fragment in the wild TT genotype, and the TC heterozygous genotype gives 3 bands at 540 bp, 470 bp, and 70 bp by electrophoresis on a 3% agarose gel (Fig. 2).

2.3. Statistical Analysis

Data was collected and analyzed by computer program IBM SPSS version 21 (SPSS Inc., Chicago, Illinois, USA). Data are presented as means ± standard deviations or percentages where appropriate. The independent t-test was used to compare the mean among quantitative data; values of $P \leqslant 0.05$ were regarded as a significant result. The genotype data were tested to fit Hardy–Weinberg equilibrium (HWE). Chi-Square test was used (the $\chi 2$ value indicates the difference between expected and observed values) for genotype count (Table 1).

Table 1 Statistical analysis data

Gene (chromosome)	SNP locus	Genotype			Allele			HWE (χ^2)
		Type	Freq.	proportion	Type	Freq.	proportion	
HBG2 (11.p15.5)	XmnI rs7482144	*CC*	61	0.87	*C*	130	0.93	=0.88
		CT	8	0.11				
		TT	1	0.01	*T*	10	0.07	
BCL11A(2.p16.2)	rs11886868	*TT*	49	0.7	*C*	21	0.15	=2.18
		TC	21	0.3	*T*	119	0.85	

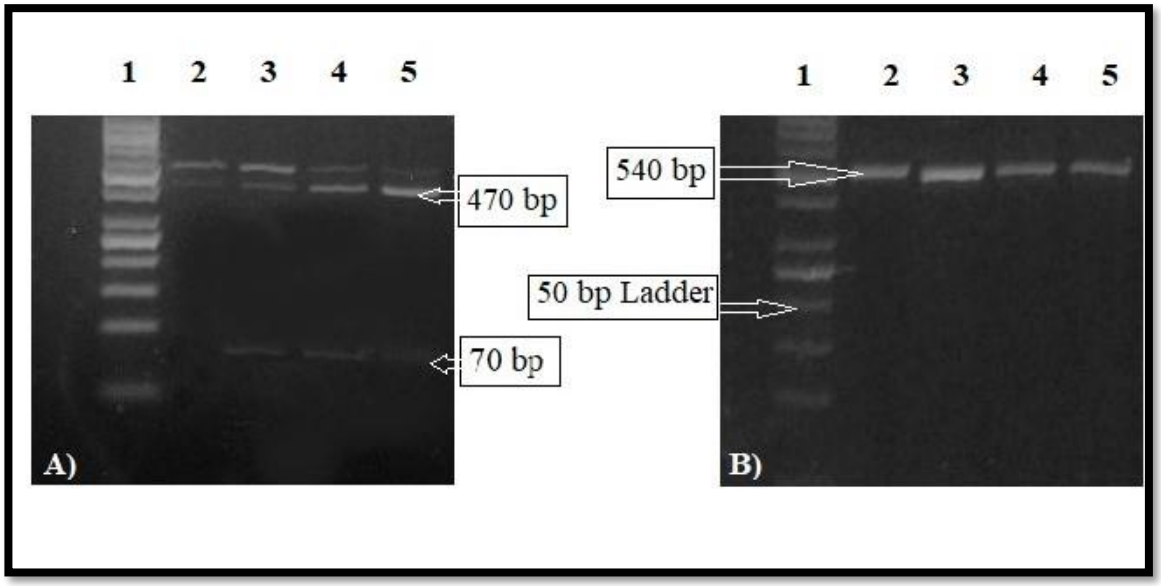


Fig. 2 BCL11A T/ C genotyping patterns on 3% agarose gel after MboII restriction enzyme digestion; A: Lane 1: 50 bp ladder and lane (2,3,4,5) show TC heterozygous genotype (3 bands at 540 bp, 470 bp, and 70 bp); B: Lane (1: 50 bp ladder and lane (2,3,4,5) show TT wild type (band at 540 bp).

2. Results

Seventy β-TM patients and 22 healthy controls were included in our study; their demographic data and routine investigations are illustrated in Table 2. P-value of ≤ 0.05 was considered significant; β-TM = Beta thalassemia major; SD = Standard deviation; No.= number; WBCs = white blood cells; RBCs = red blood cells; HCT = haematocrit; MCV= mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red distribution width; pg = picogram; fL = femtolitre; HbF= fetalhemoglobin.

Table 2 Demographic data and comparative analysis between β-TM patients and controls regarding hematologic data

	β-TM (No.=70)	**Control (No.=22)**	**P-value**
Sex:			
• Female n(%)	30 (42.9%)	8 (36.4%)	= 0.388
• Male n(%)	40 (57.1%)	14 (63.6%)	
• Age in years Mean ± SD	8.73 ± 0.5	5.60 ± 0.6	= *0.004*
Laboratory data Mean ± SD			
• WBCs (10^9/L)	9.35 ± 0.4	8.73 ± 0.6	= 0.401
• RBCs (10^{12}/L)	2.51 ± 0.7	4.49 ± 0.7	< *0.001*
• Hb (gm/dl)	5.60 ± 0.1	11.79 ± 1.6	< *0.001*
• HCT (%)	18.61 ± 5.1	35.28 ± 5.3	< *0.001*
• MCV (Fl)	75.48 ± 9.7	77.96 ± 5.9	= 0.154
• MCH (Pg)	22.68 ± 2.8	26.13 ± 2.3	< *0.001*
• RDW %	26.54 ± 7.1	15.44 ± 1.9	< *0.001*
• Platelets (10^9/L)	498.56 ± 31.4	374.55 ± 15.5	=*0.003*
• Ferritin	1303.20± 64.1	137.36 ± 10.5	< *0.001*
• HbF (%)	43.13 ± 3.1	1.00 ± 0.3	< *0.001*

The observed genotype distribution of XmnI -158 C/T and BCL11A T/C, (2p16.1) polymorphism in patients, was comparable to that predicted by Hardy-Weinberg equilibrium (χ^2 =1.34 and 2.18, respectively P-value >0.05). XmnI Polymorphism was detected in 9 of the 70 patients (the frequency of CT genotype was 11.4%, while TT genotype was 1.4%). The frequency of the T-allele was present in 7.1%of patients, while the C-allele frequency was higher (92.9%) (Table 3).

BCL11A Polymorphism was detected in 21 of 70 patients. The TC genotype was detected in 30 %, and the TT genotype frequency was 70%of patients. NoCC homozygous mutant genotype was found. The frequency of the T-allele was higher than the C-allele, which was 85% vs. 15% in patients. XmnI and BCL11A Genotypic & Allelic Frequency between β-TM patients and Controls were shown in Table 3.

XmnI SNP association with both mean RBCs count and mean Hb levels revealed that their levels were higher in CT than CC genotype with no statistically significant difference (P- value 0.830 and 0.340, respectively). At the same time, no differences were found between genotypes of BCL11A SNPs regarding RBCs count and HB levels (Table 4).

Patients were subdivided according to their Hb levels into two groups. 60 patients were less than 7 gm/dL and 10 were ≥ 7 gm/dL. By studying XmnI, the polymorphism was significantly associated with increased Hb levels. CT + TT genotypes were higher in patients with Hb level ≥ 7 gm/dl with frequency 20% versus,11.7% in patients having Hb level< 7 gm/dl (P-value 0.041). BCL11A showed that the frequency of TT genotype was (80%) in the group having Hb ≥ 7 gm/dL, which was higher than patients with Hb < 7 gm/dL (68.3%). The presence of T-allele was higher in the group with Hb ≥ 7 gm/dL than the other group with Hb level < 7 gm/dL, with no statistically significant difference between groups (Table 5).

Between patients, the proportion of heterozygous CT genotype of XmnI polymorphism was 0.11 with a mean HbF level of 53.48% higher than the wild CC genotype with a mean HbF level of 42.23 %, and this difference was statistically significant (P-Value 0.035). Also, T-allele is associated with mean HbF levels higher than C-allele with no statistical significance (P-Value 0.55). This difference was not found between genotypes or alleles BCL11Apolymorphism (Table 6).

We categorized Patients according to their HbF levels into two groups. The frequency of the XmnI polymorphism (CT + TT genotypes) in the group with HbF level ≥ 20% was higher than the frequency in the group with HbF level < 20%, and this was statistically significant (P-value 0.042). T-allele's presence in the group with HbF level ≥ 20% had frequency more than double the group with HbF level < 20%, which was statistically significant (P-value 0.036). There was no statistically significant difference between groups regarding genotypic and allelic frequencies of BCL11A polymorphism in β-TM patients (Table 7).

Haplotypes frequencies associated with mean HbF levels showed C/T haplotype had the highest frequency, 0.7865, with no difference with HbF. The T/T haplotype frequency was 0.0635 with a HbF mean difference of 19.45 and CI interval (0.18-38.73), a statistically significant P-value of 0.052 (Table 8).

Table 3 XmnI & BCL11A genotypic & allelic frequency between β-TM patients and controls (P-value of ≤0.05 was considered significant; β-TM= Beta thalassemia major; NA = not applicable; OR= odds ratio; CI= confident interval; No= number)

		β-TM (No.=70)	Control (No.=22)	OR (95% CI)	P-value
XmnI					
Genotype	CC	61 (87.1%)	17 (77.3%)	1 (Reference)	
	CT	8 (11.4%)	5 (22.7%)	0.644 (0.121-0.924)	*=0.044*
	TT	1 (1.4%)	0 (0%)	NA	NA
	CT+TT	9 (12.8%)	5 (22.7%)	1.385 (0.737-4.268)	=0.460
Allele	C-Allele	130 (92.9%)	39 (88.6%)	1 (Reference)	
	T-Allele	10 (7.1%)	5 (11.4%)	0.581 (0.097-0.898)	*=0.021*
BCL11A					
Genotype	TC	21 (30%)	16 (72.7%)	1 (Reference)	
	TT	49 (70%)	6 (27.3%)	*6.222 (2.141-18.113)*	*= 0.001*
Allele	C-Allele	21 (15%)	16 (36.4%)	1 (Reference)	
	T-Allele	119 (85%)	28 (63.6%)	*2.289 (1.081-10.685)*	*= 0.001*

Table 4 Parameters associated with Xmn1 & BCL11A polymorphisms in β-TM patients (P-value of ≤0.05 was considered significant; β-TM= Beta thalassemia major; Hb = haemoglobin; RBCs = Red blood cells; HbF= fetal hemoglobin)

Genotype	RBCs	P-value	Hb	P-value	HbF	P-value	Ferritin	P-value
XmnI								
CC	2.96 ± 0.1		6.95 ± 0.3		33.25 ± 3.2		1040.77 ± 73.9	
CT	3.14 ± 0.4	=0.830	8.06 ± 1.1	= 0.340	33.22 ± 8.8	= 0.748	963.67 ± 96.7	= 0.723
TT	2.65		4.50		11.00		533.60	
BCL11A								
TT	2.55 ± 0.7	=0.440	5.68 ± 1.6	= 0.483	42.80 ± 3.5	= 0.897	1281.88 ± 762.7	= 0.616
TC	2.40 ± 0.2		5.41 ± 1.4		43.68 ± 6.2		1352.81 ± 120.9	

Table 5 XmnI and BCL11A genotypic & allelic frequency of the studied β-TM patients according to Hb levels (P-value of ≤0.05 was considered significant; β-TM= Beta thalassemia major; NA = not applicable; OR= odds ratio; CI= confident interval; No= number; Hb = hemoglobin)

		Hb< 7 gm/dl (No.=60)	Hb≥ 7 gm/dl (No.=10)	OR (95% CI)	P-value
XmnI					
Genotypic	CC	53 (88.3%)	8 (80%)	1 (Reference)	
	CT	6 (10%)	2 (20%)	2.208 (0.378-12.89)	=0.379
	TT	1 (1.7%)	0 (0%)	NA	NA
	CT + TT	7 (11.7%)	2 (20%)	*0.802 (0.192-0.991)*	*=0.041*
Allelic	C	112 (93.3%)	18 (90%)	1 (Reference)	
	T	8 (6.7%)	2 (10%)	0.915 (0.264-3.662)	0.121
BCL11A					
Genotypic	TC	19 (31.7%)	2 (20%)	1 (Reference)	
	TT	41 (68.3%)	8 (80%)	0.539 (0.104-2.787)	= 0.461
Allelic	C-Allele	19 (15.8%)	2 (10%)	1 (Reference)	
	T-Allele	101 (84.2%)	18 (90%)	1.925 (0.277-5.491)	= 0.159

Table 6 XmnI and BCL11A genotypic frequency of β-TM patients and its association with mean HbF level (P-value of ≤0.05 was considered significant; S.E.= standard error; CI= confident interval; No= number; HbF= fetalhemoglobin)

	No. (proportion)	HbF mean %(S.E.)	Difference (95% CI)	P-value
XmnI				
Genotype				
C/C	61(0.87)	42.23 (3.32)	1 (Reference)	
C/T	8(0.11)	53.48 (8.02)	*19.39 (0.44 - 38.33)*	
T/T	1(0.01)	11 (0)	-45.10 (-94.43 - 4.23)	*0.035*
Allele				
C	130 (0.93)	43.53(3.09)	1 (Reference)	0.55
T	10(0.07)	48.76(8.50)	-5.23(-21.64 - 11.19)	
BCL11A				
Genotype				
TT	49(0.7)	42.8 (3.54)	1 (Reference)	0.26
TC	21(0.3)	43.68 (6.23)	-9.15 (-24.99 - 6.69)	
Allele				
C	21(0.15)	43.68 (6.23)	1 (Reference)	0.24
T	119 (0.85)	43.32 (3.08)	0.61(-12.45-13.67)	

Table 7 XmnI and BCL11A Genotypic & Allelic Frequency of the studied β-TM patients according to HbF Categories (P-value of ≤0.05 was considered significant; β-TM= Beta thalassemia major; NA = not applicable; OR= odds ratio; CI= confident interval; No= number; HbF= fetal hemoglobin)

		HbF< 20% (No.=17)	HbF ≥ 20 % (No.=53)	OR (95% CI)	P-value
XmnI					
Genotype	CC	16 (96%)	45 (84.9%)	1 (Reference)	
	CT	0 (0%)	8 (15.1%)	NA	NA
	TT	1 (4%)	0 (0%)	NA	NA
	CT + TT	1 (4%)	8 (15.1%)	*0.359 (0.042-0.481)*	*= 0.042*
Allelic	C	32 (94.1%)	90 (84.9%)	1 (Reference)	
	T	2 (5.9%)	16 (15.1%)	*0.352 (0.096-0.625)*	*= 0.036*
BCL11A					
Genotypic	TC	6(35.3%)	15 (28.3%)	1 (Reference)	
	TT	11 (64.7%)	38 (71.7%)	1.382 (0.433-4.410)	= 0.585
Allelic	C	6 (17.6%)	15 (13.2%)	1 (Reference)	
	T	28 (82.4%)	91 (85.8%)	1.301 (0.652-5.254)	= 0.263

Table 8: Haplotype association with HbF level in the studied β-TM patients (P-value of ≤ 0.05 was considered significant; β-TM= Beta thalassemia major; CI= confident interval; HbF= fetal hemoglobin)

Xmn1	BCL11A	Frequency	Difference (95% CI)	P-value
C	T	0.7865	0.00	---
C	C	0.1421	-8.68 (-24.23 - 6.86)	0.28
T	T	0.0635	*19.45 (0.18 - 38.73)*	*0.052*
T	C	0.0079	-65.59 (-133.5 - 2.33)	0.063

3. Discussion

β-thalassaemia/ HbF in Egyptian patients has a wide range of sickness cruelty from the unrequired regular blood transfusion to transfusion dependent.

Prediction of the disease severity is helpful for clinical management and quality of

life of the patients. This study explored the predictive SNPs for disease severity in β-thalassemia/HbF patients. Analysis of SNP interaction revealed that rs766432 (*BCL11A*), rs9399137 (*HBS1L-MYB*), and rs72872548 (*HBE1*) are the highest associated with clinical symptoms. The frequency of wild CC genotypes for the XmnI gene was (87.1%) in β-TM patients, (11.4%) for heterozygous CT, and (1.4%) for homozygous TT genotypes. To our knowledge, all other Egyptian results were in line with ours' [4, 9, 10]. Furthermore, the CC genotype was predominant in other studies [11-13]. Conversely, a higher frequency of polymorphism was reported in other studies [14, 15]. In addition, two studies were done in Iraq and Kuwait that described XmnI polymorphism in 47% and 75% of β-TI patients, respectively [16,17]. The significant variations in the genotypic distribution between this study and different studies in other countries; can be attributed to the genetic differences in these ethnic groups. Ethnicity is an important factor affecting this genetic heterogenicity. We found that XmnI polymorphism is associated with an increased RBCs count and Hb levels in β-TM patients, but it was a non-significant difference. Our patients were subdivided into two groups; 20% of patients with CT+TT genotypes had Hb

level ≥ 7gm/dl, and 11.7% less than 7gm/dl with statistically significant difference (P-value =0.041).

Others demonstrated that XmnI positive patients have higher total hemoglobin levels [10]. In addition, Iranian studies have established that T-Allele at the polymorphic site is associated with a noteworthy increase in Hb levels [15, 18].

Our results revealed that HbF level was elevated among patients with XmnI polymorphism, patients with wild CC genotypes showed mean HbF levels less than heterozygous CT genotypes, and homozygous TT, and this difference were statistically significant (P-value = 0.035). Also, when patients were categorized into two groups according to HbF levels; (15.1%) ofpatientshavingXmnI variants showedHbF level ≥20%, and only (4%) had HbF level <20%, this difference was statistically significant. It maintains the relationship between the attendance of the polymorphism and amplified HbF level between patients.

Furthermore, Tantawy et al. [4] determined that only one patient with $XmnI^{G}\gamma$ heterozygous CT genotype with significantly high HbF level (71%) [4]. Egyptian patients had higher HbF levels with CT genotype of XmnI polymorphism, but the difference was marginally insignificant (p= 0.06) [10[. Some earlier studies following our study reported that the presence of the XmnI polymorphism might contribute to the overproduction of HbF, causing mild features of β-thalassemia [9, 19, 20].

Also, a significantly higher HbF level was found in patients with at least one XmnI variant than patients without polymorphism. The TT genotype had significantly higher mean HbF than heterozygous subjects [13, 18]. Studies reported a relationship between XmnI polymorphism and increased HbF levels and enhanced severity of clinical symptoms in patients [21].

The cause of the increase in HbF level with XmnI polymorphism may be qualified to the presence of T-allele that reduces binding or interfere with the communication of transcription silencers to the regulatory sequences at β-LCR. The polymorphism bypasses the attachment of the explicit transcriptional inhibitors to the regulatory sequences of the HBG2 gene. Therefore, in erythropoietic stress conditions, the γ-globin gene is reactivated in adult life [22, 23].

These findings point that the main QTLs of the β-thalassemia phenotype, including XmnI polymorphism, potentially obstruct the binding of inhibitory transcription factors accountable for silencing HbF expression. That is chiefly significant for deliberation of targeted therapies interfering with the interaction of these transcription inhibitors with β-LCR. Several drugs, such as 5-azacytidine, erythropoietin, hydroxyurea, and butyrates, stimulate γ-globin chain production in vivo, reducing clinical strictness and symptoms in β-T patients [24]. However, other studies reported no relationship between the T-allele of XmnI polymorphism and HbF level increase [25]. That may be due to some unrevealed genetic determinants acting upstream of XmnI polymorphism.

Regarding the BCL11A SNP, the frequency of the wild TT genotype was (70%) in β-TM patients, while the heterozygous TC genotype frequency was (30%). Homozygous CC genotype was absent.

Also, others reported the homozygous mutant CC genotype in a very low frequency (4.5%), which concur with our observations. In Asia variations in genotypic frequencies were evident: 2.4–12.8% for CC, 38-42.9% for CT, and 48–54% for TT [26]. A study on 100 Egyptian sickle cell disease patients and 100 controls reported nearly absent CC mutant allele [26]. A higher frequency was found in a cohort done on 106 β-TM in France on patients of various geographical origins (Mediterranean and Asian) [27].

On the obstinately, the frequency of CC genotype was found in high frequency [8, 28-30]. These differences in the frequency of variant genotype might be due to ethnic and geographic differences [31].

According to RBCs count, Hb and HbF levels. Their mean levels were nearly the same with the presence or absence of mutant C-allele of BCL11A polymorphism. QTL on BCL11A gene (rs11886868) in β-thalassemia patients showed no significant effect on HbF levels or clinical severity. The possibility to elucidate this circumstance is that all allele mutations in β-thalassemia patients were severe (combination of β^0 and β^+) or that these patients' HbF and clinical appearance are severe [8]. The study on Egyptian SCD patients was comparable to our results according to the BCL11A SNP effect on HbF level with no significant correlation [26]. In contrast, previous studies showed that rs11886868 had a strong relationship with the increase in HbF in both normal individuals and thalassemia patients [32].

The difference in results between many studies confirms that the effect of SNPs can be different among different regions, reflecting a link between ethnicity and other genetic susceptibility factors. A group of researchers found that rs766432 had a relationship with the percentage of HbF better than rs11886868 on the BCL11A gene [33].

The relationship between XmnI and BCL11A SNPs was deliberated to recognize the effect of SNPs in modifying HbF levels in β-thalassaemic patients and alleles combination in studied SNPs haplotypes. The C/T allele (XmnI/BCL11A) was the commonest in this study (0.786). At the same time, the T/T alleles were associated with increasedHbF levels. Rujito et al. accounted that the C/C allele is the commonest even as the T/C alleles had a modifying effect onHbF levels [8]. Ethnic dissimilarities and some other genetic factors make available a justification of HbF levels change between different cross studies. The T/T allele combinations led to HbF levels increase. The result of

genetic epidemiology in our study, including the data symptomatic of those variations of SNP loci in the XmnI, and BCL11A sites, are meticulous to a specific geographical area.

3.1. Limitation of the Study

Determination of the frequency of XmnI and BCL11A polymorphism in thalassemia major and investigations of β-thalassemia sub-phenotypes from the HbF level according to the XmnI and BCL11Apolymorphism of phenotype group in thalassemia major did not give way the unsurprising result, even though some trends have appeared. Involvedness in monitoring the pediatric patients led to poor control of environmental prognostic factors. In addition, the lack of cytokine levels measurement was not done, which was due to limited funding resources. That may have impacted some results of our study. Too high for some parameters, the numeral of missing data requires additional study and further statistical analysis. The analyzed SNPs were not the most frequent or the most strongly associated with the HbF level in the pediatric population. Our results need to be further developed to support our decisive prognosis and valuable therapeutics.

4. Conclusions

XmnI polymorphism rather than BCL11A polymorphism have an altering effect on HbF construction and improved Hb levels between β-TM patients. So, it helps in identifying patients' benefits from HU therapy. Furthermore, the T/T haplotype (rs7482144 and rs11886868) is connected with higher HbF levels betweenβ-TM patients. That may assist in advising prediction and guiding therapeutics. Recognition of the genetic mechanisms of HbF production can provide crucial genetic evidence for patient lamination and prediction of the disease severity. Such information could provide more customized patient care corresponding to the expected phenotype. However, the predictive SNPs of this study might not predict disease severity in β+(severe)/β0-thalassemia and β thalassemia patients who have a higher degree of α- to non-α-globin imbalance and being the cause of the severe thalassemia.

4.1. Declarations

The authors declare that they have no conflict of interest. Consent to participate Advised consent was taken from all subjects agreeing with the 1964 Helsinki Declaration. Ethical agreement The study protocol was endorsed by the Research Ethics Committee of Paediatrics, Medical Biochemistry and Clinical Pathology Departments, Faculty of Medicine, Assiut University.

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