

Frequency of beta S globin gene haplotypes among sickle cell patients in Nigeria

Journal of International Medical Research

49(6) 1–11

© The Author(s) 2021


Article reuse guidelines:

sagepub.com/journals-permissions

DOI: 10.1177/03000605211019918

journals.sagepub.com/home/imr



Abosedede Adabale^{1,*},
Samira Batista Lobo Makanjuola^{2,6,*} ,
Akinsegun Akinbami¹, Adedoyin Dosunmu¹,
Alani Akanmu³, Farideh A Javid⁴ and
Louis C Ajonuma^{5,6}

Abstract

Objective: To determine the frequency of beta s globin gene haplotypes in Nigerian patients with sickle cell disease (SCD) and to measure their correlation with clinical and haematological characteristics.

Methods: This study enrolled patients with SCD and collected their peripheral blood for restriction fragment length polymorphism analysis in order to identify five polymorphic sites in the β -globin gene cluster.

Results: A total of 245 homozygous SCD patients (490 alleles) were included in the study. Among the analysed alleles, 426 (86.9%) had the Benin (BEN) haplotype; 19 (3.9%) had the Senegal (SEN) haplotype; 31 (6.3%) had the Cameroon haplotype; five (1.0%) had the Bantu/Central African Republic haplotype; and nine 9 (1.8%) had atypical haplotypes. No significant association was observed between the haplotypes and haematological events, although patients with the BEN/SEN haplotype showed improved red blood cell counts, haemoglobin levels and red blood cell width index. No significant association was observed between the haplotypes and the

¹Department of Haematology and Blood Transfusion, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

²Department of Pharmacology, Toxicology and Therapeutics, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

³Department of Haematology and Blood Transfusion, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria

⁴School of Pharmacy, University of Huddersfield, Huddersfield, West Yorkshire, UK

⁵Department of Physiology, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

⁶LASUCOM Molecular Biology Laboratory, Medical Research Centre, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

*These authors contributed equally to this work.

Corresponding author:

Samira Batista Lobo Makanjuola, Department of Pharmacology, Lagos State University, College of Medicine (LASUCOM), 1-5 Akinjobi Way, Ikeja, G.R.A Ikeja, Lagos, Nigeria.

Email: sbatistalobo@hotmail.com



three clinical manifestations, although patients with the BEN/SEN haplotype showed a four-fold lower frequency of painful episodes.

Conclusion: These findings suggest that the SEN haplotype combined with the BEN haplotype might contribute toward a better haematological profile and milder clinical severity in SCD.

Keywords

Sickle cell disease, haplotypes, clinical severity, haematological profile

Date received: 3 November 2020; accepted: 29 April 2021

Introduction

Sickle cell anaemia (SCA) is one of the commonest genetic disorders in the world, affecting 7% of the world's population.^{1,2} Nigeria has a prevalence rate of sickle cell trait of 24% and a prevalence rate of SCA of 2%, which means that 150 000 affected infants are born in Nigeria every year.³ Nigeria has the highest number of SCA sufferers in the world.⁴

Normal haemoglobin protein is formed by two α -globin and two β -globin subunits.⁵ In SCA, there is a mutation in the β -globin subunits that encodes the haemoglobin gene resulting in an adenine-to-thymine (A>T) nucleotide substitution and the replacement of glutamic acid with valine.⁶ SCA occurs when both haemoglobin alleles donated by each parent are mutated, resulting in a homozygous genotype HbSS or β^S .⁶

Abnormal β^S subunits stick together to form long, rigid molecules that bend red blood cells into a sickled shape.⁵ Due to the rigidity, the sickled shaped cells die prematurely, which leads to the onset of anaemia.⁵ Sickled shaped cells can also block small blood vessels, causing severe pain, organ damage and premature death.⁷ The clinical course of sickle cell disease (SCD) is unpredictable and variable. For example, from the same cohort of patients, some will die at infancy, some will develop several

complications during their teenage years and adult life such as severe anaemia, infection and acute and chronic organ failure.^{2,8} While others remain asymptomatic with near normal life expectancy.⁹ The homozygous genotype HbSS or β^S is associated with five 'classical' haplotypes, which were first identified by the presence or absence of restriction fragment length polymorphisms (RFLP) surrounding the β -globin gene.¹⁰ These were subsequently found to be characterized by strong linkage disequilibrium to the adjacent DNA sequence encoding the haemoglobin gene.^{11,12} The haplotype of the β -globin gene cluster includes two major geographic variants: Africans and Asian derivatives.^{13,14} The African derivative encapsulates the Senegalese (SEN), Benin (BEN) and Bantu (BAN) haplotypes,¹³ while the Asian derivative includes the Arabian and Indian haplotype.¹⁴

Haplotype determination has become a key focus of research in SCD due to increasing evidence that the β -globin haplotype is linked with the clinical course of disease. For example, the BAN haplotype has shown the most severe clinical course;¹⁵ while the SEN haplotype is associated with increased levels of haemoglobin F (HbF), which inhibits misshaping of the abnormal β^S and reduces sickling of erythrocytes hence presenting ameliorated symptoms.¹⁶ The BEN and Cameroon (CAM)

haplotypes are associated with an intermediate clinical course.¹⁷

A better understanding of classical β^S haplotypes is of particular relevance to anthropological and population genetic studies.^{12,18} It may also be useful for understanding the varying clinical outcomes seen among individuals with SCD.¹⁹ However, most reports from Nigeria are focused on the clinical features of the disease rather than genetic determinants of clinical severity.²⁰ It has been assumed that the variant in Nigeria is the BEN haplotype, however, neighbouring countries have the BEN haplotype.¹³ When considering the effects of intermarriages and migration, it is worth determining the β^S haplotypes for SCD in Nigeria. Understanding the distribution of the β^S haplotypes in Nigeria might be useful in deciding upon a treatment plan for a patient, which could be tailored to their specific needs. Therefore, the aim of the current study was to determine the frequency of β^S globin gene haplotypes in SCD from a cohort of Nigerian patients and to measure their correlation with clinical and haematological profiles.

Patients and methods

Patient population

This prospective cross-sectional study enrolled consecutive non-consanguineous sickle cell patients from the Haematology Clinic, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria between April 2014 and April 2015. All of the patients were of Nigerian origin. The criteria for diagnosis of homozygous genotype HbSS amongst sickle cell patients were largely based on familial sickle cell trait and to a lesser extent on events leading to initial clinical presentation. Following initial diagnosis, patients were admitted for follow-up and treatment at the Haematology Clinic. Patients selected for

this study were administered a questionnaire, followed by clinical examination and were subjected to confirmatory diagnosis using alkaline haemoglobin electrophoresis showing homozygous genotype HbSS. No prior knowledge of their clinical state was available before enrolment. The inclusion criteria were as follows: (i) all patients with SCD attending the Haematology Clinic; (ii) all patients with written informed consent. The exclusion criteria were as follows: (i) patients transfused with blood within the last 3 months; (ii) patients using hydroxyurea.

Ethical approval for the study was granted by Ethics Committee of Lagos State University Teaching Hospital (no. LREC/10/06/292 and 10/06/226). All patients provided written informed consent.

Haematological determination

Peripheral whole blood samples (12 ml) were collected into two ethylenediaminetetra-acetic acid (EDTA) containing bottles and one plain bottle without an anticoagulant. A full blood count (FBC) was conducted within 2 h of collection using one of the EDTA samples on an automated 5-part Sysmex haematology counter (Sysmex, Kobe Hyogo, Japan), which also measured haemoglobin (Hb), haematocrit (HCT), red blood cell (RBC) count and red blood cell width index (RDW). The contents of the plain bottle were centrifuged and serum collected for storage at -20°C for further analysis. The second EDTA sample was also stored at -20°C until genomic DNA was extracted.

DNA sequence analysis

Genomic DNA was extracted from peripheral mononuclear cells using the cationic surfactant method dodecyltrimmonium bromide and chloroform.²¹ Subsequently, SCD polymorphisms were investigated

using polymerase chain reaction (PCR) amplification using commercially available PCR kit FIREPol DNA polymerase (Solis BioDyne Inc, Tartu, Estonia) with seven different primers XmnI (650 base pair [bp]), HindIII_G (780 bp), HindIII_A (760 bp), HincII $\Psi\beta$ (701 bp), HincII α (590 bp), HinfI (380 bp) and HpaI (620 bp) (Creative Biogene, Shirley, NY, USA; Jena Bioscience, Jena, Germany). These primer sequences were those used as previously described.²² Each primer was tested for optimal amplification conditions. The cycling programme involved preliminary denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 120 s, annealing at 65°C for 60 s, and elongation at 72°C for 120 s, followed by a final elongation step at 72°C for 7 min. DNA amplification was confirmed by separation of PCR products on 1.5% agarose gels and visualized using ethidium bromide staining and UV light. DNA amplification was satisfactory for 245 samples. Hence a total of

1715 amplified samples were produced from seven primers and 245 samples. RFLP haplotype investigations of the β -globin gene cluster were subsequently carried out using the fast-digest restriction enzymes Hind III, Hinc II, Hinf I, Hpa I and Xmn I (Thermo Fisher Scientific Inc., Rockford, IL, USA). All 1715 amplified PCR products were digested with enzymes specific to each primer as follows: Xmn (Xmn I); Hind III (Hind III_G, Hind III_A); Hinc II (Hinc II $\Psi\beta$, Hinc II δ); Hinf I (Hinf I); and HpaI (HpaI). Gel images were used to establish if a restriction site or polymorphism was present (+) or absent (-) (Figures 1 and 2).

Phenotype classification

The clinical records of all sickle cell patients enrolled in the study were examined and the following information was extracted in order to define disease severity: (i) blood transfusion, which can be caused by episodes of acute haemolysis, pulmonary hypertension or splenic crisis; (ii) the

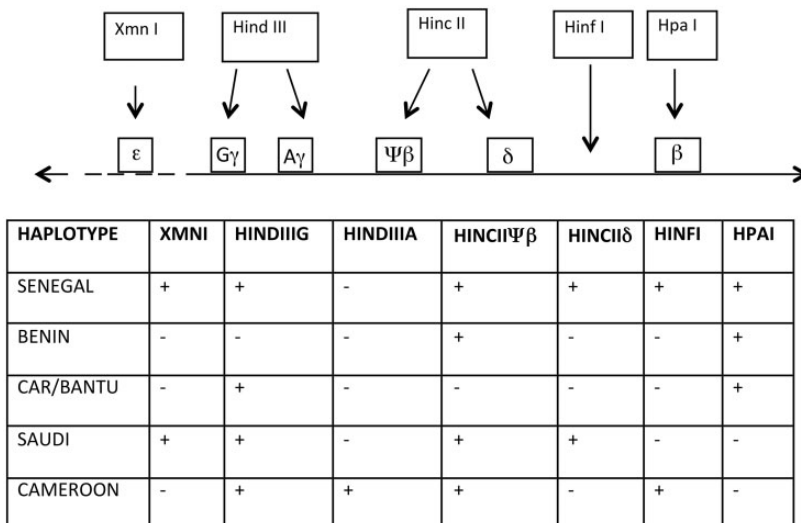


Figure 1. Classical haplotypes in the β -globin gene cluster. The figure illustrates the restriction fragment length polymorphisms (RFLP) in a 70 kilobase region around the haemoglobin gene. Five other globin synthesis genes are shown along with the approximate positions of five RFLP sites used to designate the five classical β^S haplotypes.

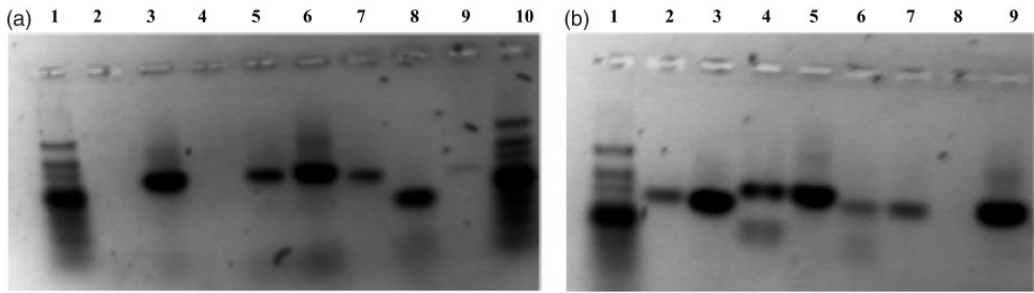


Figure 2. Representative gel picture (with contrast) showing all the primers used to determine β -globin haplotypes (a): lane 1, 1000 base pair (bp) molecular weight ladder; lane 2, negative control sample; lane 3, XmnI; lane 4, blank; lane 5, HindIII; lane 6, HindIII and HincII; lane 7, HincII; lane 8, HinfI; lane 9, HpaI; lane 10, 100 bp molecular weight ladder. Representative gel picture (with contrast) showing primers and corresponding digestion enzymes used (b): lane 1, 1000 bp molecular weight ladder; lanes 2 and 3, XmnI product undigested (650 bp); lanes 4 and 5, digested and undigested HindIII products (sizes are 340/430 and 780 bp); lanes 6 and 7, digested and undigested HindIII products (sizes are 360/400 and 760 bp); lane 8, blank; lane 9, undigested HincII primer (size is 701 bp).

number of annual hospitalizations; (iii) frequency of vaso-occlusive crises, which were clinically defined as pains in the bones, muscle, and joints that required analgesia or hospitalization. Data were gathered based on a prospective data design, where recruited patients at the Haematology Clinic were administered questionnaires by the nurses, followed by clinical examination by the consultant and were subjected to confirmatory diagnosis using alkaline haemoglobin by the laboratory staff. No prior knowledge of their clinical state was available hence reducing symptomatic bias from clinic-based population. The severity of disease expression was then correlated with haplotype distribution in this region of Nigeria.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). The prevalent haplotypes were determined and their associations with complications of SCD were assessed. Continuous data are presented as mean \pm SD. Proportions were compared using

Pearson's χ^2 -test. A P -value ≤ 0.05 was considered statistically significant. Results are reported as odds ratio (OR) with 95% confidence interval (CI).

Results

This study included 265 non-consanguineous sickle cell patients. The predominant age group were aged 16–30 years (179 of 265 patients; 67.5%) (Table 1); 72 (27.2%) were aged between 31–50 years; and four (1.5%) were older than 50 years. A higher proportion of the sickle cell patients were females (156 of 265 patients; 58.9%). The majority of the sickle cell patients were diagnosed with SCD between 3–10 years of age (111 of 265 patients; 41.9%).

Of the 265 patients that were enrolled in this study, the samples for 20 patients were lost due to failure of the PCR amplification, leaving 245 sickle cell patients with homozygous haemoglobin S variant to be assessed for their β -gene cluster haplotype. This was a total 490 alleles investigated, of which 426 alleles had the BEN haplotype (86.9%); 19 had the SEN haplotype (3.9%); 31 had the CAM haplotype (6.3%); five had the

Table 1. Demographic and clinical characteristics of the patients ($n = 265$) with sickle cell disease included in this study to investigate the beta S globin gene haplotypes in Nigeria.

Characteristic	Study cohort $n = 265$
Age group, years	
3–15	10 (3.8%)
16–30	179 (67.5%)
31–50	72 (27.2%)
>50	4 (1.5%)
Sex	
Female	156 (58.9%)
Male	109 (41.1%)
Age at diagnosis	
Up to 6 months	34 (12.8%)
7 months–2 years	52 (19.6%)
3–10 years	111 (41.9%)
11–18 years	42 (15.8%)
>18 years	9 (3.4%)
No record information	17 (6.4%)

Data presented as n of patients (%).

Table 2. The association between haematological characteristics and the beta S globin gene haplotypes in patients ($n = 217$) with sickle cell disease in Nigeria.

Characteristic	Haplotypes ^a		
	BEN/BEN $n = 169$	BEN/CAM $n = 30$	BEN/SEN $n = 18$
Hb			
≥ 7 g/dl	126 (74.6%)	20 (66.7%)	15 (83.3%)
< 7 g/dl	43 (25.4%)	10 (33.3%)	3 (16.7%)
OR (95% CI)	–	0.661 (0.42, 1.53)	2.975 (0.66, 13.39)
HCT			
$\geq 22\%$	118 (69.8%)	19 (63.3%)	15 (83.3%)
$< 22\%$	51 (30.2%)	11 (36.7%)	3 (16.7%)
OR (95% CI)	–	0.880 (3.91, 1.98)	1.223 (0.41, 3.64)
RBC count			
$\geq 2.8 \times 10^9/l$	97 (57.4%)	17 (56.7%)	12 (66.7%)
$< 2.8 \times 10^9/l$	72 (42.6%)	13 (43.3%)	6 (33.3%)
OR (95% CI)	–	1.017 (0.46, 2.23)	1.426 (0.50, 4.04)
RDW			
$\geq 60\%$	136 (80.5%)	22 (73.3%)	10 (55.6%)
$< 60\%$	33 (19.5%)	8 (26.7%)	8 (44.4%)
OR (95% CI)	–	0.688 (0.27, 1.76)	0.313 (0.11, 0.89)

Data presented as n of patients (%).

^aComplete haematological data were not available for 13 of 182 BEN/BEN patients and one of 19 BEN/SEN patients. No significant associations ($P > 0.05$); Pearson's χ^2 -test.

BEN, Benin; SEN, Senegal; CAM, Cameroon; Hb, haemoglobin; OR, odds ratio; CI, confidence interval; HCT, haematocrit; RBC, red blood cell; RDW, red blood cell width index.

BAN/Central African Republic haplotype (CAR) (1.0%); and nine had atypical haplotypes (1.8%). Among the most prevalent haplotype combinations was homozygous BEN/BEN accounting for 182 of 245 (74.3%), heterozygous BEN/CAM and BEN/SEN with 30 of 245 (12.2%) and 19 of 245 (7.8%), respectively. The least prevalent haplotypes included BEN/atypical (ATY) in eight of 245 (3.3%), BEN/CAR in five of 245 (2.0%) and ATY/CAM in one of 245 (0.4%). In 13 of 182 BEN/BEN patients and one of 19 BEN/SEN patients complete haematological and/or clinical data were not available.

Table 2 presents the analysis of the association between the β^S globin gene haplotypes and four haematological characteristics. The mean \pm SD Hb in the study cohort was 7.90 ± 1.56 g/dl. A cut-off of 7 g/dl was used as it is the established

World Health Organization cut-off value for severe anaemia.²³ The BEN/SEN haplotype recorded a higher proportion of patients (15 of 18 patients; 88.3%) with Hb values ≥ 7 g/dl compared with the BEN/BEN (126 of 169; 74.6%) and BEN/CAM (20 of 30 patients; 66.7%) haplotypes. The mean \pm SD HCT in the study cohort was $24.3\% \pm 4.9\%$. A cut-off of 22% was used, which was just above the level of 20% for severe haemolytic anaemia.¹⁹ Over 80% (15 of 18 patients) with the BEN/SEN haplotype recorded HCT values $\geq 22\%$, while 69.8% (118 of 169 patients) and 19 of 30 patients (63.3%) had the BEN/BEN and BEN/CAM haplotypes, respectively. The mean \pm SD RBC count was $2.97 \pm 0.7 \times 10^9/l$. A cut-off of $2.8 \times 10^9/l$ was used. Over 65% (12 of 18 patients) with the BEN/SEN haplotype recorded RBC counts $\geq 2.8 \times 10^9/l$, this reduced to 57.4% (97 of 169 patients) for patients with the BEN/BEN and 56.7%

(17 of 30 patients) for BEN/CAM haplotypes. The mean \pm SD RDW was 67.77 ± 10.6 . A cut-off of 60% was used. Over 80% (136 of 169 patients) of the patients with the BEN/BEN haplotype and 73.3% (22 of 30 patients) with the BEN/CAM haplotype recorded RDW $\geq 60\%$, while only 55.6% (10 of 18 patients) was recorded for the BEN/SEN haplotype.

Although no significant association was established between the haematological parameters and the various haplotypes, it was observed that patients with the BEN/SEN haplotype displayed a three-fold increased association with improved Hb levels when compared with the BEN/BEN haplotype (OR 2.975; 95% CI 0.66, 13.39), while the BEN/SEN haplotype also displayed a lower risk with increased RDW (OR 0.313; 95% CI 0.11, 0.89) compared with the BEN/BEN haplotype.

Table 3 presents the analysis of the association between the β^S globin gene

Table 3. The association between clinical manifestations and the beta S globin gene haplotypes in patients ($n = 223$) with sickle cell disease in Nigeria.

Clinical characteristics	Haplotypes ^a		
	BEN/BEN $n = 174$	BEN/CAM $n = 30$	BEN/SEN $n = 19$
Hospital admission			
Yes	54 (31.03%)	10 (33.33%)	4 (21.05%)
No	120 (68.97%)	20 (66.67%)	15 (78.95%)
OR (95% CI)	–	0.990 (0.39, 20.5)	1.668 (0.53, 5.32)
Blood transfusion			
Yes	102 (58.62%)	16 (53.33%)	10 (52.63%)
No	72 (41.38%)	14 (46.67%)	9 (47.37%)
OR (95% CI)	–	1.240 (0.56, 2.69)	1.275 (0.49, 3.29)
Pain episodes			
Yes	101 (58.05%)	17 (56.67%)	7 (36.84%)
No	73 (41.95%)	13 (43.33%)	12 (63.16%)
OR (95% CI)	–	0.999 (0.41, 2.16)	4.744 (1.904, 11.59)

Data presented as n of patients (%).

^aComplete clinical data were not available for eight of 182 BEN/BEN patients.

No significant associations ($P > 0.05$); Pearson's χ^2 -test.

BEN, Benin; SEN, Senegal; CAM, Cameron; Hb, haemoglobin; OR, odds ratio; CI, confidence interval; HCT, haematocrit; RBC, red blood cell; RDW, red blood cell width index.

haplotypes and three clinical manifestations of SCD. Hospital admission was not significantly influenced by the haplotype, with the BEN/BEN and BEN/CAM haplotypes recording higher numbers of hospitalizations compared with the BEN/SEN haplotype. Similarly, blood transfusion was not significantly influenced by the haplotype, with the BEN/SEN and BEN/CAM haplotypes recording a lower frequency of blood transfusions compared with the BEN/BEN haplotype. Pain episodes were not significantly associated with the haplotype, but painful episodes showed a four-fold lower risk with the BEN/SEN haplotype (OR 4.744; 95% CI 1.904, 11.59).

Discussion

This study analysed 490 alleles from 245 patients with SCD that had the homozygous HbSS genotype and it showed five haplotype combinations defined by RFLP analysis. The BEN haplotype was the most predominant, being recorded in 426 alleles (86.9%), while only 31 alleles (6.3%) presented with the CAM haplotype, 19 (3.9%) had the SEN haplotype and five (1.0%) had the BEN/CAR haplotype. In 1984, it was reported that within African populations with significant frequencies of sickle cell trait, three different haplotypes were present, which corresponded almost entirely to the geographic origin.¹³ For example, sickle cell patients in the mid-Western Africa region including Benin and Nigeria were homozygous for one haplotype (BEN/BEN), while the Bantu (Central African region) and those from Senegal (Atlantic-Western region of Africa) were homozygous for two other haplotypes, CAR/CAR and SEN/SEN, respectively.¹³ A later study then reported on a fourth distinct haplotype among sickle cell patients along the West coast of Africa, Cameroon CAM/CAM.²⁴ This was followed by findings of a fifth haplotype present in the Arabian

Peninsula and India.¹⁴ Those that do not correspond to the five standard haplotypes, which are commonly associated with the β -globin gene are called atypical haplotypes.²⁵ Notably, the present study demonstrated a high frequency (426 of 490 alleles; 86.9%) of the BEN haplotype. The prevalence of the BEN haplotype in the Nigerian population was reported in two previous studies, which both demonstrated a high frequency of the homozygous BEN/BEN haplotype of 93.2% and 97%.^{17,26} However, the present study is the first haplotype determination study to include patients from the Lagos region. Due to the cosmopolitan nature of Lagos, there is increased ethnic mixing, which may account for the prevalence of other haplotypes including the CAM haplotype and to a lesser extent the SEN and CAR haplotypes. In addition, previous research was not limited to homozygous sickle cell patients, but included HbAA and HbAS genotypes as well as members of the same family, which may lead to an increased prevalence of any of the haplotypes determined.¹⁷

Defining haplotypes in patients homozygous for SCD is crucial as clinical presentation can be variable hence making patient management challenging.¹⁷ The discovery of the β -globin haplotype and its association with clinical severity of the disease was therefore significant.²⁷ Some haplotypes such as the BAN haplotype is associated with the most severe clinical outcomes with a three-fold increased risk of stroke, renal failure, chronic lung disease, leg ulcers and young adult deaths.²⁸ The SEN and Arab haplotypes are associated with increased levels of HbF, which inhibits misshaping and sickling of erythrocytes, reducing attachment to vascular epithelium, hence ameliorating clinical symptoms.²⁹ While the BEN and CAM haplotypes exhibit intermediate levels of HbF and clinical severity.³⁰

In this current study, the haematological profiles were compared between three of the most prevalent haplotypes BEN/BEN, BEN/SEN and BEN/CAM. Findings in the overall cohort demonstrated reduced RBC count, Hb and HCT, which were expected due to ongoing chronic haemolysis in sickle cell patients. No significant correlation was observed between the various haplotypes and the haematological parameters, although the BEN/SEN haplotype showed an improved haematological profile with a three-fold likelihood of increased Hb levels and a two-fold likelihood of reduced RDW compared with the other haplotypes. The SEN haplotype is associated with reduced sickling of RBC due to increased HbF levels, which helps inhibit misshaping of the erythrocytes, thus the erythrocytes undergo less mechanical fragility, which improves their numbers and Hb levels.³¹ Reducing RDW is associated with rapid erythropoiesis.³² However, all SEN haplotypes in this current study were heterozygous, paired with the BEN haplotype, hence the presence of the BEN haplotype may have obscured the significant effect of the SEN haplotype on the haematological parameters.

The most common clinical manifestation of SCD is vaso-occlusive crisis, which occurs when the microcirculation is obstructed by sickled erythrocytes, causing ischaemic injury to the organ supplied and resulting in acute bone pain.^{33,34} The findings in this current study showed no significant association between frequency of painful episodes and any specific haplotype. However, patients with the BEN/SEN haplotype were four-times less likely to develop painful episodes compared with the other haplotypes. Again, because no homozygous SEN/SEN haplotypes were found in this current study, the presence of the BEN haplotype might have reduced the ameliorating effects of the SEN haplotype in terms of the clinical severity of SCD. Assessment of

HbF in patients may have contributed towards ascertaining a correlation between the SEN/BEN haplotype and the clinical manifestations, as it is probably the only parameter that is haplotype-dependent amongst sickle cell patients. If there were patients with significantly higher HbF levels, this would have potentially resulted in a milder clinical phenotype. It is therefore not surprising that although observations from this study are suggestive of a milder effect of the SEN haplotype on haematological parameters and clinical manifestations, no significant association was found between the haplotype and clinical phenotype as previously reported.¹⁷

In conclusion, SCD remains a public health threat in Nigeria. Defining haplotypes in SCD allows clinicians to predict to some extent the prognosis and to plan treatment tailored to increase HbF levels, which may in turn improve haemolytic profile and reduce clinical severity in patients with SCD.

Author contributions

Samira Batista Lobo Makanjuola: manuscript preparation, statistical analysis, literature review and DNA extraction; Abosede Adabale: literature review, research methodology and execution; Farideh A Javid: manuscript preparation and editing; Akinsegun Akinbami, Adedoyin Dosunmu, Alani Akanmu and Louis C Ajonuma: research methodology and manuscript editing.

Acknowledgement

The authors would like to thank the Lagos State University Teaching Hospital, Lagos University Teaching Hospital and the Lagos State University College of Medicine for the support provided.


Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD

Samira Batista Lobo Makanjuola  <https://orcid.org/0000-0001-7738-096X>

References

- Williams TN and Weatherall DJ. World distribution, population genetics, and health burden of hemoglobinopathies. *Cold Spring Harb Perspect Med* 2012; 2: a011692.
- Modell B and Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008; 86: 480–487.
- Anie KA, Egunjobi FE and Akinyanju OO. Psychosocial impact of sickle cell disorder: perspectives from a Nigerian setting. *Global Health* 2010; 6: 2.
- Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickled haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet* 2013; 381: 142–151.
- Marengo-Rowe AJ. Structure-function relations of human hemoglobins. *Proc (Bayl Univ Med Cent)* 2006; 19: 239–245.
- Kato G, Piel F, Reid C, et al. Sickle cell disease. *Nat Rev Dis Primers* 2018; 4: 18010.
- Steinberg MH and Rodgers GP. Pathophysiology of sickle cell disease: role of cellular and genetic modifiers. *Semin Hematol* 2001; 38: 299–306.
- Serjeant GR. Geography and the clinical picture of sickle cell disease. *Ann N Y Acad Sci* 1989; 565: 109–119.
- Akinbami A, Dosunmu A, Adediran A, et al. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Res Notes* 2012; 5: 396.
- Orkin SH, Kazazian HH Jr, Antonarakis SE, et al. Linkage of beta-thalassaemia mutations and beta-globin gene polymorphisms with DNA polymorphisms in human beta-globin gene cluster. *Nature* 1982; 296: 627–631.
- Webster MT, Clegg JB and Harding RM. Common 5' beta-globin RFLP haplotypes harbour a surprising level of ancestral sequence mosaicism. *Hum Genet* 2003; 113: 123–139.
- Fullerton SM, Harding RM, Boyce AJ, et al. Molecular and population genetic analysis of allelic sequence diversity at the human beta-globin locus. *Proc Natl Acad Sci USA* 1994; 91: 1805–1809.
- Pagnier J, Mears JG, Dunda-Belkhdja O, et al. Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa. *Proc Natl Acad Sci USA* 1984; 81: 1771–1773.
- Kulozik AE, Wainscoat JS, Serjeant GR, et al. Geographical survey of beta S-globin gene haplotypes: evidence for an independent Asian origin of the sickle-cell mutation. *Am J Hum Genet* 1986; 39: 239–244.
- Brittain HA, Eckman JR, Swerlick RA, et al. Thrombospondin from activated platelets promotes sickle erythrocyte adherence to human microvascular endothelium under physiologic flow: a potential role for platelet activation in sickle cell vaso-occlusion. *Blood* 1993; 81: 2137–2143.
- Ogedegbe HO. β -Globin gene cluster haplotype analysis as a predictor of sickle cell disease severity. *Laboratory Medicine* 2007; 38: 563–568.
- Adekile AD, Kitundu MN, Gu LH, et al. Haplotypes in SS patients from Nigeria; characterization of one atypical bS haplotype no.19 (Benin) associated with elevated Hb F and high G gamma levels. *Ann Hematol* 1992; 65: 41–45.
- Harding RM, Fullerton SM, Griffiths RC, et al. Archaic African and Asian lineages in the genetic ancestry of modern humans. *Am J Hum Genet* 1997; 60: 772–789.
- Powars D and Hiti A. Sickle cell anemia. Beta s gene cluster haplotypes as genetic markers for severe disease expression. *Am J Dis Child* 1993; 147: 1197–1202.
- Adeyemo AA, Amodu OK, Ekure AA, et al. Medical genetics and genomic medicine in Nigeria. *Mol Genet Genomic Med* 2018; 6: 314–321.

21. Gustincich S, Manfioletti G, Del Sal G, et al. A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques* 1991; 11: 298–302.
22. Sutton M, Bouhassira EE and Nagel RL. Polymerase chain reaction amplification applied to the determination of β -like globin gene cluster haplotypes. *Am J Hematol* 1989; 32: 66–69.
23. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity, www.who.int/vmnis/indicators/haemoglobin/en (2011, accessed 12 March 2020).
24. Lapoum roulie C, Dunda O, Ducrocq R, et al. A novel sickle gene of yet another origin in Africa: the Cameroon type. *Hum Genet* 1992; 89: 333–337.
25. Embury SH, Hebbel RP, Mohandas N, et al. *Sickle Cell Disease: Basic Principles and Clinical Practice*. New York: Raven, 1994.
26. Falusi A and Kulozik A. Relationship of foetal haemoglobin levels and beta s haplotypes in homozygous sickle cell disease. *Eur J Haematol* 1990; 45: 1–4.
27. Rieder RF, Safaya S, Gillette P, et al. Effect of beta-globin gene cluster haplotype on the hematological and clinical features of sickle cell anemia. *Am J Hematol* 1991; 36: 184–189.
28. Steinberg MH. Predicting clinical severity in sickle cell anaemia. *Br J Haematol* 2005; 129: 465–481.
29. Morrison JC, Whybrew WD, Bucovaz ET, et al. Fluctuation of foetal haemoglobin in sickle cell anemia. *Am J Obstet Gynecol* 1976; 125: 1085–1088.
30. Loggetto SR. Sickle cell anemia: clinical diversity and beta S-globin haplotypes. *Rev Bras Hematol Hemoter* 2013; 35: 155–1557.
31. Bailey K, Morris JS, Thomas P, et al. Fetal haemoglobin and early manifestations of homozygous sickle cell disease. *Arch Dis Child* 1992; 67: 517–520.
32. Omoti CE. The value of foetal haemoglobin level in the management of Nigerian sickle cell anaemia patients. *Niger Postgrad Med J* 2005; 12: 149–154.
33. Shapiro BS. The management of pain in sickle cell disease. *Pediatr Clin North Am* 1989; 36: 1029–1045.
34. Payne R. Pain management in sickle cell disease. Rationale and techniques. *Ann N Y Acad Sci* 1989; 565: 189–206.