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Effect of Duffy Antigen Receptor for Chemokines on Severity in Sickle Cell Disease

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Abstract

*Sickle-cell disease is among the commonest severe monogenic global disorders. At the centre of sickle cell disease physiopathology is the polymerisation haemoglobin, resulting in erythrocytes become rigid and vascular occlusion. It has been hypothesized that the Duffy glycoprotein (Fy) on erythrocytes may enhance clearance of inflammatory cytokines. This may have an impact on the initiation and development of vascular occlusion in Sickle cell disease. The aim of this study was to determine whether or not the Duffy genotype is in any way linked with the severity of clinical disease in Sickle cell disease patients. Those who were hospitalized >4 times in the previous year because of Vaso-occlusive crisis were classified as having a severe phenotype. Duffy genotypes were determined by polymerase chain reaction (PCR), then Sty1 restriction endonuclease enzyme analysis. A total of 193 participants (133 cases and 60 controls) were recruited for the study. Vaso-occlusive crises were absent in more than half (58.5%) of the cases. Vaso-occlusive crisis per year occurred three times and four times at 5.2% and 1.65%, respectively. The number of vascular occlusions per year was highest in the 18-28 years and 29-39 years age groups. All the participants were genotyped as homozygotes for Duffy null genotype (FY*B-33/FY*B-33) and categorised as Fy(a-b-). No associations between Duffy genotype and number of VOCs per year was obtained. All the samples analysed were genotyped as Duffy negative homozygous [Fy(a-b-)]. No association between Duffy genotype and number of was vascular occlusion found.*

Keywords: *Chemokine, Duffy antigen, Sickle cell, Vascular occlusion.*

Introduction

Sickle Cell Disease (SCD) is the most common genetic disorder of haemoglobin in sub-Saharan Africa [1, 2]. In Africa, it is estimated that about 200,000 children are born with the disease annually [3]. A point mutation on the 6th codon of the beta-globin gene located on the short arm of chromosome 11 produces a defective beta-globin chain, which under low oxygen tension polymerizes into long fibres that eventually lead to abnormally deformed (sickled) red cell. The red cells thus become sticky, adhere to the endothelium, and clump together, plugging micro-vessels [4] and also damage large blood vessels that can become severely stenotic or occluded. In addition, sickle red cells have a much-shortened life span making chronic haemolytic anaemia a constant

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feature of SCD. The combination of Vaso-occlusion and haemolytic anaemia contributes to the basic presentation of the disease and associated complications, of which some are life-threatening. Biomedical technology is moving very fast to use hematopoietic stem cell transplantation and gene therapy in the management of the disease in developed countries. However, only very few SCD patients have been successfully treated with hematopoietic stem cell transplantation [5] and, gene therapy has not been successful yet in curing SCD.[6] In general, the disease is managed using a combination of preventive and symptomatic therapies [7]. Two principal antigens, Fy(a) and Fy(b) are produced by the FYA and FYB codominant alleles. There are four main phenotypes: Fy(a+b-), Fy(a+b+), Fy(a–b+) and Fy(a–b–). The Duffy negative phenotype, Fy(a–b-), is uncommon among Caucasians but has a high prevalence in West Africans and in approximately 1 in every 500 African Americans [8].

The Duffy negative phenotype occurs because of a mutation in the "GATA promoter region upstream of the FY allele". This alteration stops the activity of the GATA site and prevents erythroid cell gene transcription of the Duffy glycoprotein [9].

The Duffy glycoprotein (DARC) also functions as the erythrocyte entry point for invasion by *Plasmodium vivax* and *P. knowlesi* [8]. However, the biological role of DARC is not clear; normal erythrocytes and a typical immune response is seen in persons with the null Duffy phenotype [10].

Both laboratory and animal studies have been carried out to determine DARC's functional role. Studies carried out *in vitro* that demonstrated that "red cell absorption of IL-8 may function to limit stimulation of leukocytes by IL-8 released into blood" [11] and in vivo using FY gene knockout animals [10] all point to the fact that erythrocyte Duffy antigens have "a dual role as a chemokine sink to both prevent WBC activation in the systemic circulation and impede chemokine dissemination from the blood into organs." Active research is ongoing to determine the function of cytokines as likely regulators of the complications of SCD [12]. Cytokines seem to play a role in several possible mechanisms in the pathogenesis of Vaso-occlusive phenomena in SCD. Interleukin-8, especially, appears to be engaged in the pathological process of Vaso-occlusion in SCD, and serum IL-8 levels are raised in severe Vaso-occlusive crises in SCD [13]. Increased adherence to endothelium has been demonstrated *in vitro* when sickle RBCs are exposed to IL-8 [14].

Materials and Methods

Study Design

The research was a case-control study. This design was chosen because the study was observational; it did not involve any intervention, and the course of the disease was not altered in any way. Retrospectively, a determination was made of the exposure to the risk factor of interest from each of the two study groups of individuals (cases and controls).

Study Site

The study was conducted at the Ghana Institute of Clinical Genetics, also known as Sickle Cell Clinic, located within the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. Korle-Bu Teaching Hospital was established on October 9, 1923. KBTH is currently the third-largest hospital in Africa and the leading national referral centre in Ghana [15]. Currently, the Hospital has 2,000 beds and 17 clinical and diagnostic Departments/Units. The average daily attendance is 1,500 patients and about 250 patient admissions [15].

The Ghana Institute of Clinical Genetics is a referral health facility that receives SCD patients from all over Ghana. The Institute was established in 1974 and is funded by the Ministry of Health and the Managing Trustees of Volta Aluminium Company Limited (VALCO) [16].

Sample Size Calculation

The sample size was calculated using a simple formula. Using a confidence interval of 95% and a margin of error of 2%, the calculation was as follows:

$$n = \frac{[Z]^2 P(1-P)}{E^2}$$

$$n = \frac{[1.96]^2(0.98)(1-0.98)}{0.02^2} = 189 \text{ participants.}$$

where:

- n = is estimated minimum sample size.
- E = is the allowable margin of error.
- z = is the critical z score based on the desired level of significance.
- P = Overall prevalence of Duffy negative genotype in West Africa, is 98% [17].

Data Collection

Retrieval of Patient Clinical Information

Clinical and demographic data were obtained by a structured questionnaire using information from the hospital records. Dependent variables included age, sex, and genotype. The estimated number of admissions due to sickle cell crisis was used as an indicator for the severity of the systemic disease.

Collection and Storage of Blood Specimens

Blood samples (40μl l each) from participants were spotted on Whatman 4 FTA cards (Whatman Inc., Brentford, UK) and allowed to air dry for 24 hours at room temperature (27^{o}C) and then kept in separate clean zipper bags.

Statistical Analysis

Differences between FY+ and FY– patients in demographic characteristics, clinical and overall disease severity were tested. Analyses were carried out using IBM® SPSS® Statistics version 24 for Windows and GraphPad Prism version 7.00 for Windows. Continuous variables were compared with the t-test. Chi-square tests or Fisher's exact tests were used for nominal variables. A *p*-value of less than 0.05 was considered significant. Odds ratios (ORs with 95% CIs) were used to measure the strength of the statistical associations between the outcomes and exposures.

Results and Discussion

A total of 193 subjects participated in the study and with participants' age ranging between 18 - 67 years (29.84 ± 8.85 years). The study subjects were made up of 133 (53.4%) cases, and 60 (46.6%) controls. Figure 1 shows the gender distribution of cases and controls. No statistically significant difference (p = 0.1864) was found between the ages of the cases and controls.

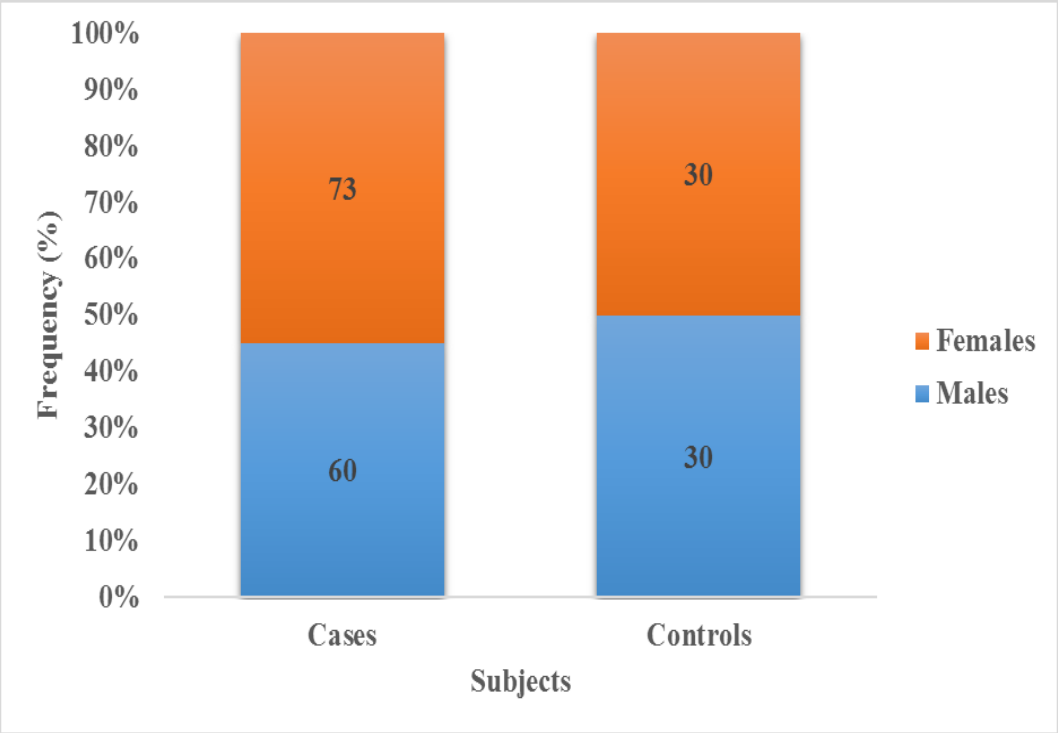

Figure. 1. Age Distribution of the Participants

Clinical History of Cases

The majority of the cases (94.7%) were Hb SS, with only a few being Hb SC (5.3%). Figure. 2 shows the clinical history of the cases. No VOCs occurred in 59 (44.4%) of the cases. Vaso-occlusive crisis per year occurred three times and four times in 7.5% and 2.2% of the cases, respectively. The number of VOCs per year was highest in the 18-28 years and 29-39 years groups (Table 1).

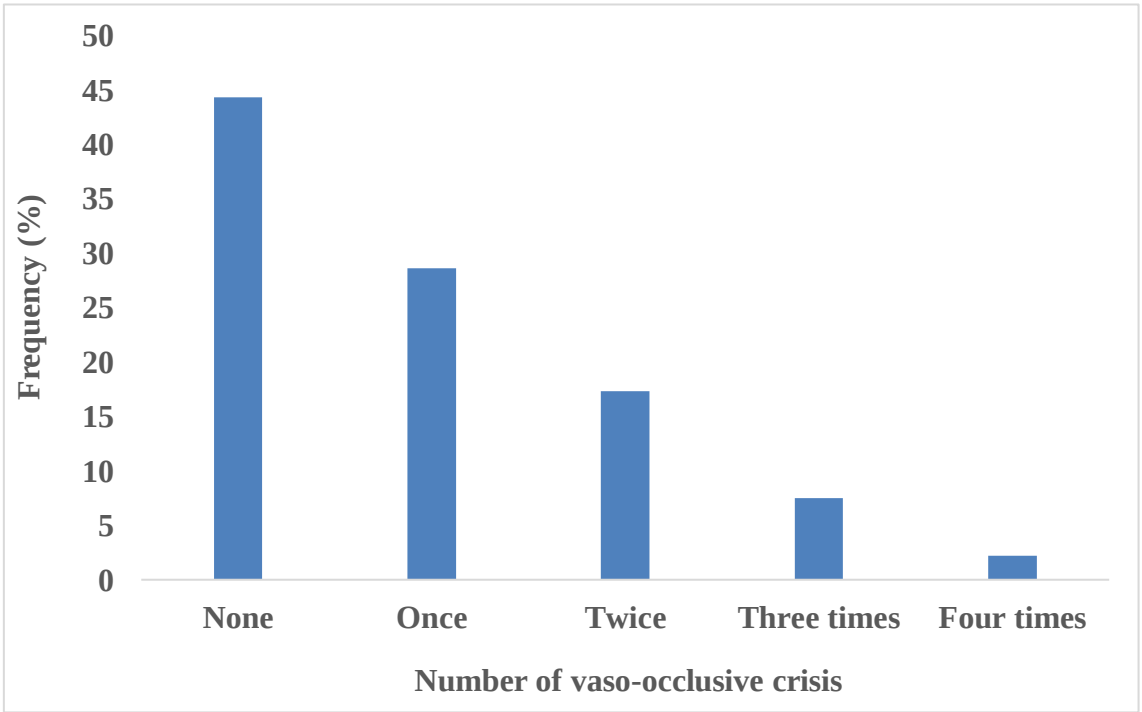

Figure. 2. Frequency of Number of Vaso-occlusive Crisis per Year

Table 1. Occurrence of Vaso-occlusive Crises per Year in Relation to Age Groups

Age group (years)	Vaso-occlusive crises/year					Total
	None	Once	Twice	Three times	Four times	
18-28	30	18	11	4	3	**66**
29-39	22	14	9	5	0	**50**
40-50	5	6	3	1	0	**15**
51-61	1	0	0	0	0	**1**
62-72	1	0	0	0	0	**1**
Total	**113**	**59**	**38**	**23**	**10**	**133**

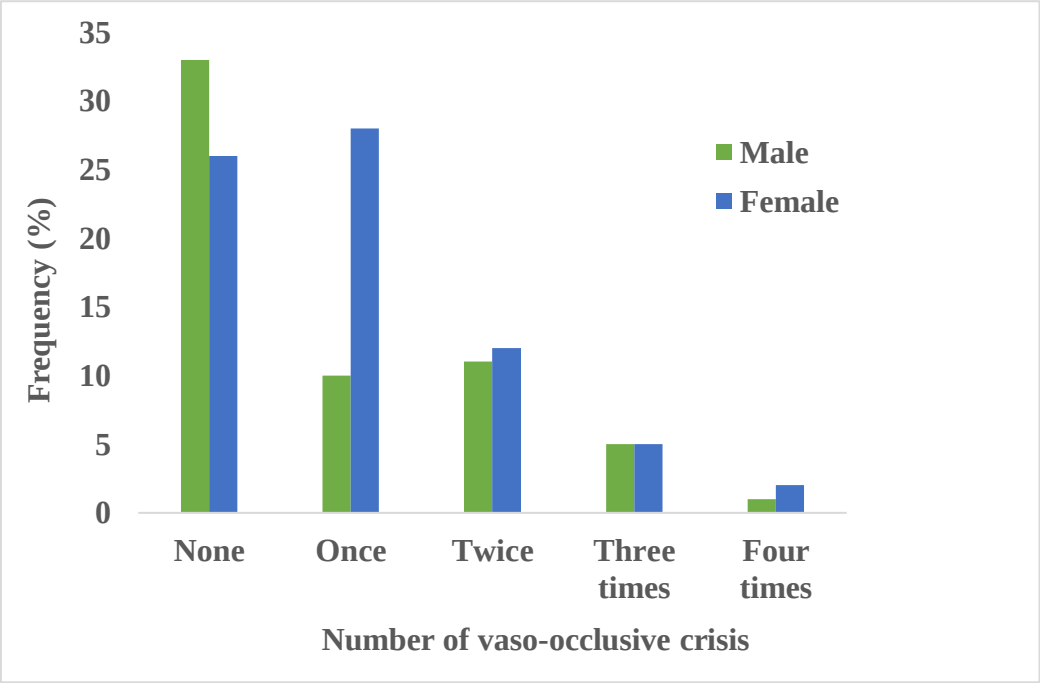

Figure 3. Gender in Relation to Number of Vaso-occlusive Crises per Year

Vaso-occlusive crises per year occurred more in females than males (Figure. 3). There was no statistically significant difference (p = 0.1463) between gender and the number of Vaso-occlusive crises per year.

Frequencies of Duffy Genotypes

All the participants were genotyped for the Duffy gene by PCR-RFLP. Restriction fragment analysis of all the amplicons yielded 82, 65, 64, and 12 bp for Duffy negative genotypes (Figure. 4). However, the fragment of 12 bp was not considered due to the low molecular weight, not visible in the gel. All the samples analysed were thus genotyped as FY*B-33/FY*B-33 (Duffy negative homozygous) being therefore classified as Fy(a-b-).

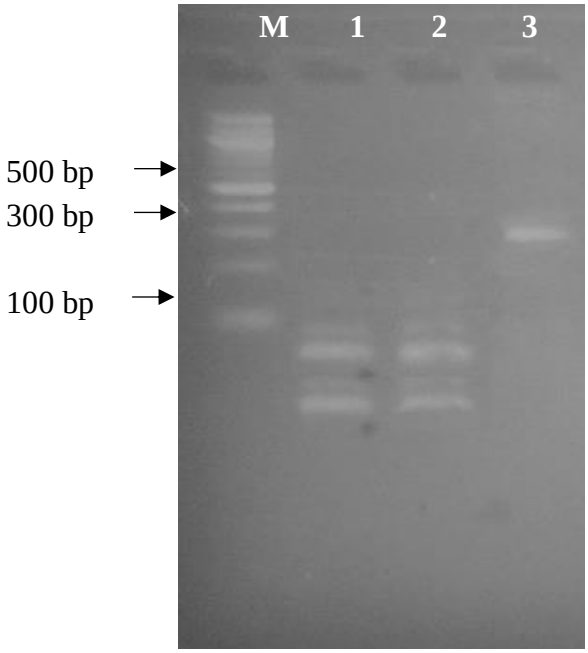

Figure 4. Ethidium Bromide-Stained 2.0% Agarose gel Electrophoregram of StyI Digestion of Amplicons obtained using the P38 and P39 Primers

Lane M = 100 bp marker (New England Biolabs Inc., Ipswich, MA, USA); Lanes 1 and 2 = PCR positives 16S rDNA genes; Lanes 3 = Undigested amplicon.

Association of Duffy Genotypes with Scd Severity

No associations (p = 0.999) between Duffy genotype and the number of vaso-occlusive crises per year were obtained.

The pathophysiology that results in complications in SCD is not fully understood, thus genetic changes likely to affect the severity of the disease carry a high value. Consequently, studies that look at how individual blood group antigens function to in ways that affect SCD expression are required. For instance, antigens of the Lutheran and LW blood groups provide adhesion receptors with several functions that studies have established to play a role in the pathological initiation of erythrocyte adhesion in SCD [18, 19].

This study looked at the likelihood of any link between the expression Duffy blood group antigen as reflected by the FY genotype at the GATA site of the FY promoter and severity of clinical disease among patients with HbSS. Investigations of the associations between biomarkers which can predict SCD severity have provided inconclusive results [20].

Sickle cell disease is characterised by a broad range of disorders, including chronic haemolytic anaemia and Vaso-occlusion of both macro-and microvasculature, resulting in continuing pain and major organs at the end getting damaged. The Vaso-occlusive crisis was absent in more than half (58.5%) of the cases, and this could be due to the effective management of the SCD patients who attend the sickle cell clinic. Crisis occurred three times and four times per year in the 18-28-year group than in any other year group. [21] in their study also reported that young adults (20-29 years) had more crises (49.9%) than other age groups.

Vaso-occlusive crises per year occurred more in females than males. However, there was no statistically significant difference (p = 0.1463) between gender and the occurrence of vaso-occlusive crises. This agrees with the study by [21], who reported more females had crises than males in the vaso-occlusive group and also a non- significant relationship between crises type and sex (p = 0.282).

All the patients tested negative for the Duffy phenotype Fy(a–b–). The Duffy negative phenotype occurs due to a GATA promoter region mutation upstream of the FY allele. This mutation stops the activity of the GATA site and prevents erythroid cell gene transcription of the Duffy glycoprotein [9]. Most West Africans and 68% of African Americans, both of whom exhibit the Duffy null phenotype, do not produce Duffy antigens on their erythrocytes [22]. Thus, the results from this study were not surprising.

The Duffy glycoprotein (Fy) on RBCs has been hypothesized to promote clearance of inflammatory cytokines, which may play a role in the pathogenesis of Vaso-occlusion in SCD. Persons with the African-type Fy (a–b–) phenotype whose RBCs lack expression of Duffy may less efficiently clear inflammatory cytokines [20]. Therefore, the Duffy-negative genotype may be associated with the more severe disease among patients with SCD. In this study, there were no associations between the Duffy genotype and the number of Vaso-occlusive crises per year, and this result did not differ from that of [24], who used a sample size that was six times the size of this study.[20] in contrast, we have found that the Duffy genotype at the GATA site may serve as a biomarker that can predict in advance damage that can be caused later on to major organs and particularly for abnormal kidney function in SCD. However, in their study, almost all the entire population was self-identified as African American while in this present study, the entire sample was identified as African or of African descent. Added to this, the final determinants in their investigations also included measures of end-organ abnormal function to assess chronic organ damage. In contrast, the relationship between the FY– genotype and end-organ abnormal function found in their study might have been as a result of a higher likelihood of other SNPs with their root from Africa or in genes located close to the FY locus.

Conclusions

All the samples analysed were genotyped Duffy negative homozygous [Fy(a-b-)]. No associations between Duffy genotype and the number of Vaso-occlusive crises were found.

Recommendation

1. Future studies will need to be conducted with a larger sample number to confirm the association between Duffy genotype and Vaso-occlusion.
2. In addition, a prospective study would be needed to assess and clarify the role of Duffy antigen expression in the development of Vaso-occlusion.

Acknowledgment

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Conflict of Interest

There is no conflict of interest.

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