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Genetic Polymorphisms of HBS1L-MYB (rs4895441 and rs9376090) in Egyptian Patients with Hemoglobinopathy

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Abstract

Objective: Study the HBS1L-MYB (rs4895441 and rs9376090) genetic polymorphisms in Egyptian patients with β-thalassemia major and sickle cell disease and its relation to Hb F and severity of the disease. **Background:** Hb F is a predominant modulator for the severity of β-thalassemia major & sickle cell disease. Genetic polymorphism in the intergenic region (HBS1L-MYB) between GTP-binding elongation factor HBS1L and myeloblastosis oncogene MYB on chromosome 6q is associated with high fetal hemoglobin levels. **Subjects and Methods:** 150 subjects were included in this study. For all studied groups: Complete blood picture and serum ferritin were evaluated. For patients, hemoglobin variants were separated by High-performance liquid chromatography. Genotyping of HBS1L-MYB (rs4895441 & rs9376090) was evaluated by real-time polymerase chain reaction technique using TaqMan probe. **Results:** AG, CT genotypes, and G, C alleles of HBS1L-MYB (rs4895441 & rs9376090) were significantly high in sickle cell patients [OR (3.400); 95% C.I (1.482 - 7.799)], (p = 0.003) & [OR (4.522); 95% C.I (1.854 - 11.029)], (p = 0.001) respectively. Also, a significant association was detected between polymorphisms and disease severity. However, in β-thalassemia major, no significant association was detected. **Conclusion:** In sickle cell disease patients, Genetic polymorphisms in HBS1L-MYB (rs9376090 & rs4895441) affect the level of Hb F which could improve the prognosis of these patients.

Keywords

Hemoglobinopathy, Beta-Thalassemia, Sickle Cell Disease, HBS1L-MYB Polymorphism, Polymerase Chain Reaction

1. Introduction

Hemoglobinopathies are inherited diseases caused by defects in globin chain synthesis; they include thalassemia syndromes, sickle cell disease & globin chain variants [1]. β-thalassemia results from numerous mutations or rarely deletions of the β globin gene on chromosome 11. These mutations primarily point mutations that affect transcriptional control, translation, and splicing of the Hb B gene and gene product [2].

β-thalassemia is characterized by chronic anemia and iron excess due to blood transfusion and increased gastrointestinal absorption [3]. A comparison of Egyptian β-thalassemia patients with their healthy peers revealed that the former reported lower growth parameters, lower physical, mental, schoolwork, and overall quality of life scores [4].

The intensity of β-thalassemia may be mitigated by the production of Hb F as it may alter the imbalance of the α/β globin chain [5].

Sickle cell disease (*SCD*) is considered as one of the commonest gene disorders of human beings. In Africa, more than 200,000 infants are born yearly with sickle cell anemia [6]. Sickle cell disease is an inherited genetic disorder, resulting from homozygous and compound heterozygote mutation in the β globin gene as a single base-pair point mutation (GAG to GTG) results in the substitution of glutamic acid (hydrophilic) to Valine (hydrophobic) in the 6^{th} position of the β-chain of hemoglobin resulting in hemoglobin S formation [7].

The increase of Hb F can decrease the severity of SCD because of its ability to inhibit the polymerization of Hb S [8].

Hb F expression is affected by several quantitative trait loci (QTL) within and outside the β globin gene cluster. The HBG2, BCL11A & HBS1L-MYB are well-known major modifier loci [9]. HBS1L-MYB genes are expressed in the precursor cells of the erythroid. HBS1L encodes a protein with apparent GTP binding activity and is included in different cellular processes while MYB encodes a transcription factor for erythroid differentiation in hematopoiesis [10]. Disturbance in the interval between the HBS1L and MYB suppresses MYB expression and gives rise to the increased level of Hb F [7]. Single nucleotide polymorphisms (SNPs) in the Gγ-globin gene promoter in BCL11A regions and HBS1L-MYB intergenic polymorphism (HMIP) are thought to be associated with Hb F levels. Strongly associated markers in HMIP block 2, are either in intron 1a (rs9376090, rs9399137, rs9402685 and rs11759553), or directly upstream of the 5 UTR of HBS1L exon 1a (rs4895440, rs4895441, rs9376092, rs9389269, rs9402686, rs11154792 and rs9483788) [11].

With the increasing prevalence of β-thalassemia and SCD among Egyptian children, regardless of traditional genetic factors, there are no data related to the association of HBS1L-MYB intergenic polymorphism with these conditions. Thus, this study was done to investigate the association of HBS1L-MYB (rs 9376090 & rs 4895441) genetic polymorphisms in Egyptian patients with β-thalassemia major & sickle cell disease and its relation to Hb F and disease

severity.

2. Subjects and Methods

This case-control study was conducted in the Clinical Pathology Department, Menoufia University and, Pediatric Department, Ain-shams University from September 2018 to December 2019. One hundred fifty subjects were included and divided into three groups:

Group [1] (*β*-thalassemia major patients): included 50 patients with *β*-thalassemia major diagnosed by HPLC and under regular blood transfusion.

Group [2] (sickle cell patients): included 50 sickle cell disease patients, diagnosed by HPLC and under hydroxyurea therapy.

Group [3] (controls): included 50 apparently healthy subjects, age and gender-matched with patients.

Informed written consent was attained from subjects who participated in this study. The study was approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

For all subjects: Complete history taking, complete blood count, serum ferritin and genotyping of HBS1L-MYB (rs4895441 & rs9376090) by real-time polymerase chain reaction technique were done. Also, separation of hemoglobin variants by high-performance liquid chromatography (HPLC) for patients.

Samples collection: Before the planned blood transfusion (for *β*-thalassemia major patients) and under complete aseptic conditions, 5 ml of venous blood were collected, and then divided as follows: 2 ml were collected into an EDTA tube for CBC and HPLC, another EDTA tube with 2 ml blood for genotyping of HBS1L-MYB gene. The remaining 1 ml was collected in a plain tube for measurement of serum ferritin level.

Laboratory investigations: Serum ferritin was done by the auto-analyzer **(HITACHI Cobas e 411 High-Technologies Corporation Tokyo, Japan).** Complete blood count was done by Sysmex1 XN-1000 Automated Hematology Analyzer **(Sysmex Corporation, Japan).** Hb separation and quantification was carried out using HPLC **"Arkray, ADAMS™ A1c HA8180T analyzer" (Kyoto, Japan)** and Bio-Rad D10-Hemoglobin A1C Testing system **(Bio-Rad Laboratories, Hercules, CA, USA).**

3. Determination of HBS1L-MYB (rs 9376090 and rs 4895441) Genotypes by Real Time-PCR

1) Genomic DNA extraction: [12]

The genomic double-stranded DNAs were extracted from whole blood using (Thermo Scientific Gene Jet. Whole Blood Genomic DNA Purification Mini Kit) supplied by **(Thermo Fisher Scientific, USA).** The purity and concentration of extracted DNAs were observed with a spectrophotometer **(Implen NanoPhotometer™ N60 UV/VIS spectrophotometer, Germany)**. The eluted genomic DNA was aliquoted and stored at −80˚C until the performance of PCR.

2) TaqMan SNP genotyping assay: [13]

Genotyping of **HBS1L-MYB (rs 9376090 and rs 4895441)** polymorphism was performed using ABI 7500 real-time PCR system **(Applied Biosystem, Foster City, California, USA).** Fluorescent labeled probes, TaqMan probes **(Thermo Fisher Scientific, USA)** [VIC/FAM]:

(rs4895441):

CTGGGGAGAAAGACTCTTTGTAAAGT**[A/G]**TACATGAGCAGAACTGAG TAAGT.

(rs9376090):

AGCTAAGTCTAGCTGAGTGTTAG**[C/T]**GGGGGATACTGCCAGGAACAA ATGA.

PCR reaction was done by adding 30 µ Master mix **(Applied Biosystems, Foster City, CA)**, 3.75 µ primer, extracted DNA (according to measured DNA concentration), and 11.25 µ sterile deionized water into PCR wells, PCR was done by the following conditions: initial denaturation at 94˚C for 5 minutes, 40 cycles (94˚C for 30 sec for denaturation then 62˚C for 1 minute for annealing) and for final extension 7 minutes at 72˚C min.

4. Statistical Analysis

Results were collected, tabulated, and statistically analyzed by an International Business Machines (IBM) compatible personal computer with Statistical Package for the Social Sciences (SPSS) version 23 (SPSS Inc. Released 2015.IBM SPSS statistics for windows, version 23. 0, Armnok, NY: IBM Corp.) Data were expressed as mean ($\bar{x}$), standard deviation (SD), Number (No), and percentage (%). Kruskal Wallis, Chi-square, Mann Whitney, Fischer's Exact, Post hoc tests were used. Two-sided P-value of ≤0.05 was considered statistically significant.

5. Results

In β-thalassemia major patients, age of disease presentation was ranged from (6 - 11 months). Thirty-seven β-thalassemia major patients were on regular blood transfusion every 3 weeks, while thirteen of them were on regular blood transfusion every 5 weeks. In SCD, the age of disease presentation was (9 - 36 months) and the numbers of crises were (1 - 8/year). There was a statistically significant increase in serum ferritin in β-thalassemia than controls, also a significant decrease in Hb level in β-thalassemia compared to other groups (Table 1). HPLC for Hb distribution in β-thalassemia major and sickle cell disease patients showed a significant difference between the two groups regarding Hb A and Hb F (Table 2).

The independent segregation genotype for the two SNPs in the controls was in agreement with the Hardy-Weinberg equilibrium ($p > 0.05$) as (AA) and (TT) were the wild types of HBS1L-MYB (rs4895441 and rs9376090) respectively. AG, CT genotypes and, G, C alleles of HBS1L-MYB (rs4895441 and rs9376090) polymorphisms, were significantly higher in sickle cell disease patients. Also sig-

nificant difference was detected between sickle cell disease and thalassemia patients regarding (rs9376090) with no significant difference regarding (rs4895441) (Table 3(a)). In β-thalassemia major, no significant difference was detected between β-thalassemia major patients and controls regarding HBS1L–MYB (rs4895441 & rs9376090) genotyping and allele's frequency (Table 3(b)).

While in SCD, (Table 3(c)) comparing CT, AG, genotypes of rs9376090 & rs4895441 between SCD and control; CT and AG were significant in SCD (P = 0.001), [OR (4.522); 95% C.I (1.854 - 11.029)] & (P 0.003), [OR (3.400) & 95% C.I (1.482 - 7.799)] respectively. Also C and G alleles frequency of both genes were significantly high in SCD (P = 0.001) [OR (3.500); 95% C.I (1.595 - 7.679)] & (P 0.030) [OR (2.092); 95% C.I (1.066 - 4.108)] respectively.

Table 1. Comparison between the studied groups according to demographic and laboratory data.

Parameters	Thalassemia (n = 50)	Sickle cell disease (n = 50)	Controls (n = 50)	p value	Post hoc P value.
Age/(years)					
Mean ± SD.	7.6 ± 4.0	7.3 ± 3.9	8.2 ± 3.5	0.344	-
Median (Min. - Max.)	7.5 (2.5 - 13)	6 (3 - 14.0)	7 (2 - 15)		
Gender Male	28(56.0%)	30 (60%)	26 (52%)	0.723	-
Female	22 (44.0%)	20 (40%)	24 (48%)		
Ferritin (ng/ml)					$p_1 = 0.001^*$
Mean ± SD.	3004.7 ± 1845.4	223.3 ± 120.0	91.5 ± 55.8	0.001*	$p_2 = 0.001^*$
Median (Min. - Max.)	2541.8 (658 - 8504)	74 (10 - 210)	71.5 (20.0 - 231.2)		$p_3 = 0.828$
Hb (gm/dl)					$p_1 = 0.001^*$
Mean ± SD.	7.6 ± 1.4	9.8 ± 1.6	12.3 ± 0.88	0.001*	$p_2 = 0.001^*$
Range	4.7 - 10.3	5.8 - 11	10.9 - 13		$p_3 = 0.001^*$

SD: standard deviation, **p**: p-value for comparing between the studied groups. *: Statistically significant at p ≤ 0.05. **p1**: p-value for comparing **Thalassemia** and **Sickle cell disease**. p_2: p-value for comparing **Thalassemia** and **controls. p_3**: p-value for comparing **Sickle cell disease** and **controls**.

Table 2. Comparison between Thalassemia and Sickle cell groups according to HPLC results.

Parameters	Thalassemia (n = 50)	Sickle cell disease (n = 50)	P value
HB F%			
Mean ± SD	16.8 ± 14.4	8.0 ± 5.9	<0.001*
HB A2%			
Mean ± SD	3.3 ± 0.95	2.9 ± 1.2	0.060
HB A%			
Mean ± SD	80.2 ± 14.5	30 ± 12.4	<0.001*
HB S%			
Mean ± SD		59.5 ± 21.6	-

SD: standard deviation, **p**: p-value for comparing between the studied groups. *: Statistically significant at p ≤ 0.05.

Table 3. (a): Distribution of HBS1L-MYB (rs9376090 and rs4895441) genotypes and allele frequency among the studied groups; (b): Comparison between Thalassemia and control groups regarding HBS1L-MYB (rs9376090 and rs4895441) genotypes and allele frequency; (c): Comparison between Sickle cell disease and control regarding HBS1L-MYB (rs9376090 and rs4895441) genotypes and allele frequency.

(a)

Parameters	Thalassemia (n = 50)	Sickle cell disease (n = 50)	Controls (n = 50)	P value
rs9376090				
TT	41 (82%)	23 (46%)	40 (80%)	$p_1 = 0.001^*$
CT	6 (12%)	26 (52%)	10 (20%)	$p_2 = 0.13$
CC	3 (6%)	1 (2%)	0 (0%)	$p_3 = 0.001^*$
Allele frequency				$p_1 = 0.004^*$
T	88 (88%)	72 (72%)	90 (90%)	$p_2 = 0.65$
C	12 (12%)	28 (28%)	10 (10%)	$p_3 = 0.001^*$
rs4895441				
AA	28 (56%)	20 (40%)	34 (68%)	$p_1 = 0.06$
AG	20 (40%)	30 (60%)	15 (30%)	$p_2 = 0.44$
GG	2 (4%)	0 (0%)	1(2%)	$p_3 = 0.008^*$
Allele frequency				$p_1 = 0.33$
A	76 (76%)	70 (70%)	83 (83%)	$p_2 = 0.22$
G	24 (24%)	30 (30%)	17 (17%)	$p_3 = 0.03^*$

P: p value for comparing between the studied groups. **p_1**: p-value for comparing between **Thalassemia** and **Sickle cell disease**. **p_2**: p-value for comparing between **Thalassemia** and **controls**. **p_3**: p-value for comparing between **Sickle cell disease** and **controls**. *: Statistically significant at $p \leq 0.05$.

(b)

Parameters	Thalassemia (n = 50)	Controls ® (n = 50)	p	OR (95% C.I)
rs9376090				
TT®	41 (82%)	40 (80%)	-	1.000
CT	6 (12%)	10 (20%)	0.337	0.585 (0.194 - 1.762)
CC	3 (6%)	0 (0%)	0.243	-
Allele frequency				
T®	88 (88%)	90 (90%)	---	1.000
C	12 (12%)	10 (10%)	0.651	1.227 (0.504 - 2.986)
rs4895441				
AA®	28 (56%)	34 (68%)	-	1.000
AG	20 (40%)	15 (30%)	0.257	1.619 (0.702 - 3.734)
GG	2 (4%)	1 (2%)	0.591	2.429 (0.209 - 28.200)
Allele frequency				
A®	76 (76%)	83 (83%)	----	1.000
G	24 (24%)	17 (17%)	0.220	1.542 (0.770 - 3.089)

OR: Odds ratio, ®: Reference type, **CI:** Confidence interval, **LL:** Lower limit, **UL:** Upper Limit, **p**: p-value for comparing between the studied group, *: Statistically significant at $p \leq 0.05$.

(c)

Parameters	Sickle cell disease (n = 50)	Controls* (n = 50)	p	OR (95% C.I)
rs9376090				
TT*	23 (46%)	40 (80%)	-	1.000
CT	26 (52%)	10 (20%)	<0.001*	4.522 (1.854 - 11.029)
CC	1 (2%)	0 (0%)	0.375	-
Allele frequency				
T*	72 (72%)	9 (90%)	--	1.000
C	28 (28%)	10 (10%)	<0.001*	3.500 (1.595 - 7.679)
rs4895441				
AA*	20 (40%)	34 (68%)	-	1.000
AG	30 (60%)	15 (30%)	0.003*	3.400 (1.482 - 7.799)
GG	0 (0%)	1 (2%)	1.000	-
Allele frequency				
A*	70 (70%)	83 (83%)	----	1.00
G	30 (30%)	17(17%)	0.030*	2.092 (1.066 - 4.108)

OR: Odds ratio, *: Reference type, **CI:** Confidence interval, **LL:** Lower limit, **UL:** Upper Limit, **p**: p-value for comparing between the studied group, *: Statistically significant at $p \leq 0.05$.

In β-thalassemia major patients, there was no significant association between HBS1L-MYB (rs 9376090 and rs 4895441) genotypes and age of disease presentation, frequency of blood transfusion, splenectomy, Hb level, and Hb F (Table 4). Meanwhile, in the SCD group, CT genotype of HBS1L-MYB (rs 9376090) was significantly associated with a lower number of crises and lower Hb S level, however, no significant association was detected between this polymorphism and age of disease presentation and Hb F, while AG genotype of HBS1L-MYB (rs4895441) was significantly associated with lower number of sickling crises, higher age of disease presentation, higher Hb F and lower Hb S levels (Table 5).

6. Discussion

β-thalassemia and SCD are serious autosomal hereditary disorders that occur in several populations around the world. β-thalassemia is associated with genetic mutations that contribute to a reduced or total loss of expression of the β-globin gene. While in the SCD, mutation of the β-globin gene induces alterations in protein structure [14]. In β-thalassemia, the accumulation of unbound alpha-globin chains precipitates in the erythrocyte precursors resulting in bone marrow destruction resulting in ineffective erythropoiesis. As well, excess unbound alpha-globin chains cause membrane damage in mature red thalassemia cells leading to hemolysis [15]. SCD pathogenesis starts when hemoglobin deoxy-sickle molecules are polymerized leading to the deformation of the red blood cell structure. Due to environmental and genetic factors, clinical phenotypes are extremely variable in both diseases, ranging from relatively mild to severe anemia [14].

Table 4. Relation of HBS1L-MYB (rs9376090 & rs 4895441) genotypes with clinical and laboratory features of thalassemia patients.

Parameters	Age of presentation "months" mean ± SD	Frequency of blood transfusion		Splenectomy		Hb	Hb F%
		Regular (every 3 w)	Regular (every 5 w)	Yes	No	mean ± SD	mean ± SD
				rs 9376090			
TT	6.6 ± 1.4	34 (82.8%)	7 (7.2%)	33 (80.5%)	8 (19.5%)	7.8 ± 1.34	17.2 ± 15.0
CT	6.5 ± 1.2	2 (33.3%)	4 (66.6%)	4 (66.7%)	2 (33.3%)	7.3 ± 1.1	10.1 ± 7.5
CC	6.6 ± 1.1	1 (33.3%)	2 (66.6%)	2 (66.7%)	1 (33.3%)	6.5 ± 1.05	18.3 ± 16.5
P value	0.737	0.469		0.663		0.259	0.620
				rs 4895441			
AA	6.7 ± 1.4	22 (78.6%)	6 (21.4%)	21(75.0%)	7 (25.0%)	7.4 ± 0.96	17.8 ± 14.9
AG	6.5 ± 1.3	14 (70%)	6 (30%)	17 (85.0%)	3 (15.0%)	8.05 ± 1.7	14.3 ± 13.4
GG	7.0 ± 1.4	1 (50%)	1 (50%)	1 (50.0%)	1 (50.0%)	7.09 ± 0.57	17.5 ± 23.3
P Value	0.848	0.095		0.442		0.278	0.678

SD: standard deviation, P**:** p-value for comparing between the studied groups, **Hb:** hemoglobin.

Table 5. Relation of HBS1L-MYB (rs 9376090 and rs 4895441) genotypes with clinical and laboratory features of sickle cell disease patients.

Parameters	Age of presentation "months" mean ± SD	number of crisis/year mean ± SD	HB F% mean ± SD	HB S% mean ± SD
		rs 9376090		
TT	21.6 ± 18	3.6 ± 1.9	8.4 ± 6.5	68.3 ± 18.4
CT	16.8 ± 12	2.7 ± 1.03	7.1 ± 5.6	54.4 ± 22.6
CC	36	2.0	1.5	47.0
P value	0.35	0.03 *	0.583	0. 031*
		rs 4895441		
AA	21.6 ± 13	4.1 ± 2.2	4.7 ± 5.0	63.7 ± 24.8
AG	32.4 ± 19	1.8 ± 0.98	9.4 ± 6.04	47.8 ± 23.4
P value	0.02 *	0.001 *	0. 013 *	0. 022 *

SD: standard deviation. **p:** p-value for comparing between the studied group, **Hb:** hemoglobin.

General genetic HbF modifiers influence disease outcomes such as frequency of painful episodes, the incidence of end-organ complications, the efficacy of hydroxyurea therapy, and life expectancy [16]. Polymorphisms in the intergenic region between HBS1L and MYB are identified as HBS1L-MYB Intergenic Polymorphism (HMIP) is significantly related to variability in expression of Hb F. The stimulation of Hb F expression can offer alternate treatment for the improvement of these diseases severity [17].

This research investigated HBS1L–MYB (rs 4895441 & rs 9376090) gene polymorphisms in Egyptian patients with hemoglobinopathy (Beta thalassemia major & sickle cell disease) and its relation to Hb F and severity of the disease.

In *β*-thalassemia major, age of disease presentation was (6 - 11 months), 74% of patients were on regular blood transfusion every 3 weeks and 26% every 5 weeks. These results were in agreement with Tubman *et al.* [18] who reported that *β*-thalassemia major patients remain asymptomatic until 3 - 6 months of age or more when Hb F production falls and adequate Hb A can't be produced. According to blood transfusion, Langhi *et al.* [19] indicated that *β*-thalassemia major patients received regular blood transfusion throughout life, usually administered every 2 - 5 weeks depending on the transfusion needs of each individual. While in SCD patients' age of disease presentation was (9 - 36 months) and the number of crises was (1 - 8/year). Strouse, [20] reported that SCD patients during the first 6 months of life are protected by elevated levels of Hb F and when reduced symptoms of SCD appear and clarified that the frequency of crises is extremely variable as crises have many triggers as hypoxemia, dehydration & change in body temperature. In *β*-thalassemia major, the serum ferritin level was significantly high. Taher and Saliba [21] explained this increase in transfusion-dependent thalassemia patients by the process of iron loading secondary to transfusion therapy and in non-transfusion–dependent thalassemia patients by ineffective erythropoiesis, enhanced intestinal absorption, and hepcidin suppression.

In SCD, AG genotype & G allele of HBS1L-MYB (rs4895441) were significantly higher than controls. Also, AG genotype was significantly associated with higher age of disease presentation, lower number of crises, higher Hb F, and lower Hb S level. These results were in agreement with Cardoso *et al.* [11] who demonstrated a significant association between AG genotype in African SCD patients and higher Hb F level, better clinical picture, and lower incidence of pain. Also, Menzel *et al.* [22] reported that polymorphisms in HBS1L-MYB (rs4895441) explained 9.2% of the variation in Hb F present in sickle cell disease patients.

No significant difference was observed between *β*-thalassemia major patients and controls regarding HBS1L–MYB (rs4895441) gene polymorphism and allele frequency and no association was detected between gene and disease severity (age of disease presentation, frequency of blood transfusion, splenectomy and Hb F). This agreed with Nguyen *et al.* [23] who explained that the high level of ineffective erythropoiesis observed in beta-thalassemia major could mask or inactivate the biological expression of HBS1L-MYB polymorphisms, another explanation that the levels of multiple cytokines and/or chemokines are much higher in beta-thalassemia patients than normal subjects, these small molecular weight substances in peripheral blood are likely to exert diverse effects on erythropoiesis including globin gene expression. On the contrary, Cyrus *et al.* [17] reported a significant association between beta-thalassemia major and HBS1L-MYB (rs 4895441 and rs 9376090) genetic polymorphism in Saudi Arabian patients. This difference may be due to marked heterogeneity in the preva-

lence of the HBS1L-MYB allele among ethnic groups. In SCD, CT genotype & C allele of HBS1L-MYB (rs 9376090) were significantly higher when compared to other groups. Also, CT genotype was significantly associated with a lower number of crises & lower Hb S levels. Sales *et al.* [24] reported a significant impact of HBS1L-MYB (rs 9376090) polymorphism on sickle cell disease phenotype and the minor allele of HBS1L-MYB (rs 9376090) was associated with decreasing the odds of having a stroke by 3.7 folds. On the contrary, Wonkam *et al.* [25] reported a non-significant association between sickle cell disease severity and HBS1L-MYB (rs9376090) polymorphism. This difference may be due to marked heterogeneity in the prevalence of the HBS1L-MYB allele among ethnic groups. No significant difference was detected between β-thalassemia major patients and controls regarding HBS1L-MYB (rs 9376090) gene polymorphism and allele frequency and no significant association was detected between gene polymorphism and age of disease presentation, frequency of blood transfusion, splenectomy, Hb level, the severity of disease nor Hb F. This result was in agreement with Yunyan *et al.* [26], while Cyrus *et al.* [17] reported a significant difference in HBS1L-MYB (rs9376090) genotyping between β-thalassemia major patients and controls.

7. Conclusion

Lastly, we concluded that the severity of certain genetic disorders could be controlled by the expression of many genes that could decrease disease severity. In SCD, genotypes and alleles distribution of HBS1L-MYB (rs4895441&rs9376090) were associated with lower sickling crises numbers, higher Hb F, and lower Hb S. So this finding supports the development of innovative therapeutic targets.

Recommendations

Other studies including a large sample size and more HBS1L-MYB gene SNPs to obtain more conclusive results about the distribution of the HBS1L-MYB gene in Egyptian patients with hemoglobinopathy are recommended.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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