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ORIGINAL ARTICLE

Assessment of *MTR* Rs1805087 SNP as Possible Modifier of Sickle Cell Disease Severity in a Nigerian Population

Évaluation du SNP MTR Rs1805087 en Tant Que Modificateur Possible de la Gravité de la Maladie à Cellules Sickle dans une Population Nigérienne

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ABSTRACT

BACKGROUND: Sickle cell disease is the commonest genetic disorder in Nigeria, affecting 2–3% of an estimated population of 160 million people. The role of genetic mutations in folate cycle genes, and the variable phenotypic expressions constituting disease severity, needs to be critically examined.

OBJECTIVE: This study was carried out to establish the pattern of *methionine synthase* gene mutations (rs1805087 SNP), and its possible association with disease severity in adults with sickle cell anaemia in Lagos, Nigeria.

METHODOLOGY: This is a cross-sectional study of seventy (70) subjects with sickle cell disease (HbSS) matched for age and gender with known apparently healthy haemoglobin genotype AA (HbAA) subjects, as cases and controls respectively. Structured questionnaires were used to obtain demographic, clinical and other phenotypic data needed to compute disease severity. Pattern of *MTR A2756G gene mutation and* homocysteine assay (Hcy) were assessed by Polymerase Chain Reaction and Enzyme- linked Immunosorbent Assay respectively. Full blood count analysis of participants was done using the KX-21 Automated Analyzer (Sysmex Corporation, Japan).

RESULTS: The mutant genotypes *MTR* 2756 AG/GG were recorded in 46.4% (n =55) of subjects with disease severity score >7. Elevated plasma homocysteine (HHcy) was significantly associated with disease severity among HbSS subjects (OR=17.2, CI: 3.490-86.079; p=0.0001). Conversely, no significant association was observed with the mutant genotypes *MTR* 2756 AG/GG and disease severity (p>0.05).

CONCLUSION: While HHcy is significantly associated with phenotypic expression of HbSS, the *MTR* 2756 SNPs did not appear to independently influence homocysteine level or disease severity in HbSS subjects. **WAJM 2022; 39(11): 1198–1204.**

Keywords: Methionine synthase gene, Sickle cell disease, Sickle cell disease severity, Homocysteine.

RÉSUMÉ

CONTEXTE: La drépanocytose est la maladie génétique la plus répandue au Nigeria, affectant 2 à 3 % d'une population estimée à 160 millions de personnes. Le rôle des mutations génétiques dans les gènes du cycle du folate, et les expressions phénotypiques variables constituant la gravité de la maladie, doivent être examinés de façon critique.

OBJECTIF: Cette étude a été menée pour établir le schéma des mutations du gène de la méthionine synthase (rs1805087 SNP), et son association possible avec la gravité de la maladie chez les adultes atteints de drépanocytose à Lagos, au Nigeria.

MÉTHODOLOGIE: Il s'agit d'une étude transversale de soixante-dix (70) sujets atteints de drépanocytose (HbSS) appariés pour l'âge et le sexe avec des sujets connus apparemment sains de génotype d'hémoglobine AA (HbAA), comme cas et contrôles respectivement. Des questionnaires structurés ont été utilisés pour obtenir des données démographiques, cliniques et autres données phénotypiques nécessaires au calcul de la gravité de la maladie. Le profil de la mutation du gène MTR A2756G et le dosage de l'homocystéine (Hcy) ont été évalués respectivement par réaction en chaîne par polymérase et par test immunologique enzymatique. L'analyse de la formule sanguine complète des participants a été effectuée à l'aide de l'analyseur automatisé KX-21 (Sysmex Corporation, Japon).

RÉSULTATS: Les génotypes mutants MTR 2756 AG/GG ont été enregistrés chez 46,4 % (n =55) des sujets présentant un score de gravité de la maladie > 7. L'homocystéine plasmatique élevée (HHcy) était significativement associée à la gravité de la maladie chez les sujets HbSS (OR=17,2, CI : 3,490–86,079 ; p=0,0001). À l'inverse, aucune association significative n'a été observée entre les génotypes mutants MTR 2756 AG/GG et la gravité de la maladie (p>0,05).

Conclusion : Alors que l'HHcy est significativement associée à l'expression phénotypique de l'HbSS, les SNP MTR 2756 ne semblent pas influencer indépendamment le niveau d'homocystéine ou la gravité de la maladie chez les sujets HbSS. **WAJM 2022; 39(11): 1198–1204.**

Mots clés: Gène de la méthionine synthase, Drépanocytose, Gravité de la drépanocytose, Homocystéine.

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Abbreviations: HbAA, Haemoglobin; **AA** Phenotype; **HbSS,** Haemoglobin; **SS** Phenotype (Sickle Cell Anaemia); **Hcy,** Homocysteine; **HHcy,** Hyperhomocysteinaemia; **MTR,** Methionine Synthase Gene; **SNPs,** Single Nucleotide Polymorphisms.

INTRODUCTION

Sickle cell disease has long been recognized as a disorder of significant public health importance[1] The disease is endemic in sub-Saharan Africa, India and the Middle-East.[1] More than 75% of sickle cell disorders are documented to occur in sub-Saharan Africa.[1] Sickle cell disease is the commonest genetic disorder in Nigeria where it is projected to affect 2-3% of an estimated population of 160 million people, with about 25% of adults having the sickle cell trait.[2–4]

Disease severity score presents a possible tool that may be used as a prognostic index in sickle cell disease. However, a clear consensus on the modalities for evaluating disease severity, is still a subject of debate.[5] Many authors on this subject matter, have opined that an interplay of multiple biological, genetic and environmental factors may affect phenotypic characteristics, and ultimately disease severity in sickle cell disease.[5] Because it is difficult to quantitatively define the concept of disease severity in sickle cell disease, many researchers have resulted to the use of multiple variables, which are combinations of clinical events and laboratory reports.[5,6] This has led to the formulation of different models for indicating disease severity in sickle cell disease. A study conducted among persons with SCA in south-western Nigeria over a one-year period, assessed a total of 15 parameters to reflect each patient's present state, their state during the previous one year, and lifetime complications[6]. However, the most referenced severity scoring models adopted in many studies from Nigeria[7] (with few modifications) is one reported by Hedo, *et al*.[8] (Table 1). However, as a limitation, this study model did not consider genetic polymorphisms that may also underlie or modulate some of the observed laboratory and clinical outcomes that were factored into the computation of the disease severity model in these category of patients.[9]

Folate metabolism is key to the sustenance of increasing haemopoetic activities in sickle cell disease. Methionine synthase, is a rate limiting folate cycle enzyme required in the generation of physiological methyl donors (methionine) which is required for subsequent re-methylation of harmful biochemical intermediates (homocysteine), methylation of lipids and modification of various gene expression mechanisms.[10] Methionine synthase is a cytoplasmic enzyme that requires methylcobalamin for activity; and two polymorphisms in the methionine synthase gene have been commonly reported.[10] A point mutation, where aspartic acid is replaced by glycine at nucleotide position 2756 (MTR 2756A>G). The influence of MTR 2756A>G on total homocysteine plasma levels is still controversial. Normal methionine synthase activity is responsible for the regeneration of methionine from homocysteine, maintaining the level of circulating folate and methionine and possibly preventing accumulation of homocysteine. In the presence of methionine synthase gene mutation, there is a possibility for the accumulation of homocysteine which theoretically, can worsen the clinical presentations of sickle cell disease. This is because homocysteine has been widely documented to cause significant endothelial damage and vasculopathy.[11,12,13]

Very few studies, if any, have attempted to link *Methionine synthase* gene polymorphisms with sickle cell anaemia sub-phenotypes among African populations. Therefore, this study aimed to establish the pattern of *Methionine synthase* gene mutations among adults with sickle cell anaemia, and determine the role of *Methionine synthase* gene polymorphism as a contributory factor in their differential phenotypic expression and disease severity.

SUBJECTS, MATERIALS AND METHODS

Study Site and Population

This study was carried out at the Haematology Clinic of the Lagos State University Teaching Hospital, (LASUTH) Lagos, Nigeria. The tertiary center provides multidisciplinary healthcare service for the cosmopolitan city of Lagos state (with a projected population of over 21million people), and its environs. The study recruited patients with sickle cell anaemia (HbSS) >18years, diagnosed by haemoglobin electrophoresis at alkaline pH (study group), while apparently healthy age and sex matched individuals with no known medical conditions (control group) were recruited from consenting members of staff and medical students with Haemoglobin (Hb) phenotype AA.

Ethical Considerations

Ethical approval was obtained from the Health Research and Ethics Committee of LASUTH prior to commencement of the study. A written informed consent was obtained from all the participants after due explanation of study objectives and procedures. In order to guarantee confidentiality, participants were given code numbers and all their electronic records were protected using passwords.

Inclusion and Exclusion Criteria

Persons living with sickle cell anaemia, who had been screened by the Haemoglobin S solubility test and diagnosed by cellulose acetate electrophoresis at pH 8.4, and aged ≥18years, were recruited into the study. Only HbSS subjects in steady state were included in the study (steady state was defined as crisis free period extending from at least three weeks since the last clinical event and three months or more since the last blood transfusion, to at least one week before the start of a new clinical event).[14] Control group participants were Haemoglobin AA persons as confirmed by cellulose acetate electrophoresis at pH 8.4. All other Hb genotypes apart from HbSS; persons with: acute febrile illness, hypertension, diabetes mellitus and other known systemic diseases, including smokers, alcoholics and intravenous drug users were excluded from this study.

Study Method

Pretested, structured questionnaires were administered by the researcher on all participants to obtain relevant clinical and demographic data. Information on the subjects and their medical history including disease complications were retrieved from their hospital records.

Assessment of Disease Severity Score

The disease severity score was assessed using a modified scoring system by Hedo *et al* (Table 1).[8] This scoring system has also been used by other researchers in Nigeria.[7] In this

study, the disease severity state was calculated based on 11 parameters derived from physical examination, laboratory investigations and findings retrieved from hospital records. The parameters analyzed were number of crisis per year; number of blood transfusions per year; presence of: acute chest syndrome, osteomyelitis, renal failure, heart failure, avascular necrosis of the femoral head, pneumonia, pigment gallstones and jaundice, dehydration and low haemoglobin levels. The total severity score was computed as ≤7 for low severity, and >7 for high severity.[7]

Determination of Plasma Homocysteine Level

Plasma samples were separated from whole blood by centrifugation (3000 *g* for 10 min at 4°C) and stored at –80°C until analyses. The plasma from the EDTA sample was used for the estimation of homocysteine based on Enzyme Immunoassay technique using the AxisR-homocysteine EIA kit (LOT No: 802896074, Axis-Shield Diagnostics Ltd, Scotland, United Kingdom). Final reading was done by ELX 800TM absorbance microtiter reader at 450nm (Biotek Ltd, UK serial No: 205808). Detection range for the plasma homocysteine was 2–50 µmol/L.

Determination of Methionine Synthase Gene Polymorphism

DNA Extraction

Extraction of DNA from whole blood was done by spin column method using Jena Bioscience Blood-Animal-Plant DNA preparation kit (Thuringia, Germany). The concentration, purity and yield of the DNA samples were checked using Unico 2100 Spectrophotometer (Unico, USA) at 260nm and 280nm respectively. Eluted DNA samples were stored at –20°C till they were used.

PCR Amplification and Restriction Digestion

Polymerase chain reaction **(**PCR) was performed in a 25 µl reaction volume containing 50 µg of DNA, 0.2mM dNTP mix, 1X Complete Buffer (Jena Biosciences, Germany), 0.04U/µl High Yield taq polymerase (Invitrogen, USA) and 0.5 µM of each target primer pair. Thermal cycling was done at 94°C for 2 minutes; 94°C for 30 seconds; 56°C for 30 seconds; 72°C for 30 seconds; and 72°C for 2 minutes; steps 2 to 4 were repeated 35 times. Primer pairs used for *MTR* A2756G polymorphic variants located on exon 25 of the *MTR* gene were identified after amplification with the following primers as described by Al Farra: [15]

MTR Locus: – forward primer – 52 CATGGAAGAATATGAAGATATTAGAC32

MTR Locus: – reverse primer – 52 GAACTAGAAGACAGAAATTCTCTA32

Restriction digestion of the PCR products for *MTR* gene amplicons were done using *the* Hae III restriction enzyme.

Genotyping

Gel electrophoresis was carried out with a 2% agarose gel. The gel plate together with the gel block, were placed in an electrophoretic tank with a 1X TAE Buffer. A mid-range ladder by Jenna Biosciences (Germany) containing DNA fragments from 100bp to 1kb was loaded into the first well of the 2% agarose gel block, while 10µL of PCR product obtained was loaded into the remaining wells of the gel. This was subjected to electrophoresis at 80v for 90 minutes. After which, the DNA bands were observed using Ultraviolet light from a trans-illuminator.

The *MTR* gene amplicon was digested with Hae III restriction enzyme to yield the following visible fragments: genotype AA (211bp only), genotype AG (211bp, 131bp and 80bp), and genotype GG with 131bp and 80bp (plate 1).

Data Analysis

Statistical analyses were performed using SPSS 23.0 software (Armonk NY: IBM Corp. USA) Kolmogorov-Smirnov test was used to assess normality of data distribution for continuous variables and Box plots for identification of outliers. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data. Categorical variables were expressed as percentages, and Chi-square test was used to compare proportions for categorical variables.

RESULTS

MTR 2756 Genotypic and Allelic Distribution between Participant Groups

The *MTR* 2756 AA genotype was demonstrated in 50% (35 of 70) of the HbAA subjects (Table 2), while *MTR* 2756AG genotype and *MTR*2756GG were observed in 35.7% (n=25) and 14.3% (n=10) of the HbAA subject group. In the HbSS study group, the genotype frequencies for *MTR* 2756 AA, AG and GG were 57.1% (n = 40), 32.9% (n= 23) and 10% (n=7) respectively. No significant difference was observed in the genotype distribution of methionine synthase gene for both control and study group (p=0.623).

Of the two alleles associated with the *MTR* 2756 locus, the allelic frequencies for A and G in the control group were 67.9% and 37.1% respectively. These did not differ significantly from the allelic frequencies of 73.6% for A allele and 26.4% for G alleles as observed in the HbSS study group (p = 0.348).

Association between Disease Severity in HbSS Subjects and Plasma Homocysteine

Elevated plasma Hcy (> 12.45 µmol/L) was observed in 86.7% (n=13), of HbSS subjects with disease severity score > 7 (Table 3). Conversely, normal plasma Hcy level was observed in 72.8% (n= 40) of subjects with disease severity score ≤ 7. Elevated plasma Hcy was significantly associated with disease severity among HbSS subjects (OR=17.2, CI: 3.490-86.079; p=0.0001).

Association between Disease Severity in HbSS Subjects and *MTR* 2756 Genotype

The *MTR* 2756 AA genotype was observed in 53.6 (n=15) of those with disease severity score >7, and in 59.6% (n = 25) of HbSS subjects with the severity score of ≤7 (Table 3). The mutant genotype GG was recorded in 3.6% (n =1) of subjects with disease severity score >7 and in 14.3% (n=6) of subjects with disease severity score ≤7. Disease severity was neither associated with Heterozygous mutant (*MTR* 2756 AG) SNP (OR=0.550, CI: 0.195-1.554; p=0.258), nor the homozygous mutant (*MTR* 2756 GG) SNP (OR= 3.600, CI: 0.394–32.873; p=0.395).

Association between Disease Severity in HbSS Subjects and *MTR* Mutation using the Dominant Genetic Study Model (AA vs AG + GG):

Table 1: Modified Disease Severity Scoring System for Sickle Cell Anaemia

Clinical And Laboratory Features	Score	Score
Crisis number(s) per year? 0–1 [0]	2–3 [1]	≥4 [2]
Previous blood transfusion:		
Yes/No If yes, how many times per year?	1–2 [1]	≥3 [2]
Acute chest syndrome	Yes [1]	No [0]
Osteomyelitis	Yes [1]	No [0]
Renal Failure	Yes [1]	No [0]
Anaemic Heart Failure	Yes [1]	No [0]
Avascular necrosis of femoral head	Yes [1]	No [0]
Pneumonia	Yes [1]	No [0]
Pigment gallstone & Jaundice	Yes [1]	No [0]
Dehydrated	Yes [1]	No [0]
Anaemia Hb ≥10g/dl [0]	Hb≥8<10g/dl [1] Hb≥4<6g/dl [3]	Hb≥6<8g/dl [2] Hb<4g/dl [4]
Total Severity Score: 21		

Author: Hedo, *et al.,* 1993

Table 2: *MTR* 2756 Genotypic and Allelic Distribution between Participant Groups

MTR 2756 Genotypes	Control Group HbAA n (%)	Study Group HbSS n (%)	χ^2	p
A A	35(50.0)	40(57.1)	0.946	0.623
A G		25(35.7)	23(32.9)	
G G		10(14.3)	7(10)	
Allelic Frequencies				
A	95(67.9)	103(73.6)	0.874	0.349
G	45(32.1)	37(26.4)		

Table 3: Association between Disease Severity in HbSS Subjects and Plasma Homocysteine Across *MTR* SNP Study Models

Hcy Levels	Disease Severity Score n (%)			
	<7	>7	OR(CI)	p
Hcy Level				
Normal Plasma Hcy (≤12.45µmol/L)	40(72.8)	15(27.2)	0.06(0.012–0.287)	0.0001
Elevated plasma Hcy (> 12.45 µmol/L*)	2 (13.3)	13 (86.7)	17.32(3.490–86.079)	0.0001
MTR Genotype				
A A	25 (59.5)	15(53.6)	–	
A G	11 (26.2)	12(42.8)	0.550(0.195–1.554)	0.258
G G	6 (14.3)	1 (3.6)	3.600(0.394–32.873)	0.395
Dominant Model				
A A	25(62.5)	15(37.5)	–	
AG+GG	17 (56.7)	13(43.3)	1.274(0.486–3.345)	0.624
Recessive Model				
G G	6(83.3)	1(16.7)	0.222 (0.025–1.956)	0.230
A A + A G	36(57.1)	27(42.9)	–	

** 95th percentile value of plasma homocysteine value for control group.*

Using the Dominant genetic study model (AA vs AG+GG), 37.5% (n=15) of HbSS subjects with genotype AA had disease severity score >7 (Table 3). In HbSS subject population with either heterozygous or homozygous mutant *MTR* genotypes (AG + GG), 43.3% (n= 13) had disease severity score >7. The presence of *MTR* genotypes AG or GG did not significantly contribute to disease severity score of HbSS subjects (OR=1.274, CI: 0.486–3.345; p=0.624).

Association between Disease Severity in HbSS Subjects and *MTR* Mutation using the Recessive Genetic Study model (GG vs AA+AG)

In the recessive study model (GG vs AA+AG) as shown in Table 3, HbSS subjects with the mutant homozygous GG genotype had 16.7% (n=1) of the total number of subjects with disease severity score >7. Conversely, HbSS subjects with either homozygous wild type genotype (AA) or the heterozygous genotype (AG), constituted 42.9% (n= 27) of subjects with disease severity score >7. However, the association between homozygous *MTR*2756 GG genotypes and disease severity among HbSS subjects in the recessive study model, was not statistically significant (OR= 0.222, CI: 0.025-1.956; p=0.230).

Association between *MTR* 2756 Genotype and Plasma Homocysteine Level in HbSS Subjects

Of the HbSS subjects with *MTR* genotype AA (Table 4), 27.5% (n= 11) had elevated plasma Hcy compared to the elevated plasma Hcy level observed in 3 (13%) of HbSS subjects with genotype AG and 1 (16.7%) of HbSS subjects with genotype GG respectively. The association between plasma hcy and presence of *MTR* genotypes AG and GG was not statistically significant (OR =0.406, CI: 0.115–1.431; p=0.239 and OR= 0.439, CI: 0.047–1.431; p=0.659 respectively).

Association between Plasma Homocysteine and Haemoglobin Phenotype of Participants

The proportion of HbSS subjects with elevated plasma Hcy (Table 4) was 21.4% (n=15) which was significantly

Table 4: Association between Plasma Homocysteine, Hb Phenotype and *MTR* SNPs

Genotype	Plasma Hcy Level (µmol/L)			p
	Normal (≤12.45µmol/L)	Elevated (>12.45)	OR(CI)	
A A	29(72.5)	11(27.5)	–	
A G	20 (87.9)	3(13.0)	0.406(0.115–1.431)	0.239
G G	6 (83.3)	1 (16.7)	0.439 (0.047–1.431)	0.659
Hb Phenotype				
HbAA	67(95.7)	3(4.3)		
HbSS	55 (78.6)	15 (21.4)	6.091(1.677–22.125)	0.003

Table 5: Comparing Mean Haematological Parameters, Plasma Hcy and Mean Severity Score of HbSS Participants with and without *MTR* 2756 Mutation

MTR 2756 Genotype	Hb (g/dl)	WBC (x10^9/L)	plt (x10^9/L)	Hcy (umol/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hcy (µmo/L)	Severity
AA	10.3 ±3.8	9.59 ±4.7	356.0 ±132.2	11.14 ±3.6	82.8 ± 6.8	27.1 ±2.7	32.7 ±1.3	9.2 ±1.0	3.7 ±1.9
AG+GG	12.3 ±3.21	8.95 ± 5.7	323.6 ±136.6	12.55 ± 6.5	83.8 ±5.3	27.5 ±2.1	32.5 ± 1.2	9.5 ±1.10	4.2 ± 1.9
p-value	0.003	0.475	0.157	0.350	0.344	0.384	0.335	0.886	0.319

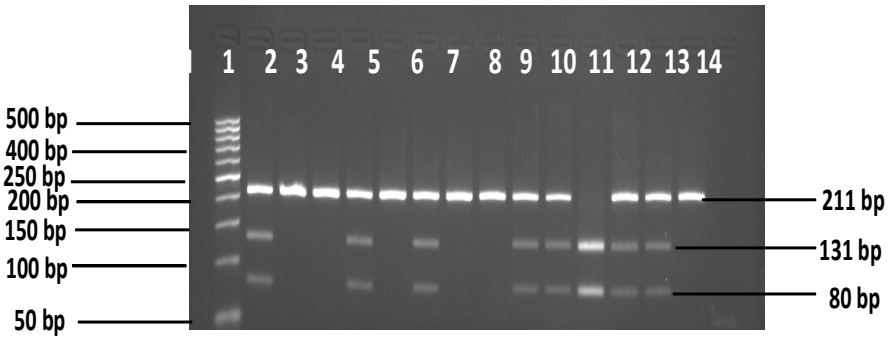

Plate 1: Representative Agarose Gel Electrophoresis of MTR Gene Polymorphism

M: Molecular weight marker (50-500 bp)
Lanes 1, 4, 6, 9-10, 12-13 represent: AG genotype (211, 131, and 80 bp)
Lanes 2-3, 5, 7-8 represent AA genotype (211 bp)
Lane 11 represents GG genotype (131 and 80 bp)
Lane 14 represents undigested PCR product
Lane 15 represents non-template control.

higher than proportion of HbAA subjects with elevated plasma Hcy (4.3%). HbSS was significantly associated with increased risk of elevated hcy (OR= 6.091, CI: 1.677–22.125; P= 0.003).

Comparing the Mean Haematological Parameters and Homocysteine of HbSS Participants with and without *MTR* 2756 Mutation

In Table 5, the haemoglobin concentration of HbSS subjects with wild type *MTR* 2756 genotype (12.3±3.2g/dl) was significantly higher than haemoglobin concentration for HbSS subject with mutant *MTR* 2756 genotypes (10.5±3.8g/dl, P = 0.003). However, the mean values of platelet count (356±132.2 x 10^9/l); MCV (82.8±6.8fl); MCH (27.1±27pg) and MCHC (32.7±1.3g/dl) for HbSS subjects with the wild type *MTR* genotype did not differ significantly (P >0.05) from the mean of platelet count (323.6±136.6 x10^9/l); MCV (83.8±5.3fl); MCH(27.5±2.7pg);MCHC (32.5±1.2g/dl) and the Hcy concentration (9.5±1.1 µmo/ L) for the HbSS subjects with mutant *MTR* genotypes. Likewise, the total white cell count for HbSS subjects with genotype AA (9.59x10^9/L) did not vary significantly from mean values of 8.95 x10^9/L observed for HbSS subjects with either *MTR* genotype AG or GG (p=0.475). Similarly, the mean severity score of 4.2 obtained for HbSS subjects with mutant *MTR* genotypes (AG+GG), did not differ significantly from severity scores of 3.7 observed for HbSS subjects with MTR genotypes AA.

DISCUSSION

Multiple factors (including metabolic, environmental and genetic) contribute to the phenotypic expression and variations in individuals with sickle cell anaemia.[6,8,14] The *MTR gene mutation of A2756G is one* of such genetic polymorphisms whose deleterious effects on folate metabolism have not been fully evaluated as possible genetic modifiers of sickle cell disease severity among African populations. In this study, the frequencies of the A2756G mutation (AG and GG) observed among subjects with sickle cell disease did not differ significantly from the normal population. Similarly, allelic frequency distribution for The *MTR gene* polymorphism were similar for both study groups. There is paucity of published data on the prevalence of *MTR* gene mutation among Nigerians, and even within and outside the African continent. Results from this study revealed a higher prevalence of A2756G mutation among healthy HbAA populations from Nigeria compared to genotype frequencies reported in a study from Burkina Faso which is one of the few African countries with documented *MTR* polymorphism.[16] However, the *MTR* genotype frequencies obtained in this study were more closely related to findings among Pakistani populations.[17]

Thrombosis may contribute significantly to the pathogenesis of sickle-cell anaemia related complications. In addition, the kidneys play an important role in the metabolism of homocysteine, which has been widely acknowledged in literature as a major thrombogenic end product of folate metabolism.[18,19,20]

Plasma homocysteine levels can be influenced by genetic factors. Theoretically, Altered functioning of catalytic enzymes in Hcy metabolic pathways caused by gene mutations may lead to inhibition of certain pathways and elevation of plasma Hcy level.[21] Though, some studies have documented increasing homocysteine levels in *MTR* 2756 polymorphism.[18,22] However, this study could not establish an association between *MTR* SNP and elevated Hcy among the HbSS subjects studied.

Theoretically, genetic polymorphisms of the *MTR* gene could result in alteration of *MTR* enzyme activity leading to elevated homocysteine levels as possible risk factor for thrombo-embolic complications in HbSS patients. Similarly, this study, observed elevated homocysteine levels in individuals with HbSS. The association between *MTR* A2756G polymorphism and hyperhomocysteinaemia (HHcy) has not been established in all studies where vasculopathy is shown to be associated with elevated homocysteine. Sun *et al* in a study of 424 subjects, could not establish an association between *MTR* A2756G polymorphism and occurrence of hyperhomocysteinaemia.[23] However, Laraqui *et al*. in a related study, reported that *MTR* rs1805087 (A2756G) G allele contributed a 2.0-fold risk for HHcy in some individuals with coronary angiopathy in their study population.[7] Additionally, a positive correlation between plasma homocysteine levels and frequency of vaso-occlusive crisis and disease severity has been previously documented.[24] Even though this study reported higher mean values of plasma homocysteine in subjects with HbSS, this observed hyperhomocysteinaemia could not be associated with the presence of the mutant genotypes for the *MTR* A2756G SNPs. Though the study further supported a significant association between HbSS disease severity and elevated plasma homocysteine, a relationship between disease severity and presence of *MTR* mutation could also not be established in the study. This may lay credence to a report by Sebastiani *et al*, in a candidate gene association study involving 1265 African-American with sickle cell disease where *MTR* gene was not listed as part of the 45 candidate genes identified as HbSS disease modifiers.[25] Therefore, in this study population, it is possible that sub-optimal levels of folic acid intake, dietary defects or impaired folate utilization, an impaired renal function combined with other genetic mutations affecting folate or homocysteine metabolism not explored in this study, could be responsible for the increased serum levels of homocysteine observed among HbSS subjects in this study.

Conversely, significant reduction in Haemoglobin (Hb) level was observed in subjects with the wild type *MTR* genotype (AA) compared to HbSS subjects with the mutant genotype(AG+GG). This finding has not been widely reported. However, Yameogo *et al* and Mirgal *et al*, working independently in Burkina Faso and India respectively, reported significant increases in the malaria parasite load of individuals with the *MTR* AA genotype compared to individuals with the mutant genotypes.[16,26] This higher susceptibility to malaria parasite infection could significantly contribute to the lower Hb reported among HbSS subjects with the wild type *MTR* genotype in Nigeria, which geographically falls under the malaria endemic zone.

The presence of mutant *MTR* gene did not significantly affect other red cell indices; WBC or platelet count among HbSS subjects in this study. Literature on the correlation between haematological parameters and *MTR* SNPs are sparse. The Burkinabe study also, could not report significant association between *MTR* gene, platelet and white cell count in their study.[16]

CONCLUSION

Evidence from these data show that the prevalence of *MTR A2756G* mutations among individuals with sickle cell anaemia were not significantly different from those of controls. Individuals with sickle cell anaemia had significantly higher mean serum homocysteine which appear to correlate positively with disease severity scores. And individuals with *MTR A2756G* mutations might develop more severe anaemia.

Limitations of the Study

This study estimated serum homocysteine levels among the subjects as an indirect index of folate lack, the effects of other nutrient factor deficiency such as Vitamin B_{12} and riboflavin were outside the scope of this work. Furthermore, in the folate metabolism pathway, there are other metabolic enzymes and co-factors, which are coded by genes and whose mutations could result in elevated serum homocysteine levels which may hypothetically contribute to the severity or differential phenotypic expression in adults with phenotype HbSS but were also outside the scope of this study.

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Duality of Interest

The authors hereby declare no conflict of interest.

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