EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 8/ November 2017

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Integrin alpha2 [ITGA2] gene C807T allele Polymorphism and Clinical Severity of Sickle Cell Disease among Sudanese patients

ALAAEDDIN M. ELZUBEIR[1]
Department of Haematology and Immunohaematology
Faculty of Medical Laboratory Sciences
Sudan International University - Sudan
ABEER EDRIS
ESRAA ELHASSAN
HIBA ALI
REEM SULIMAN
ROWIDAH ZUMRAWY
TUMADER IBRAHIM
OSMAN A. SADDIG
Department of Haematology and Immunohaematology
Faculty of Medical Laboratory Sciences
University of Khartoum - Sudan
NAZIK ELMALAIKA O. S. HUSAIN
Department of Pathology, Faculty of Medicine and Heath Sciences
Omdurman Islamic University, Sudan
ABU ELGASIM A. ELKAREEM
Department of Histopathology, Faculty of Medical Laboratory Sciences,
Sudan University of Science and Technology, Sudan
HIND M. AHMED
Department of Pediatric, Gaafar Ibn-Auf Paediatric Tertiary Hospital
Khartoum, Sudan
NOAH M.
Department of Molecular Biology, Nile University Khartoum, Sudan

Abstract:

Background: *Sickle cell disease (SCD) vaso-occlusive crisis are still responsible for high morbidity and early mortality. Integrins, a family of cell surface receptors, interact with vascular cell adhesion molecule-1 and fibronectin, leading to vaso-occlusion.*

[1] Corresponding author email: ala_masad@yahoo.com

Objectives: *The present study aimed to investigate the correlation between the Integrin Alpha 2 (ITGA2) C807T (rs1126643) polymorphic loci and clinical severity of homozygous SCD in Sudanese patients, as well as hematologic variables.*

Methods: *Human venous blood samples were collected from homozygous SCD patients admitted to referral Hematology Clinic (n=133, 'patients') and apparently healthy individuals (n=112, 'controls'). Blood was genotyped by polymerase chain reaction-restriction fragment length polymorphism. Complete blood counts were measured by hematology analyzer.*

Result: *The genotype and allele frequencies of ITGA2 C807T were found to be significantly different between patients and controls (p=0.002). Relative risk analysis of allele frequency showed that patients with the T allele were 5.4 times more likely to suffer from hemolytic crisis, vaso-occlusive and ischemic stroke rather than patients with the C allele. As expected Hematologic parameters; Hb, RBCs and PCV were all significantly higher in controls than in patients when compared between severity groups P. value (0.000, 0.045, and 0.034), respectively.*

Conclusion: *ITGA2 C807T (rs1126643) polymorphism is associated with more complications and crisis, with the T allele that appears to deliberate increased susceptibility to ischemic stroke and vasoocclusive crisis in Sudanese patients with homozygous SCD.*

Key words: Sickle Cell Disease, SCA, Clinical Severity, ITGA2 C807T allele polymorphism.

INTRODUCTION

Sickle Cell Disease (SCD) is a group of autosomal recessive genetic blood disorders arises from a single amino acid substitution in the sixth codon of the ß-globin gene. In deoxygenated area, the abnormal hemoglobin S polymerizes and causes rigid and sickle-shaped red blood cells. Sickle

haemoglobin (HbS) is the most common haemoglobinopathy worldwide.(1)

The pathogenesis of sickle cell disease is depends on the increased adhesiveness of sickle cells to the endothelial layer of blood vessels causing vaso-occlusion resulting in chronic organs damage in SCD. Individuals with SCD suffer a wide range of complications: increased susceptibility to infections, chronic hemolytic anemia, recurrent periodic acute vaso-occlusive events and chronic damage affecting almost every organ system.(2)

Sickle cell disease crises (painful crisis or vaso-occlusive crisis), are still responsible for high morbidity and early mortality. Blood transfusions remain the basis of therapy for all severe acute crises.(3)

Integrins are heterodimeric transmembrane glycoprotein consist of an alpha and a beta subunit. They composed of a large extracellular domain, a transmembrane domain and a short cytoplasmic domain. The extracellular domain is able to bind to different extracellular matrix ligands, such as collagens, bronectin, Laminin and vitronectin Integrins play an important role as cell adhesion receptors and signaling receptors. Integrin-mediated adhesion to extracellular proteins can activate numerous cytoskeletal-associated proteins, such as paxillin, tensin and intracellular signaling proteins such as FAK (focal adhesion kinase, a non-receptor protein tyrosine kinase).(4)

Integrins, a family of cell surface receptors, are composed of non-covalently linked type 1 transmembrane glycoprotein subunits α and ß [5-8]. They mediate the connection between extracellular matrix (ECM) and intracellular actin cytoskeleton, which may lead to the activation of related signaling pathways and cause changes of cell function.(5)

Mammalian genomes contain 18 alpha subunit and 8 beta subunit genes, and to date 24 different combinations have been identified at the protein level. Although some subunits appear only in a single heterodimer, 12 integrins contain the beta1 subunit, and five contain alpha These molecules generates ligand-specific outside-in signals to modulate neutrophil apoptosis, a critical control point in the resolution of inflammation and specially ITGA2 gene C807T allele it has postulated to be a key factor in ischemic stroke.[6, 7]

A study by Wei YS, *et al.,* founded that the ITGA2 gene C807T allele polymorphism was associated with ischemic stroke, the ITGA2 gene C807T polymorphism may affect ischemic stroke through plasma lipid and lipoprotein levels.[8]

Another study by Marie- Claude Drupes *et al*., demonstrated that sickle reticulocytes express improved levels of alpha 4 beta 1 integrin (a4b1) which interacts basically with vascular cell adhesion molecule-1 and fibronectin, leading to vaso-occlusion.[9]

Another one connected the integrin as a crucial factor for triggering vaso-occlusion done by J.-P. Cartron et al revealed that reticulocytes from SCD patients express higher levels of a4b1 integrin and CD36, and that under hydroxyurea (HU) therapy, bothcell adhesion to extracellular matrix (EC) proteins and the levels of these adhesion molecules are reduced.[10]

So, according to findings above our goal in this study was to investigate the correlation between the ITGA2 C807T polymorphism and clinical severity of SCD in Sudanese patients, as well as of hematologic variables.

MATERIAL AND METHODS:

This is a descriptive analytical case- control, facility-based study, conducted at Gaafar Ibn-Auf Paediatric Tertiary Hospital, as a part of previous work started from March /2016 up to April /2017. The local ethics committee at Sudan University of Science and Technology and Khartoum State Ministry of Health approved the research conducted in accordance with World Medical Association (WMA) Declaration of Helsinki (2008).

Subjects:

The study population comprised; patients with Sickle cell disease, regardless to age, gender and ethnic group. The diagnoses of patients with sickle cell were confirmed using cellulose acetate electrophoresis having Hb SS disease. Clinical data collected by enclosed questionnaire and recording form and authorized clinician who carries out the clinical examination. After informed consent a sample of venous blood (5mL) collected under specific condition into two EDTA container (whole blood) one for DNA extraction and the second for CBC and electrophoresis**.** Apparently Healthy clinical controls were selected to be similar as sex, age group and residence place with the patients.

Genomic DNA extraction

To extract leukocyte genomic DNA we used a small amount of whole blood quickly with (innuPREP) whole Blood DNA (Mini Genomic DNA extraction Kit) which was then stored at −80°C until use.

DNA genotyping:

The detection of integrin alpha-2, (ITGA2) genes was based on examination of the size of the polymerase chain reaction (PCR) products.

The ITGA2 C807T (rs1126643) polymorphism genotyping has been achieved by *polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP) technique, using PCR following protocol.(11)

PCR amplification and primer design:

Each amplification reaction (total volume 25 µl L) contained the primer sequences for detecting the polymorphism 5'-TTCAGCTCTCAGCCAGCTTC-3' (forward primer) and 5'-TGCACTGAATCCCACTTGTGA-3' (reverse primer) (designed by primer 3 with aid of computer software Serial Cloner 2.6 program). 1µl of forward primer, 1 µl of reverse primer and 2 µl of DNA were added to the other PCR components needed for the reaction (Maxime PCR PreMix, i-Tag iNtRON BIOTECHNOLOGY South Korea). Initial denaturation in 95°C for 5 min, denaturation 30 cycles in 94°C for 1 min, annealing in 60°C for 1 min, extension in 72°C for 1 min, and then final elongation in 72°C for 10 min were done.

Restriction digestion

PCR amplification products were digested with restriction enzymes to identify polymorphisms; 3 µl products were digested with AvaII (Thermo-Fisher, Waltham, Massachusetts, USA) followed by PAGE (8%). The genotypes have been established after ethidium bromide staining. To identifyITGA2, reactions were performed at 37°C for 3 hours, and then products were identified by electrophoresis on an 8% polyacrylamide gel stained with ethidium bromide. A gel documentation imaging system was used to visualize bands.

Laboratory investigations:

Automated hematology analyzer (Sysmex KX21N) was used as standard laboratory methods to determine haemoglobin concentration, hematocrit concentration, red cell count, total white blood cells count, platelets count, red cell indices, and reference neutrophils and lymphocytes values at the time of presentation**.**

Statistical analysis:

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) IBM analytics US, for Windows software version 23.0 Means, standard deviations (SD), and percentages were determined. Means ± SD were compared using independent t-test or one-way analysis of variance (ANOVA) as appropriate. Ratios were compared using the Pearson Chi squared (χ^2) test. The odds ratio (OR) was used in order to compare distributions of alleles and genotypes between patients and healthy individuals. The relationship between disease severity and continuous variables such as age and laboratory findings was assessed using Pearson correlation analysis. With P value less than (0.05) were considered statistically significant.

RESULTS

All clinical parameters of the subjects were recorded before polymorphism genotyping. The age of respondents ranged forms 1 to 37 years; 6-7 years is most common age group. Vaso-occlusion and sequestration diseases were detected for 79 % of the SCD patients and those without crisis about 21% of all selected, hematological values as showed in **Table 1.**

RFLP for the ITGA2 C807T polymorphism produced three possible allele combinations: CC (two bands, 414 bp and

275 bp, CT (three bands, 689 bp, 414 bp, and 275 bp; or TT (one band, 689 bp), as shown in **figure 1.**

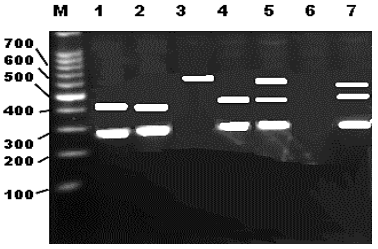

Figure 1. Genotyping of the ITGA2 C807T polymorphism was performed by PCRRFLP; lane M: DNA marker; lanes 1, 2, and 4: CC genotype; lanes 3: TT genotype; lanes 5 and 7: CT genotype and; lanes 6 is Negative control

Table2: Frequencies Genotype for ITGA2 C807T				
Sample		**Frequency N**	**Percent%**	**P. value**
Patients	CC	**17**	**12.78**	**0.0021**
	CT	**23**	**17.29**	
	TT	**93**	**69.92**	
Clinical severity High % of allele expression	CC	**Mild severity**	**93**	**0.000**
	CT	**Moderate severity**	**70**	
	TT	**Severe cases**	**90**	
Controls	CC	**91**	**82.72**	**0.0021**
	CT	**12**	**10.90**	
	TT	**7**	**6.36**	

The distribution of ITGA2 genotypes within both the control and patient groups reached by using Hardy-Weinberg equilibrium. However, χ^2 and correlation analyses revealed significant differences between the two groups in the distribution of the ITGA2 C807T genotype and allele frequencies ($p<0.05$). Furthermore, relative risk analysis of allele frequency showed that patients with the T allele were 5.4 times more likely to suffer from hemolytic crisis, vaso-occlusive and ischemic stroke rather than patients with the C allele (**Table 2**).

DISCUSSION

Integrins are adhesion molecules and in their nature that can mediate interactions among cells or between cells and the extracellular matrix. These proteins can aid white blood cells and platelets in adhering to the vascular endothelium, thereby facilitating the vascular occlusion.[12] As a member of the integrin family of adhesion molecules, ITGA2 is able to mediate cell-cell, cell-matrix, and cell-matrix-cell adhesions. This protein, therefore, plays an important role in the physiological and pathological processes of inflammatory reaction, immune response, thrombosis, etc.[13, 14] Studies have associated ITGA2 polymorphisms with vaso-occlusion. [15–17]

Because findings of correlations between ITGA2 polymorphism and vaso-occlusion and ischemic stroke have been conflicting, we assessed ITGA2 C807T polymorphisms in 133 SCD patients and 121 healthy controls among Sudanese population. To understand the potential mechanistic contribution of the ITGA2 C807T polymorphism to stroke, we also investigated differences in blood parameters among study population.

Unsurprisingly, patients suffering from SCD exhibited lower levels of Hb, RBCs, and HCT than controls.

Further, the ITGA2 C807T polymorphism affected clinical severity levels, as shown in the results the TT levels were significantly higher in patients with severe crisis when compared with patient carrying the CT allele and there was higher level of CC allele in mild cases. Therefore, individuals with increased TT allele, may increase the likelihood of ischemic stroke as well as vaso-occlusive and hemolytic crisis.

CONCLUSION

In summary, our findings suggest that these gene polymorphisms of ITGA2 C807T in particular, the T allele may be a hereditary susceptibility allele for development of complications in Sudanese patients with SCD, especially vaso-occlusion and cerebral stroke.

We recommend including wide variety of genes and sub-genes in such types of studies and investigations in future.

ACKNOWLEDGEMENT

We are so acknowledged to our supervisors who supplied us and guided us through this epic work. We also thank Gaafar Ibn-Auf Paediatric Tertiary Hospital; University of Khartoum, Faculty of Medical Laboratory Sciences, Institute of Endemic Diseases, Sudan University of Science and Technology for their permissions to carry out this work.

Table 1: Clinical Severity of Sickle Cell Disease among study population

Clinical Data		Severity of Disease			Total	P.value
		Mild	Moderate	Severe		
Gender	Female	7	23	46	**76**	0.456
	Male	7	12	38	**57**	
Age (mean) years		7.2	8.2	6.8	**7.2**	**0.001**
Stroke		2	18	67	**87**	**0.000**
Sequestration/vasoocclusion		4	21	81	**106**	**0.000**
Hemolytic Crisis		14	35	84	**133**	*a
Leg ulcer		0	1	3	**4**	0.768
Muco-skeletal pain		9	35	79	**123**	**0.000**
Acute Chest Syndrome		8	23	76	**107**	**0.001**

*Bolded values indicate statistical significance. *a No statistics are computed because Hemolytic Crisis is a constant

Table 2: Relationship between laboratory findings and disease severity

Laboratory parameters	Mild disease N=14 (mean)	Moderate disease N=35 (mean)	Severe disease N=84 (mean)	P
TWBCs ($\times 10^9$/L)	12.2	13.8	15.1	**0.007**
Neutrophils ($\times 10^9$/L)	6.8	7	7.5	**0.000**
Lymphocyte($\times 10^9$/L)	5.9	5.7	5.1	**0.001**
RBCs ($\times 10^{12}$/L)	2.6	2.7	2.3	**0.045**
Haemoglobin (g/dL)	7.0	8.2	7.3	**0.000**
Haematocrit (%)	22.7	22.9	21.3	**0.034**
MCV(fL)	89	86.3	94	**0.017**
MCH(pg)	32.4	30.7	34.3	**0.000**
MCHC(g/dL)	35.8	35.8	36.5	**0.001**
Platelet($\times 10^9$/L)	572.6	450	525	**0.044**

N: Number of cases, TWBCs, total white blood cells count; RBCs, red blood cells count; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin; **Bolded values** indicate statistical significance.

REFERENCES:

1. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *The Lancet*. 2010 Dec 17; 376(9757):2018-31.
2. Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HH, Elbashir MI, Crawford M. Effect of omega-3 (n− 3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. *The American Journal of Clinical Nutrition*. 2013; 97(1):37-44.
3. Alaaeddin M. Elzubeir *et al*, Association of L-Selectin gene polymorphism with Clinical Severity of sickle Cell Disease among Sudanese Patients in Khartoum. *International journal of multidisciplinary and current research.* 2016 July. Vol.4:637-640
4. Shakibaei M, Thilo JO, de Souza P, Rahmanzadeh R, Merker Hj. Signal transduction by ß1 integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. *Biochemical Journal.* 1999 Sep 15; 342(3):615-23.
5. Liu J, Cheng S, Zhang Y, Li H, Huang J, Zhang P. Association between polymorphisms in the integrin gene predicted microRNA

binding sites and bladder cancer risk. *Int J ClinExp Med*. 2014 Jan 1; 7(11):4398-405.

6. Humphries JD, Byron A, Humphries MJ. Integrin ligands at a glance. *Journal of cell science.* 2006 Oct 1;119(19):3901-3
7. Jian-Xia Lu, Zhong-Qian Lu, Shao-lan Zhang, Juan Zhi, Zheng-Ping Chen, and Wan-Xiang Wang, 2014 Mar, Polymorphism in Integrin ITGA2 is Associated with Ischemic Stroke and Altered Serum Cholesterol in Chinese Individuals *Balkan Med J*. (1): 55–59. doi: 10.5152/balkanmedj.2013.7993
8. Wei YS, Lan Y, Liu YG, Meng LQ, Xu QQ, Xie HY. Association of the integrin gene polymorphisms with ischemic stroke and plasma lipid levels. *Chinese journal of medical genetics*. 2009 Apr;26(2):211-5.
9. Marie-Claude Durpes, Marie-Dominique Hardy-Dessources, Wassim El Nemer, Julien Picot, Nathalie Lemonne, Jacques Elion, Monique Decastel. Activation State of α4β1 Integrin on Sickle Red Blood Cells Is Linked to the Duffy Antigen Receptor for Chemokines (DARC) Expression. *The journal of biological chemistry*. 2011 Jan 286,3057-3064.
10. Cartron JP, Elion J. Erythroid adhesion molecules in sickle cell disease: effect of hydroxyurea. Transfusion *clini queet biologique.* 2008 Mar 31;15(1):39-50
11. Xu-Guang Gao, Yang Huo, Xian-Zeng Liu, Zhi-Ping Teng, Gene Polymorphism of Platelet Glycoprotein I b in Chinese Patients with Large- and Small-Artery Subtypes of Ischemic Stroke *Eur Neurol* 2005;54:73–77
12. Hellewell PG. Adhesion molecule strategies. Pulm Pharmacol Ther. 1999;12:137–41.
13. Jacquelin B, Rozenshteyn D, Kanaji S, Koziol JA, Nurden AT, Kunicki TJ. Characterization of Inherited Differences in Transcription of the Human Integrin alpha 2 Gene. *J Biol Chem*. 2001;276:23518–24.
14. Vijayan KV, Liu Y, Dong JF, Bray PF. Enhanced activation of mitogen-activated protein kinase and myosin light chain kinase by the Pro33 polymorphism of integrin beta 3. *J Biol Chem*. 2003;278:3860–7.

15. Payne KE, Bray PF, Grant PJ, Carter AM. Beta3 integrin haplotype influences gene regulation and plasma von Willebrand factor activity. *Atherosclerosis.* 2008;198:280–6.
16. Manginas A, Tsiavou A, Chaidaroglou A, Giamouzis G, Degiannis D, Panagiotakos D, et al. Inflammatory cytokine gene variants in coronary artery disease patients in Greece. *Coron Artery Dis*. 2008;19:575–82.
17. Giusti B, Gori AM, Marcucci R, Sestini I, Saracini C, Paniccia R, et al. Role of glycoprotein Ia gene polymorphisms in determining platelet function in myocardial infarction patients undergoing percutaneous coronary intervention on dual antiplatelet treatment. *Atherosclerosis.* 2008;196:341–8.