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Relationship between Polymorphism of *HBS1L-MYB* (RS66650371) Gene, Fetal Hemoglobin and Disease Severity in Patients with Sickle Cell Disease

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Introduction

Sickle Cell Disease (SCD) is a monogenetic blood disorder characterized by clinical heterogeneity which can be influenced by a combination of genetic, epigenetic, and environmental factors [1]. More than 70% of cases of Sickle Cell Disease (SCD) worldwide are caused by Sickle Cell Anemia (SCA) [2]. Sickle-cell disease is a major global public health issue and the most common life-threatening genetic disorder worldwide. However, despite being the most prevalent genetic disease in Africa, the serious health and sociodemographic consequences are largely ignored [3].

It has been estimated that approximately 90% of the world's SCD population lives in these three countries: Nigeria, India, and the Democratic Republic of Congo [4], where the disease affects 2% of the population and carriers (sickle cell trait) account for 10% to 30% of all cases [5]. In Nigeria alone, there are estimated to be 150,000 babies born with SCD every year, which is 20 per 1000 births [4].

In SCD, the oxygen-carrying protein hemoglobin is abnormally altered in red blood cells, resulting in an autosomal recessive genetic blood disorder. Those with HbS develop deformed red blood cells (erythrocytes), which become hard and sticky under stress and resemble a C-shaped farm tool called a 'sickle'. Due to the short lifespan of sickle cells, anemia is often caused by the constant shortage of red blood cells [5,6].

In order to establish a clinical classification based on severity, it is necessary to search for factors responsible for the clinical variability of SCD. By using this classification, management can be optimized and follow-up matched to each patient's actual risk [7]. As a result, understanding the genetics underlying the heritable sub-phenotypes of SCD, specifically for each population, would be useful for prognosis and could help to tailor therapeutic interventions. Numerous studies have been devoted to genetic modulating factors of SCD [8]. Fetal Hemoglobin (HbF) concentration is one of the major modifiers of the Sickle Cell Disease which directly affects the sickle erythrocyte and is a major modulator of the phenotype of the disease [9]. Fetal Hemoglobin (HbF) concentration is the most powerful modifier of the clinical and hematological features of the Sickle Cell Disease (SCD) [10]. Understanding the genetics underlying the heritable sub phenotypes of sickle cell anemia would be prognostically useful. This could inform personalized therapeutics, and might help the discovery of new “drug gable” pathophysiologic targets [1].

Five typical haplotypes have been described across the β-globin gene cluster based upon the pattern of specific restriction fragment-length polymorphisms across the region [11]. Four haplotypes are associated with HbS in Africa (Benin, Bantu/Central African Republic, Senegal and Cameroon) and the fifth is thought to have arisen in India and/or the Arabian Peninsula (Arab/Hindu) [12]. It has been suggested that these haplotypes also have an effect on the severity of the disease through their genetically determined effect on HbF level [13]. Very low HbF and hematocrit are associated with SCD individuals carrying Bantu (Central African Republic) haplotype and they mostly present with severe clinical complications [14]. Meanwhile, clinical presentations of individuals with Senegal or Arab–India haplotype are usually mild due to high levels of HbF and hematocrit. Clinical course of persons with Benin haplotype is in between the Bantu and Arab/India haplotypes. Benin haplotype, associated with low HbF level, is the predominant haplotype in Nigeria with a frequency of 93.2% to 97% [15]. The mean HbF among Nigerian SCD patients is about 6% [13].

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Genetic association at the three principal loci have been identified, SNPs in major loci that are associated with different HbF levels in patients with SCD and in healthy adults. The BCL11A gene is located on chromosome 2 (2p16) and the *HBS1L-MYB* intergenic region on chromosome 6 (6q23) [9].

The *HBS1L-MYB* Intergenic Polymorphisms (HMIP) are present in three Linkage Disequilibrium (LD) blocks with most of the effect on HbF levels and numbers of F cells contributed by the second block [16]. Among the causes of unusually high HbF levels are deletions or single base substitutions in the HBB gene cluster and Single Nucleotide Polymorphisms (SNPs) in the genes. *HBS1L-MYB*: known main HbF sub-loci in this region, *HMIP-2A* (tagged by *rs66650371*). The SNP rs66650371, the functional 3-bp deletion in the MYB enhancer, is one of the most significant SNPs in the HMIP region [17,18].

Methodology

This was a cross-sectional hospital-based study to investigate the fetal hemoglobin variant *HBSIL-MYB* in individuals with sickle cell anemia and their phenotypic presentations with a view of providing explanations on strategy for reducing the burden of the disease in Nigeria. All suspected homozygous sickle cell patients attending Sickle cell clinics of Adeoyo Hospital and University College Hospital from age thirteen above in the period of the study was enrolled, 260 blood samples of Sickle cell Patients were collected for this study.

Hematological and Hemoglobin analyses

A total of 7 ml blood sample was collected from each participant and dispensed into Ethylene Diamine Tetra Acetic acid (EDTA), for laboratory analysis. The Packed Cell Volume (PCV) was measured to determine the proportion of the volume occupied by the Red Blood Cells to the volume of the whole blood. The PCV was determined using the hematocrit reading device [19]. Fetal Hemoglobin Concentration (HbF), was determined using Betke method [20]. The quantity of hemoglobin is measured to the proportion of alkali-resistant (fetal) hemoglobin is then calculated as a percentage of the fetal hemoglobin present.

Molecular analysis

DNA extraction was done from archived buffy coat by using Da An Gene Kit, following the manufacturers protocol. ARMS-PCR reaction was carried out in 25 µL total volume to amplify the DNA segment [21]. ARMS is based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. The Amplification-Refractory Mutation System (ARMS) is a simple method for detecting any mutation involving single base change or small deletions.

Statistical analysis

Data obtained was statistically analyzed using Statistical Package for Social Sciences (SPSS) software version 24.0. Chi-square was used to determine the association between HbF and the genetic variants. P values ≤ 0.05 at 95% confidence interval was considered significant. Descriptive analysis was used to present the frequencies of outcomes. Genetic association analysis was performed by multiple regression, with age and sex as covariates (SPSS v. 20).

Results

The genetic variant rs66650371 (*HBS1L-MYB*) was genotyped from 260 SCA patients, and its effect on HbF levels, phenotypic variables and PCV were investigated. The mean age is 23.0 ± 0.6 (SE).

Table 1 shows the prevalence of Vaso-occlusive crises among SCD patients across one year. Higher HbF levels have been associated to benefit some complications of the disease than others, examples of some of its benefit include, reduced rate of acute painful episodes, fewer leg ulcers, less frequent acute chest syndromes and reduced disease severity [22]. These Vaso-occlusive variables were the bench mark used to determine how severe and how frequent participants experience crises in this study. About 59.2% of the population experiences chest symptoms at least once in a year while only 15.2% experiences leg ulcers. The high frequency of sickle crises amongst the participants is similar to what has been reported in a previous study in Nigeria, which revealed that there is an overall increase in the frequency of Vaso-occlusive crises among SCD patients [23]. This situation is consistent with the trend in some countries such as Britain and Saudi Arabia [24,25].

Table 2 shows the relationship between Vaso-occlusive variables, HbF and PCV values among SCD patients. The HbF Concentration ranged from 1.8% to 12%, the mean HbF level of patients in this study was 4.9 ± 2.4 percent, which is lower than what has been reported in a study in Ife, Nigeria [26]. The mean HbF was 9.9 ± 6.0 percent. This difference may be as a result of the different methods used in the estimation of fetal hemoglobin levels in both studies, or the mean age of the populations, for instance, report from Ife showed the age range recruited for their study was 1 to 15 years, at this stage, fetal hemoglobin levels are not yet stable, this might account for the discrepancy in the mean fetal hemoglobin levels of both studies. The mean Hbf of this study is close to a study that evaluated the fetal hemoglobin concentration levels of individuals in Ibadan (5.16 ± 4.04) [27]. There was no statistical difference in the mean HbF

Table 1: Prevalence of Vaso-occlusive crises in SCD patients.

Number of Crises in a year, N=260	Chest pain N=260	Leg Ulcer N=260
Once = 130 (50%)	Yes = 154 (59.2%)	Yes = 41 (15.8%)
Twice = 26 (10%)	No = 106 (40.7%)	No = 219 (84.2%)
Quarterly = 104 (40%)		

Table 2: Relationship between Vaso-occlusive Variables, HbF and PCV.

Vaso-occlusive Variables	Low HbF	High HbF	Low PCV	High PCV
Leg Ulcer, Yes	68.30%	31.7%	71.5%	28.5%
No	53.00%	47.0%	55.5%	44.5%
Chest Pain, Yes	71.40%	28.5%	74.5%	25.5%
No	41.1	58.8%	30%	70%

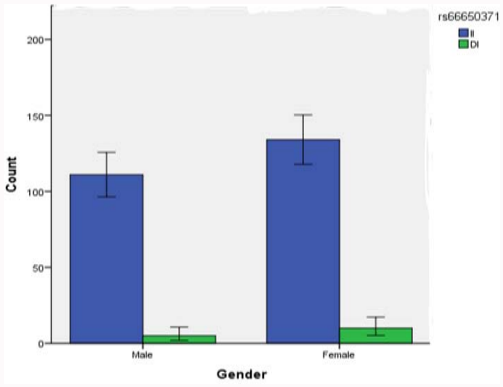

Figure 1: The relationship between gender and the SNP (rs66650371).

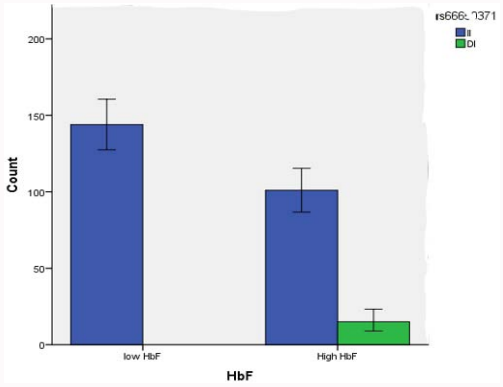

Figure 2: The relationship between the rs66650371 SNP and HbF levels.

levels of males and females. Although, males had higher HbF levels compared to females.

PCV count is also significantly increased in patients with high HbF levels than in patients with low HbF levels which is also in line to a study done in Tanzania [28], how the beneficial effects of elevated HbF seen in their study reduced anemia, leukocytosis and thrombosis. Increased PCV counts reduces anemia, which is common to SCD patients. Also, the average mean of males PCV was higher compared to that of females.

Figure 1 shows the relationship between gender and the SNP (rs66650371). The HbF-boosting allele frequency of rs66650371 in Chromosome 6 (HMIP) showed that 15 out of 260 SCD patients showed the rs66650371 gene, which is a 3bp deletion in position 276bp in gel electrophoresis, while the remaining 245 showed insertion at position 207bp. This indicates an allelic frequency of 3% in the total population (II=245, DI=15). In agreement to the first genetic study on fetal hemoglobin findings in Nigeria, the 3bp-deletion of rs66650371 on chromosome 6 showed an allelic frequency of 3% in the population. Although, about 38% of the total SCD patients had an elevated level of HbF with the absence of the rs66650371 SNP. In this study, more females were recruited than the males, this shows the rs66650371 has a higher frequency in the female cohort compared to the males, although, there was no significant difference. Hence gender is not likely of direct functional significance in the rs66650371 SNP.

Figure 2 shows the relationship between the rs66650371 SNP and HbF levels. There was a significant difference between the hematological factors (HbF and PCV) with rs66650371 SNP, patients with rs66650371 SNP had an increased level of fetal hemoglobin, and about 80% of them also had increased level of PCV counts, this is agreement to the genetic study of fetal hemoglobin in SCD in Nigeria [15]. Also, there was a reduced severity of the disease in patients with rs66650371, more than two-third of the patients with rs66650371 experiences mild crises about once a year, while the others experiences about twice in a year.

Conclusion

The present study demonstrated the presence and beneficial effect of fetal hemoglobin, and HBSIL-MYB gene with SCD patients. The number of Vaso-occlusive crises, such as acute chest pain, leg ulcer is significantly indirectly proportional to elevated HbF levels. Patients with reduced HbF levels, have repetitive number of Vaso-occlusive crises in a year. The 3bp deletion of rs66650371 has been associated with elevated levels of HbF and mild Vaso-occlusive crises among SCD patients in Ibadan, Nigeria.

Facilities for early and regular quantification of fetal hemoglobin should be made available in Sickle cell Clinics and Hospitals in Nigeria.

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