Association of *BCL11A* Genetic Polymorphisms with Fetal haemoglobin Level in Sudanese Patients with Sickle Cell Anaemia

Shireen Zuhdi Nimer[1*], Elshazail Widaa Ali[1] and Hiba Khalil[2]

1   Faculty of Medical Laboratory Sciences, Alneelain University, Sudan
2   Alneelain Stem Cell Center, Alneelain University, Sudan

*Corresponding author:
Shireen Zuhdi Nimer

✉ shereen.almaaas100@gmail.com

Faculty of Medical Laboratory Sciences, Alneelain University, Sudan.

Tel: 00966531546162

Abstract

Background: Fetal Hemoglobin (HbF) level is the major genetic modulator of the hematologic and clinical features of sickle cell anaemia. Fetal hemoglobin genes are regulated genetically. Recently, the *BCL11A* gene was identified as a regulator of HbF level. The aim of this study to investigate the association of *BCL11A* genetic polymorphisms (rs11886868) with HbF Level in Sudanese patients with sickle cell anaemia (Hb SS).

Materials and Methods: A cross-sectional observational study included 71 Sudanese patients with sickle cell anemia (Hb SS). Patients under hydroxyurea and those with a history of blood transfusion for at least three months were excluded. Genotyping for rs11886868 was determined using PCR/Sequencing. Fetal hemoglobin level for each patient was quantified and it was compared with Genotype and allele frequencies.

Results: Genetic variants were detected on *BCL11A* gene rs11886868, rs766432, rs144866206, rs766431. Genotype of rs1886868 and rs766432 found to have a statistically significant effect on the HbF level (p-value <0.0001), rs766432 (p-value 0.042) and also found to have significant effects on the clinical severity of sickle cell anaemia, but rs144866206 and rs766431 showed a statistically insignificant association on HbF level (p-value 0.173 and 0.546respectively).

Conclusion: The *BCL11A* genetic polymorphisms (rs11886868, rs766432) in Sudanese patients with sickle cell anaemia were associated with HbF levels and complication of this disease.

Keywords: Sickle cell anaemia; Fetal haemoglobin; Polymorphism of *BCL11A*

Introduction

Sickle Cell Anaemia (SCA) is autosomal recessive genetic haemoglobinopathy disorders [1]. Worldwide, more than 300 000 infants are born with sickle cell disease each year, takes place nearly 67% in Africa [2]. Sickle cell anaemia is caused by one point mutation in the beta chain of haemoglobin, is caused by the substitution of an Adenine (A) for a Thymine (T) in the sixth codon of the beta-globin gene, leading to the substitution of glutamic acid for valine and to the production of Haemoglobin S (HbS) [3]. Sickle cell anaemia affects the structure of erythrocytes by altering the normal biconcave shape of a crescent. During this process, the hemoglobin S (HbS) mutation leads to haemoglobin polymerization and deoxygenation, resulting in sickling, abnormal adhesion of leukocytes and platelets, inflammation, hypercoagulation, hemolysis, and hypoxia, in addition to microvascular obstruction and finally organ damage

[4]. One important factor that has been described as influencing the clinical course and hence disease severity in the SCA is fetal Hemoglobin F level (HbF) [5]. The levels of fetal Hemoglobin F (HbF) influence the severity of sickle cell anaemia, due to its ability to inhibit HbS polymerization. Fetal hemoglobin genes are regulated genetically. Genetic studies have identified three major quantitative trait loci (QTLs) (Xmn1-HBG2, HBS1L-MYB intergenic region on chromosome 6q23, and *BCL11A* on chromosome 2p16) that account for 20-50% of the common variation in HbF levels in patients with SCA. *BCL11A* is regulated and maintain HbF silencing in human adult erythroid cells [6]. The *BCL11A* protein occupies sites within the Locus Control Region (LCR) and intergenic regions of the β-globin locus. Interestingly, all the reported significant Single Nucleotide Polymorphisms (SNPs) of *BCL11A* reside within a region of 14 kb in intron 2 of the *BCL11A* gene [7,8]. Determine the effect of Single Nucleotide Polymorphisms (SNPs) in *BCL11A* with HbF level variation in sickle cell patients will be beneficial

to improve the prediction of one's ability to produce HbF. This study aimed to investigate the association of *BCL11A* genetic polymorphisms (rs11886868) with HbF level in Sudanese patients with sickle cell anaemia.

Material and Methods

Samples and clinical data

This study was a cross sectional observational included Sudanese patients with sickle cell anaemia (Hb SS) attend at the Ahmad Kassem hospital in Khartoum, in 2017, we excluded patients under hydroxyurea and those with a history of blood transfusion for at least three months. This study was approved by the Faculty of Medical Laboratory Sciences, Alneelin University, and informed consent was taken from each participant before sample collection for children, the consent was taken from their parents. Clinical data was extracted from parents and medical files. It is included age, gender and complications. Venous blood samples were collected from each patient for all subjects in EDTA blood tube for haematological and molecular analysis. Complete Blood Count (CBC) was performed by automated haematology analyzer (Sysmex KX21N, Japan), and HbF level was measured by automation capillary electrophoresis (Sebia minicap, France).

DNA extraction and PCR

The genomic Deoxyribonucleic Acid (DNA) extraction was from whole blood samples using (G-spin TM Total Kit –INtRON, Korea) according to the manufacturer's protocol. The identification of polymorphisms on the *BCL11A* gene was performed by (PCR) amplification of these gene regions: rs11886868 using primers Forward: CACACCATGGATGAATCCAGA and Reverse: TGGTGCTACCCTGAAAGACGG. Polymerase Chain Reaction (PCR) was performed on a volume of 20 µL consisted of 3 µL genomic DNA template, 2 µL forward and reverse primer and 15 µL distilled water, added to master mix ready to use (iNtRON Biotechnology, Korea). The thermo cycling condition of PCR were 35 cycles of denaturation (95°C for 30 seconds), annealing (61°C for 60 sec), and extension step (72°C for 90 sec). A preheating step at (95°C for 10 minutes) and final extension step for (7 min at 72°C). The PCR product separated using 2% agarose gel electrophoresis and visualized with ethidium bromide and fragments were visualized by use of the gel documentation system (Syengene, Japan).

DNA sequence analysis

PCR products were then purified and doubly sequenced (forward and reverse), sequencing was based on Sanger's chain termination method by Beijing Genomic Instituted (BGI), China.

Bioinformatics analysis

The chromatogram sequences were visualized through Finch TV program version (1.4.0). Reference sequences of the *BCL11A* gene have accession numbers NG_01196. Were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) and subjected to multiple sequence alignment using Bio Edit software version (7.2.5.0).

Data analysis

Patients' hematological and clinical data and together with molecular data were analyzed by the Statistical Package for Social Sciences (IBM SPSS statistics for windows, version 22.0).

Results

Overall, 71 patients with sickle cell anaemia (HbSS) were enrolled; 38 (54%) of them were males and 33 (46%) were females. Patients' ages were ranged from 1 to 16 years (Mean ± SD: 6.42 ± 4.32). In general, the HbF level was ranged between 1% and 35.7% (Mean ± SD 11.3 ± 7.5). **Table 1** summarizes the description of demographic, hematological and clinical data of patients with sickle cell anaemia (HbSS). In this study, the total of 4 genetic variants was detected on *BCL11A* gene rs11886868, rs766432, rs144866206, rs766431. Mutations positions and allele frequencies for each mutation was displayed in **Table 2**.

The minor allele in the rs11886868 and rs766432 was "G", accounting for (35% and 37% respectively) of the total chromosomes. The minor allele" G" was associated with increased HbF levels and genotypes containing the minor allele exhibited significantly high of HbF levels. In 44866206 had 12% of minor allele" C". Allele "C" increased the Hb-F levels in genotypes, but statically insignificant. Genotype distribution in **Tables 3 and 4**.

Discussion

Patients with sickle cell anaemia, typically demonstrate clinical phenotypes whose severity is inversely proportional to the degree of preservation of HbF expression. Polymorphisms of

Table 1 Hematological, demographic, and clinical data of studied population.

Frequency		Percentage (%)	Mean ± SD of HbF	p-value
Gender				
Male	38	(54%)	9.95 ± 6.80	0.104
Female	33	(46%)	12.86 ± 8.03	
Age in years				
1-5	36	50.7%	12.76 ± 8.51	
6-10	21	29.6%	10.77 ± 6.05	0.008
>10	14	19.7%	8.35 ± 6.08	R=-0.312
complication				
Yes	58	(82%)	9.425 ± .69	
no	13	(18%)	19.70 ± 9.11	< 0.0001
Degree of anaemia				
Moderate anaemia	41	(59%)	13.87 ± 8.41	0.019
Severe anaemia	29	(41%)	9.63 ± 6.44	

Table 2 Frequencies of the genotypes of the *BCL11A* variants.

SNP	Allele change	Chr:position	Minor Allele Factors
rs11886868	G → A	2:65388	0.35 (G)
rs766431	C → T	2:65426	0.01 (T)
rs766432	G → T	2:65664	0.37 (G)
rs144866206	T → C	2:65717	0.12 (C)

Table 3 Mean of HbF levels in SCA patients across polymorphism.

rs11886868	GG	GA	AA	*p-value*
Hb-F	15.7 ± 8.5	14.7 ± 8.11	7.89 ± 4.79	<0.0001
rs766431	CC	CT	-	
Hb-F	11.21 ± 7.37	14.50 ± 10.41	-	0.546
rs766432	GG	GT	TT	
Hb-F	15.80 ± 9.47	10.21 ± 7.42	10.19 ± 5.52	0.042
rs144866206	TT	TC	CC	
Hb-F	10.44 ± 7.46	14.02 ± 7.30	14.20 ± 6.78	0.173

Table 4 Distribution of genotype frequencies and allele frequencies of SCA patients according to complication.

Genotype	Complication		*p-value*	OR (CI L-CI Up)
	Yes	No		
rs766432				
GG	8 (14%)	6 (46%)		
GT	21 (36%)	25 (31%)	0.024	1.9 (0.15-25.2)
TT	29 (50%)	3 (23%)	0.008	1.8 (0.12-6.7)
rs144866206				
TT	48 (83%)	7 (54%)		
TC	9 (15%)	6 (46%)	0.048	0.234 (0.12-0.67)
CC	1 (2%)	0 (0.0%)	0.024	0.45 (0.32-0.87)
rs766431				
CC	56 (97%)	13 (100%)	0.656	0.81 (0.72-0.91
CT	2 (3%)	0 (0.0%)		
rs11886868				
GG	10 (17.2%)	6 (46.2%)		
GA	14 (24.1%)	3 (23.1%)	0.468	1.82 (0.36-9.22)
AA	34 (58.6%)	4 (30.8%)	0.027	5.10 (1.19-21.7)

BCL11A are one of the most important factors in variation in HbF levels in patients with SCA. Several SNPs have been identified in *BCL11A* intron-2 as the most highly associated with the HbF level in African people, including rs11886868 [9]. These SNPs lie within an erythroid enhancer and act combinatorial to influence *BCL11A* regulate [10]. Also, our study in the Sudanese patients showed rs11886868 was associated with HbF levels. Additionally, this study detected another independent effect of SNPs (rs766432, rs766431 and rs144866206) on HbF Levels. The rs11886868 genotype has a wide distribution that varies in populations throughout the world. In this study, the minor allele frequency "G" of rs11886868 (0.35) a statically significant with increased HbF levels (p-value<0.0001) has a similar frequency with the populations of Cameron and Tunis and African Americans. Allele "G" is widespread in Indonesia and Sardinians (0.88) [11]. The difference in the frequency of variant genotype might be on account of ethnic differences. Our study indicates that the minor allele "G" in rs766432 has a frequency (0.37) agree with various populations, including whites in Europe, the population of white Americans, and Asian populations and associated with increased

HbF levels [12]. The minor allele frequency "C" in rs766431 and "T in "rs144866206 was (0.12 and 0.01 respectively) these alleles increase HbF, but no statically significant (0.173 and 0.546 respectively), while in rs144866206 genotypes (CT and CC) containing mutant allele C was statically significant with complication and severity disease (p-value=0.048 and 0.024). The association of" C" in rs766431 and "T " in rs144866206 in *BCL11A* in SCA patients, not mentioned in any previous studies for comparison.

The results of this study are capable of correcting the underlying cause of the disease, alleviate its symptoms and offer the opportunity to therapy, and that is done by Gene Therapy (GT) based on autologous transplantation of genetically modified stem cells is a promising therapeutic option for patients with sickle cell aneamia. The objective of the therapy is the introduction of nucleic acids into cells that will modify gene expression in order to prevent, halt, or reverse the pathological process. Gene therapy is done with different strategies (e.g. CRISPR/Cas system).

Conclusion

The present study revealed that genotypes for SNPs (rs11886868 and rs766432) in the *BCL11A* gene were associated with HbF levels and complications in sickle cell anemia. This is finding can help improve the treatment of patients with SCA complications and help to improve the prediction of one's ability to produce HbF and future treatment of sickle cell anemia.

Acknowledgment

References

1   Patrick TMG, Hernandez AG, Russell W (2017) Sickle cell anemia in sub-Saharan Africa: advancing the clinical paradigm through partnerships and research. Blood 129: 155-161.

2   Sabahelzain MM, Hamamy H (2014) The ethnic distribution of sickle cell disease in Sudan. Pan Afr Med J 18: 13.

3   Kaur M, Dangi CBS, Singh M (2013) An Overview on Sickle Cell Disease Profile. Asian J Pharm Clin Res 6: 25-37.

4   Bitoungui VJN, Pule GD, Hanchard N, Ngogang J, Wonkam A (2015) Beta-Globin Gene Haplotypes Among Cameroonians and Review of the Global Distribution: Is There a Case for a Single Sickle Mutation Origin in Africa?. OMICS 19:171-179.

5   Mpalampa L, Ndugwa CM, Ddungu H, Idro R (2012) Foetal haemoglobin and disease severity in sickle cell anaemia patients in Kampala, Uganda. BMC Blood Disord 12: 11-17.

6   Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, et al. (2008) Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 322:1839-1842.

7   Cardoso GL, Diniz IG, Silva AN, Cunha DA, Silva Junior J, et al. (2014) 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, Molecules and Diseases 53: 176-179.

8   Xu J, Bauer DE, Kerenyi MA, Vo TD, Hou S, et al. (2013) Corepressor-dependent silencing of fetal hemoglobin expression by BCL11A. Proceedings of the National Academy of Sciences of the United States of America 110: 6518–6523.

9   Fanis P, Kousiappa L, Phylactides M (2014) Genotyping of BCL11A and HBS1L-MYB SNPs associated with fetal haemoglobin levels: a SNaPshot minisequencing approach. BMC Genomics 15: 108.

10   Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, et al. (2013) An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science 253-257.

11   Lantip R, Basalamah M, Siswandari W, Setyono J, Wulandari G, et al. (2016) Modifying effect of XmnI, BCL11A, and HBS1L-MYB on clinical appearances: A study on b-thalassemia and hemoglobin E/b-thalassemia patients in Indonesia. Hematol Oncol Stem Cell Ther 9: 55-63.

12   Sedgewick AE, Timofeev N, Sebastiani P, So JCC, Ma ESK, et al. (2008) BCL11A is a major HbF quantitative trait locus in three different populations with β-hemoglobinopathies. Blood Cells Mol Dis 41: 255-258.