Received: 30 March 2017 | Accepted: 25 April 2017

DOI: 10.1002/jcla.22264

RESEARCH ARTICLE

WILEY

Protein Z and Endothelin-1 genetic polymorphisms in pediatric Egyptian sickle cell disease patients

Mervat M. Khorshied[1] | Nohair S. Mohamed[1] | Rania S. Hamza[1] | Rasha M. Ali[1] | Mona K. El-Ghamrawy[2]

[1]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

[2]Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence
Mervat Mamdooh Khorshied, Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.
Email: mervatkhorshied@hotmail.com

Background: Sickle cell disease (SCD) is a monogenic disease associated with multisystem morbidity. Vasculopathy caused by delicate imbalance between coagulation and endothelial systems plays a pivotal role in disease course. As Protein Z and Endothelin-1 genetic polymorphisms may increase the thrombotic risk, the aim of the current work was to verify the possible impact of Protein Z (PROZ G79A) and Endothelin-1 (EDN1 G5665T) polymorphisms on the clinic-laboratory features of the SCD in a cohort of Egyptian pediatric patients.

Methods: Genotyping of Protein Z G79A and Endothelin-1 G5665T was carried out by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) assay for 100 SCD patients and 100 controls.

Results: Protein -Z G79A polymorphism was not associated with vascular complications in the studied SCD patients. Endothelin-1 G5665T polymorphism was associated with pulmonary dysfunction (pulmonary artery hypertension and acute chest syndrome) and severe vaso-occlusive crises (VOC).

Conclusion: Endothelin-1 G5665T polymorphism could be considered as a molecular predictor for pulmonary dysfunction and severe VOC in SCD. Further researches with larger cohorts are recommended to understand the pathophysiology of SCD and to explain the inter-patients' variability of disease severity.

KEYWORDS

Egypt, Endothelin-1 G5665T, protein Z G79A, rs3024735, rs5370, sickle cell disease

1 | INTRODUCTION

Sickle cell disease (SCD) is an inherited, autosomal recessive, monogenic hemoglobinopathy.[1] Although this disease is caused by a single base pair mutation in the hemoglobin β-chain gene, persons with SCD can experience varying severity of a number of complications, including vaso-occlusive episodes that can result in acute chest syndrome (ACS), pain, stroke, chronic ischemic organ damage, disability, and early death.[2] SCD shows marked variability in disease severity. The patho-physiology underline complications in SCD remains incompletely understood. This could be attributed to co-existing environmental, psychosocial, and genetic factors. However, such known causes of variation are still insufficient to fully explain the clinical heterogeneity of the disease. Accordingly, identification of genetic alterations that may modulate disease severity is of great interest.[3]

Vasculopathy and endothelial dysfunction are the most important contributors to many long-term complications of SCD as cerebral strokes, renal dysfunction, and pulmonary hypertension.[4] Protein Z (PZ), a vitamin K-dependent glycoprotein synthesized in the liver.[5,6] PZ level is mandatory for controlled inhibition of activated coagulation factor X (FXa), the key player of the common coagulation pathway.[7] PZ binds PZ protease inhibitor (ZPI), a member of the serpin- super family

Abbreviations: EDN1, genetic variants of the Endothelin-1 gene; ET-1, Endothelin-1; ETRA, Endothelin receptor type A; ETRB, Endothelin receptor type B; PAH, Pulmonary artery hypertension.; PZ, Protein Z; SCD, Sickle cell disease; SNP, single-nucleotide polymorphism; VOC, Vaso-occlusive crisis.

of protease inhibitors forming the PZ-ZPI complex which inhibits FXa on phospholipid surfaces in the presence of calcium. This in turn reduces the formation of prothrombinase complex, resulting in inhibition of thrombin generation.[6,8] Single-nucleotide polymorphisms (SNPs) in Protein Z gene were reported to be associated with decreased PZ plasma level, thus affecting its anti-coagulant capacity and increasing the risk of thrombotic tendency as ischemic stroke, cardiovascular diseases, retinal vessel occlusion, pregnancy-related complications, and vaso-occlusive crises (VOC) in SCD patients.[7-11]

Endothelin-1 (ET-1) is one of the most potent endogenous vasoconstrictors known. It acts through type A (ETRA) and B (ETRB) receptors, leading to cell proliferation and vasoconstriction.[12] Endothelial dysfunction increases ET-1 production, leading to vascular hypertrophy, atherogenesis, and in the kidney, glomerulosclerosis.[13-15] Endothelin-1 (EDN1) gene polymorphism with G-to-T substitution at nucleotide 5665 in exon 5 (rs5370; corresponding to a Lys/Asn change at codon 198) was found to be associated with increased vascular reactivity,[16] hypertension in obese populations,[17] heart failure,[18] malignant arrhythmias in adults with structural cardiac disease,[19] pre-eclampsia [20], and pulmonary artery hypertension.[21]

As previous studies in SCD reported that pro-thrombotic conditions associated with altered PZ and ET-1 levels may influence, at least in part, the susceptibility to vascular complications in sickle patients, the aim of the current study was to clarify the possible impact of coagulation imbalance and/or endothelial dysfunction (Protein Z [PROZ G79A; rs3024735] and Endothelin-1 [EDN1 G5665T; rs5370] polymorphisms) on the clinical course of the disease in a cohort of pediatric Egyptian SCD patients.

What is Known

- Sickle cell disease (SCD) is a monogenic disease characterized by clinical variability.
- Vasculopathy is a major complication in SCD.
- Protein Z and Endothelin-1 genetic polymorphisms may increase the thrombotic risk.

What is New

- There was no significant difference noticed between pediatric SCD patients having the wild genotype or the polymorphic genotypes of Protein Z G79A polymorphism.
- Endothelin-1 G5665T polymorphism could be considered as a molecular predictor for pulmonary dysfunction and severe VOC in SCD.

2 | MATERIALS AND METHODS

The present study included 100 sickle cell disease patients who were attending Pediatric Hematology Clinic of El-Mounira Children Hospital, Cairo University. They were 53 males and 47 females. Their ages ranged between 3 and 18 years. Hundred age- and sex-matched un-related healthy children with normal hemoglobin electrophoresis were included in the study as a control group. Informed consents were obtained from the parents or caregivers of participants in advance. The study was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University and all procedures performed were in accordance with the 1964 Helsinki declaration. Complete history taking, clinical, and laboratory evaluation were done at presentation and follow-up visits. Quantitative assessment of different types of hemoglobin for diagnosis SCD was performed by hemoglobin electrophoresis, high-performance liquid chromatography, and column chromatography. For genotypic analysis, genomic DNA was extracted from peripheral blood leucocytes by QIAamp genomic DNA purification kit (Fermentas Life Sciences, Canada) according to manufacturers' instructions. Samples were stored in elution buffer at −20°C until use. Genotyping of Protein Z G79A and Endothelin-1 G5665T polymorphism was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. Forty samples were randomly chosen and subjected to re-genotyping for quality control. The results of genotyping were interpreted blindly and found to be 100% concordant.

2.1 | Genotyping of Protein Z (PROZ G79A; rs3024735) SNP

The following primers were used: Forward primer: 5′-TAA CAC CAT AGA CAG AGT CCG ATA TTC GC -3′ and Reverse primer: 5′-ATG AAC TCG GCA TTA GAA CAT GGT TGG AA-3′. Amplification of the target gene was performed using the following program: initial denaturation at 94°C for 4 min followed by 32 cycles of 94°C for 60 s, 57°C for 60 s, and 74°C for 60 s, and a final extension step at 74°C for 7 min. The amplified product (320 bp) was resolved by 2% agarose gel electrophoresis. The PCR product was digested with 10 U with ***HpaII*** (Fermentas life sciences, USA) in the manufacturer's buffer at 37°C overnight, resulting in two bands of 221 and 99 bp for polymorphic type (A) allele, and an unrestricted single band of 320 bp band for the wild type (G) allele.[3]

2.2 | Genotyping of Endothelin-1 (END1 G5665T; rs5370) SNP

To amplify the target part of the gene, the following primer pair was used: F: 5′-TCT TGC TTT ATT AGG TCG GAG ACC-3′ and R: 5′-TTT GAA CGA GGA CGC TGG TC-3′. The thermocycler program and amplification conditions were initial heating at 95°C for 10 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 61°C for 1 min, extension at 72°C for 1 min and 30 s, and final extension step at 72°C for 10 min. PCR products of 262 bp were digested with 2U of ***Cac8I*** (Ferementas, Lithuania) restriction endonuclease at 37°C for 2 h. The wild type (G) allele was restricted into two fragments of

TABLE 1 Clinical and laboratory data of SCD patients

Item	Number
Gender Male/female	53:47
Pulmonary dysfunction	47/100
Acute chest syndrome	16/47
Pulmonary artery hypertension (PAH)	31/47
Renal dysfunction	40/100
Osteonecrosis	8/100
Central nervous system complications (TIAs & Strokes)	10/100
Leg ulcers	8/100
Vaso-oclussive crisis (VOC)	84/100
Moderate to severe VOC (requiring emergency visit or hospitalization/year)	
Range	0-17
Median	5
Mean ± SD	4.1± 4.7
Priapism	6/100
Hand foot syndrome	24/100
Splenic status	
Splenomegaly	19/100
Splenectomized	21/100
Hepatomegaly	16/100
Gall stones	6/100
Frequency of blood transfusion (units of blood/year)	
Range	0-20
Median	4
Mean±SD	5.6 ± 4.7
Transfusion dependency	
Frequent (>4 times blood transfusion/year)	56/100
Infrequent (1-3 times blood transfusion/year)	23/100
Sporadic (infection, preoperative, occasional)	21/100
Type	
Sickle cell anemia (Hb-SS)	70/100
Sickle/beta thalassemia (Hb-Sβ)	30/100

Laboratory data				
	Item	**Range**	**Mean±SD**	**Median**
Hematological data	Hb (g/dl)	3.9-11.8	7.698±1.6	7.8
	PLT×10^3/cm^3	31-1476	363±211.2	355.0
	TLC×10^3/cm^3	2.4-21.8	10.0±4.4	9.3
	Reticulocytic count (%)	0.4-31.1	8.2±6.8	6.2
Hemoglobin electrophoresis (%)	HbA	0-74	21.4±19.5	15.2
	HbF	0-42.4	16.4±11.9	17.2
	HbS	6-98.0	68.0±18.4	70.9
	HbA2	1.0-9.3	2.4±1.1	2.1
Ferritin (ng/ml)		30-8679	1034.3±1451	560.0
Bilirubin (mg/dl)	Total	0.5-5.2	2.2 ± 0.9	2.0
	Direct	0.1-2.2	0.36 ± 0.25	0.30
A/C ratio (mg/g)		11-4096	454.7±538.7	300

Hb, hemoglobin; PLT, Platelets; TLC, total leukocytic count; A/C, Albumin/Creatinine ratio.

TABLE 2 Protein Z (PROZ G79A; rs3024735) and Endothelin-1 (END1 G5665T; rs5370) polymorphism in SCD patients and control

Genotypes		Control (n %)	SCD patients (n %)	OR (95% CI)	*P*-value
PROZ G79A	GG	78/100 (78%)	73/100 (73%)	1 (Reference)	
	GA	22/100 (22%)	27/100 (27%)	1.21 (0.687-2.51)	.41
	AA	0/100 (0%)	0/100 (0%)		
	G- allele	0.89	0.86	1.26 (0.63-2.3)	
	A-allele	0.11	0.14		.45
END1 G5665T	GG	69/100 (69%)	64/100 (64%)	1 (Reference)	
	GT	28/100 (28%)	29/100 (29%)	1.12 (0.6-2.1)	.73
	TT	3/100 (3%)	7/100 (7%)	2.52 (0.62-10.15)	0.51
	GT & TT	31/100 (31%)	36/100 (36%)	1.25 (0.7-2.26)	.45
	G- allele	0.84	0.82	1.33 (0.81-2.5)	.25
	T-alleler	0.16	0.18		
Combined genotypes PROZ/END1	GG/GG	53 (53%)	47 (47%)	1 (Reference)	
	GG/GT & TT	25 (25%)	26 (26%)	1.173 (0.597-2.303)	.64
	GA/GG	16 (16%)	17 (17%)	1.198 (0.545-2.634)	.65
	GA/GT & TT	6 (6%)	10 (10%)	1.879 (0.635-5.566)	.26

OR, Odds ratio; 95%CI, 95% Confidence interval.

208 amd 25 bp, while the polymorphic allele was not restricted giving single band of 228 bp.[22]

2.3 | Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) statistical package version 17. For numerical data, parametric data were expressed as mean, standard deviation, and range, while nonparametric data were expressed as median and interquartile range. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Nonparametric numerical data were analyzed using Mann-Whitney test. Correlation analysis was performed by Spearman's rank correlation. Unconditional logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for risk estimation. *P*-values less than .05 were considered significant. Chi-square (v2) test was performed to assess deviation from Hardy-Weinberg equilibrium (HWE).

3 | RESULTS

The demographic data of SCD patients is presented in Table 1. The frequency of Protein Z G79A and Endothelin-1 G5665T genotypes in SCD patients and controls is presented in Table 2. Genotypic distribution of the studied SNPs in the studied population was in accordance with the Hardy-Weinberg equilibrium (*P*>.05). The distribution of Endothelin-1 (EDN1 G5665T) and Protein Z (PROZ G79A) polymorphic genotypes did not differ between SCD patients and controls. Comparison between patients having the wild or the polymorphic genotypes of Protein Z (PROZ G79A) revealed no statistically significant difference between the two groups regarding their gender, clinical, or laboratory characteristics (Tables S1 and S2). Endothelin-1 G5665T polymorphic genotypes was associated with pulmonary dysfunction (pulmonary hypertension and acute chest syndrome) and severe frequent VOC (Tables S3 and S4).

4 | DISCUSSION

Sickle cell disease patients suffer from acute and chronic vascular complications. Vascular occlusion is a major pathophysiologic event in SCD, leading to a diverse group of morbidities such as painful crisis, acute chest syndrome, stroke, aseptic necrosis of bones, priapism, leg ulcers, and proliferative retinopathy.[23] The variable clinical spectrum of SCD is multi-factorial. It could be the consequence of multiple events and genetic susceptibility that goes beyond the occurrence of a single amino acid substitution in the beta globin chain of hemoglobin. It could be attributed in part to co-existing coagulation imbalance and/or endothelial dysfunction.

Protein Z (PZ) is a vitamin K-dependent plasma protein. Recent studies have suggested an association between Protein Z gene polymorphism, low plasma PZ levels, and thrombotic tendency.[24] Furthermore, the combination of protein Z deficiency with other thrombophilic factors as Endothelin-1 gene polymorphism may increase the thrombotic risk. The aim of the current work was to clarify the possible association between Protein Z G79A and Endothelin-1 G5665T polymorphisms and the clinic-pathological features of SCD in a cohort of pediatric Egyptian SCD patients.

Regarding of Protein Z G79A polymorphism, 73% of SCD patients had the wild genotype (GG), 27% had the heteromutant genotype (GA),

while none of the patients had the homomutant genotype (AA). These frequencies go with that previously reported in Bahraini patients being 65%, 29%, and 5% for the GG, GA, and AA genotypes, respectively.[25] SCD complications may be modified by genetic and acquired risk factors for vasculopathy and several studies have reported that Proein Z G79A polymorphism is associated with thrombotic complications. In the present work, patients harboring the polymorphic genotypes were compared to those with the wild genotype and there was no statistically significant difference between the two patients' groups regarding their clinical or laboratory features. Although the frequency and severity of VOC were higher in patients having the variant genotypes, the difference between the two patients' groups did not reach a statistically significant level. This could be attributed to the sample size of our study, or the unreported attacks of VOC missed by the patients or their caregivers. The study of Mahdi and colleagues [25] reported that the polymorphic genotypes were significantly higher in SCD patients with VOC, and this SNP could be considered as molecular risk factor for vaso-occlusion.

Endothelin-1 (EDN-1) gene polymorphisms have been investigated and incriminated in the pathogenesis of various vascular diseases. The mutant T allele of a G5665T polymorphism was found to be associated with higher plasma ET-1 levels.[16,26-28] In our study, 64% of SCD patients had the wild genotype (GG) of Endothelin-1 G5665T polymorphism, 20% had the heteromutant genotype (GT) and 7% had the homomutant genotype (TT). These frequencies were close to that reported in African, African American, and in Indian SCD patients.[3,11,29] There was no statistical difference in the distribution of the polymorphic genotypes between SCD patients and controls. This goes with that previously reported in Indian and African population.[3,29] However, the study of Navarro and colleagues [11] has found a statistically significant difference in the genotypic and allelic frequencies of END1 G5665T SNP between African American sickle patients and controls. The difference between the studied populations potentially reflects the role of ancestry.

Acute chest syndrome (ACS) and painful vaso-occlusive crises (VOC) are the most common causes of hospitalization in sickle cell disease. Several studies have suggested the involvement of Endothelin-1 in the pathogenesis of these two sickle cell anemia-specific complications.[30,31] Furthermore, Endothelin-1 (rs5370) gene polymorphism has been implicated in progression of chronic glomerulosclerosis,[26] pulmonary hypertension [27], and vaso-occlusive episodes correlated with pain history.[28] In our study, statistical comparison between patients having the wild and the polymorphic genotypes revealed that pulmonary dysfunction in the form of pulmonary hypertension and acute chest syndrome, and severe vaso-occlusive attacks requiring hospitalization were more frequent among patients harboring the polymorphic genotypes. On the contrary, the study of Thakur et al. [3] has shown that ET-1 G5665T SNP has no significance among native African SCD. In the current study, the polymorphic genotypes were significantly higher among male patients. However, Thakur et al. [3] reported that the distribution of the polymorphic genotypes was not sex-linked.

In conclusion, Endothelin-1 (EDN1 G5665T) polymorphism could be considered as a molecular predictor for severe VOC and pulmonary complications in pediatric Egyptian SCD patients. Larger confirmatory studies and wide scale analysis are recommended to confirm our results, and further researches in this field are required to identify more genetic variants that could help in understanding the molecular mechanisms underlying the pathophysiology of the disease and to explain the clinical variability between SCD patients.

AUTHORS' CONTRIBUTION

Prof. Mervat Khorshied and Prof.Nohair Soliman were responsible for study design. Prof. Mervat Khorshied and Dr. Rania Hamza were responsible for performing the genotypic analysis of the candidate SNPs. Prof. Mona Ghamrawy and Dr. Rasha Mahmoud were responsible for sample collection and revising patients' data. Prof. Mervat Khorshied was responsible for the statistical analysis and manuscript submission. All authors participated in manuscript writing.

REFERENCES

1. Santos MN. Haptoglobin: an emerging candidate for phenotypic modulation of sickle cell anemia? *Rev Bras Hematol Hemoter*. 2015;37(6):361-3. https://doi.org/10.1016/j.bjhh.2015.08.009. Epub 2015 Oct 9.
2. Pakbaz Z, Wun T. Role of the hemostatic system on sickle cell disease pathophysiology and potential therapeutics. *Hematol Oncol Clin North Am*. 2014;28:355-374. https://doi.org/10.1016/j.hoc.2013.11.011. Review.
3. Thakur TJ, Guindo A, Cullifer LR, et al. Endothelin-1 but not endothelial nitric oxide synthase gene polymorphism is associated with sickle cell disease in Africa. *Gene Regul Syst Bio*. 2014;25:119-26. https://doi.org/10.4137/GRSB.S14836.
4. Rees DC, Gibson JS. Biomarkers in sickle cell disease. *Br J Haematol*. 2012;156:433-45.
5. Vasse M. Protein Z, a protein seeking a pathology. *Thromb Haemost*. 2008;100:548-556.
6. Almawi WY, Al-Shaikh FS, Melemedjian OK, et al. Protein Z, an anticoagulant protein with expanding role in reproductive biology. *Reproduction*. 2013;146:R73-R80.
7. Zeng Y, Zhang L, Hu Z, et al. Association of protein Z and factor VII gene polymorphisms with risk of cerebral hemorrhage: a case-control and a family-based association study in a Chinese Han population. *J Genet*. 2016;95:383-8.
8. Sofi F, Cesari F, Fedi S, et al. Protein Z: "light and shade" of a new thrombotic factor. *Clin. Lab*. 2004;50:647-652.
9. Erez O, Hoppensteadt D, Romero R, et al. Preeclampsia is associated with low concentrations of protein Z. *J Matern Fetal Neonatal Med*. 2007;20:661-7.
10. Topalidou M, Effraimidou S, Farmakiotis D, et al. Low protein Z levels, but not the intron F G79A polymorphism, are associated with unexplained pregnancy loss. *Thromb Res*. 2009;124:24-27.
11. Navarro KG, Agyingi SE, Nwabuobi CK, et al. Polymorphism of the endothelin-1 gene (rs5370) is a potential contributor to sickle cell disease pathophysiology. *Genes Diseases*. 2016;3:294-298.
12. Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med*. 2003;139:137-47.
13. Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. *Am J Physiol Renal Physiol*. 2003;285:F579-89.
14. Hocher B, Schwarz A, Reinbacher D, et al. Effects of endothelin receptor antagonists on the progression of diabetic nephropathy. *Nephron*. 2001;87:161-9.

15. Gomez-Garre D, Ruiz-Ortega M, Ortego M, et al. Effects and interactions of endothelin-1 and angiotensin II on matrix protein expression and synthesis and mesangial cell growth. *Hypertension*. 1996;27:885-92.
16. Iglarz M, Benessiano J, Philip I, et al. Preproendothelin-1 gene polymorphism is related to a change in vascular reactivity in the human mammary artery in vitro. *Hypertension*. 2002;39:209-13.
17. Tiret L, Poirier O, Hallet V, et al. The Lys198Asn polymorphism in the endothelin-1 gene is associated with blood pressure in overweight people. *Hypertension*. 1999;33:1169-74.
18. Colombo M, Ciofini E, Paradossi U, et al. ET-1 Lys198Asn and ET (A) receptor H323H polymorphisms in heart failure. A case-control study. *Cardiology*. 2006;105:246-52.
19. Kozak M, Izakovicova Holla L, Krivan L, et al. Endothelin-1 gene polymorphism in patients with malignant arrhythmias. *J Cardiovasc Pharmacol*. 2004;44(suppl):S92-5.
20. Aggarwal PK, Jain V, Srinivasan R, et al. Maternal EDN1 G5665T polymorphism influences circulating endothelin-1 levels and plays a role in determination of preeclampsia phenotype. *J Hypertens*. 2009;27:2044-50.
21. Benza RL, Miller DP, Barst RJ, et al. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. *Chest*. 2012;142:448-456.
22. Lichy C, Kropp S, Dong-SI T, et al. A common polymorphism of the protein Z gene is associated with protein Z plasma levels and with risk of cerebral ischemia in the young. *Stroke*. 2004;35:40-45.
23. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Rev*. 2007;21(1):37-47.
24. Ghinoi A, Boiardi L, Atzeni F, et al. Protein Z G79A and A-13G gene polymorphisms in Italian patients with Behçet's disease. *Clin Exp Rheumatol*. 2009;27(2 Suppl 53):S23-8.
25. Mahdi N, Abu-Hijleh TM, Abu-Hijleh FM, et al. Protein Z polymorphisms associated with vaso-occlusive crisis in young sickle cell disease patients. *Ann Hematol*. 2012;91:1215-20. https://doi.org/10.1007/s00277-012-1474-6.
26. Tharaux PL. Endothelin in renal injury due to sickle cell disease. *Contrib Nephrol*. 2011;172:185-199.
27. Li C, Mpollo MS, Gonsalves CS, et al. Peroxisome proliferator-activated receptor-a-mediated transcription of miR-199a2 attenuates endothelin-1 expression via hypoxia-inducible factor-1a. *J Biol Chem*. 2014;289:36031-36047.
28. Smith TP, Schlenz AM, Schatz JC, et al. Modulation of pain in pediatric sickle cell disease: understanding the balance between endothelin mediated vasoconstriction and apelin mediated vasodilation. *Blood Cells Mol Dis*. 2015;54:155-159.
29. Bingül İ, Aydıngöz İE, Vural P, et al. The Evaluation of Endothelin-1 and Endothelin receptor type A gene polymorphisms in patients with vitiligo. *Indian J Dermatol*. 2016;61:118. https://doi.org/10.4103/0019-5154.174076.
30. Morris CR, Kuypers FA, Larkin S, et al. Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. *J Pediatr Hematol Oncol*. 2000;22:515-20.
31. Graido-Gonzalez E, Doherty JC, Bergreen EW, et al. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. *Blood*. 1998;92:2551-5.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Khorshied MM, Mohamed NS, Hamza RS, Ali RM, El-Ghamrawy MK. Protein Z and Endothelin-1 genetic polymorphisms in pediatric Egyptian sickle cell disease patients. *J Clin Lab Anal*. 2018;32:e22264. https://doi.org/10.1002/jcla.22264