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Protective Effect of HLA-DQB1 Alleles Against Alloimmunization in Patients with Sickle Cell Disease

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Abstract

Background—Alloimmunization or the development of alloantibodies to Red Blood Cell (RBC) antigens is considered one of the major complications after RBC transfusions in patients with sickle cell disease (SCD) and can lead to both acute and delayed hemolytic reactions. It has been suggested that polymorphisms in HLA genes, may play a role in alloimmunization. We conducted a retrospective study analyzing the influence of HLA-DRB1 and DQB1 genetic diversity on RBC-alloimmunization.

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Study design—Two-hundred four multi-transfused SCD patients with and without RBC-alloimmunization were typed at low/medium resolution by PCR-SSO, using IMGT-HLA Database. HLA-DRB1 and DQB1 allele frequencies were analyzed using logistic regression models, and global p-value was calculated using multiple logistic regression.

Results—While only trends towards associations between HLA-DR diversity and alloimmunization were observed, analysis of HLA-DQ showed that HLA-DQ2 (p=0.02), -DQ3 (p=0.02) and -DQ5 (p=0.01) alleles were significantly higher in non-alloimmunized patients, likely behaving as protective alleles. In addition, multiple logistic regression analysis showed both HLA-DQ2/6 (p=0.01) and HLA-DQ5/5 (p=0.03) combinations constitute additional predictor of protective status.

Conclusion—Our data suggest that particular HLA-DQ alleles influence the clinical course of RBC transfusion in patients with SCD, which could pave the way towards predictive strategies.

1. Introduction

Red Blood Cell (RBC) transfusion remains the main treatment for acute complications of Sickle Cell Disease (SCD) [1-3] and is increasingly used as a preventive therapy. The vast majority of patients with SCD have experienced at least one RBC transfusion by the time they reach adulthood [4-6]. Alloimmunization, the development of alloantibodies against major and minor RBC antigens, is a common and serious side effect of RBC transfusion. Susceptibility to RBC-alloimmunization is dependent on several factors including the degree of antigen disparity between the donor and recipient, as well as genetic factors [6-12]. Alloimmunization can lead to both acute and delayed hemolytic transfusion reactions, some of which can be lethal [13]. Eighteen to 47% of multiply transfused SCD patients develop RBC-alloantibodies in their lifetime [6, 7]. Transfusion of cross-matched incompatible blood, when no other options exist, may result in increased hemolysis with substantial morbidity or even mortality [14]. In such cases, the patient either has to forgo the beneficial and potentially life-saving benefits of transfusion, or receive incompatible blood and risk the effects of hemolysis. RBC alloimmunization therefore, has considerable medical importance and consequence [15-17].

The adaptive immune response reacts to AB0-matched donor RBC antigens only if CD4 T-cells are activated upon interaction with peptides presented by HLA class II genes. Several studies have shown that in specific ethnic groups, the intrinsic immunogenicity of a given RBC antigen is, in part, correlated with the presence of particular HLA class II alleles [18-23]. In one study, those patients with HLA–DRB1*01 and DRB1*15:03 alleles experienced a higher rate of alloimmunization while HLA-DRB1*09:01 variant was found mostly in alloantibody negative patients [20, 22]. Recent studies demonstrated the association of HLA-DQB1 alleles with anti-platelet antibody production [24-26] pointing out the capacity of the encoded molecules to efficiently present platelet antigens to T-cells that can result in alloimmunization. In this present study, we analyzed the distribution of HLA-DRB1 and HLA-DQB1 alleles in SCD patient with and without RBC alloantibodies. Our results suggest that specific HLA-DQB1 alleles confer protection against alloimmunization which could impact both the understanding of alloimmunization and the management of RBC transfusions in SCD patients.

2. Patients and Methods

2.1. Patients

Genomic DNAs from 204 African-American SCD patients were genotyped at low/medium resolution for HLA-DRB1 and -DQB1 polymorphism. All patients were homozygous for $Hb\beta^{s-s}$. One hundred thirty seven patients were enrolled at Children's National Health System [8]. Repository DNA was obtained from 67 patients participating in the Pulmonary Hypertension and the Hypoxic Response in the SCD (PUSH) study [27] from the following medical centers: Howard University Hospital (n=38), University of Michigan (n=16) and Fairfax Hospital in Northern Virginia (n=13). Blood samples were collected at the time of consent and DNA was extracted using a Qiagen DNA extraction kit (Qiagen, CA). In accordance with the Declaration of Helsinki, patients were enrolled after informed consent and applicable patient assent were obtained following the approved Institutional Review Board research protocol at each institution.

2.2. Red Blood Cell Transfusion Protocol

All patients received prestorage leukodepleted RBC in accordance with a standard protocol. Prior to 2010, at Children's National Medical Center, all patients, not on specific research protocols, received cross-matched compatible ABO and RhD matched RBCs. Since 2010, all patients received rhesus -C, -E and -K antigens (CEK) matched RBCs. Upon development of the first and subsequent persistent R B C alloantibody, RBCs are matched for -C, -E and -K antigens and antigen-negative for the detected antibodies. At the time of two RBC-allo-antibodies detection, full phenotypic matching is performed. Data for patient's age, gender and units of received RBC transfusions were available for 188, 199 and 204 patients (Table 1).

2.3. HLA-DRB1and -DQB1 typing

DNA was extracted using standardized procedures. HLA low/medium resolution typing for HLA-DRB1 and HLA–DQB1 alleles was performed using PCR-sequence specific oligonucleotide (SSO) Luminex LABTYPE®SSO kits designed to recognize all the broad specificities based on the sequence databases from IMGT/HLA Database (database version 3.17.0) (http://www.ebi.ac.uk/ipd/imgt/hla/probe.html) [28, 29].

Luminex 100 flow analyzer identified HLA alleles via HLA visual 1.0 software by referring to HLA typing template data for DRB1 and DQB1 provided by manufacturer (OneLambda, Inc. CA). The software will then assign the detected alleles into their corresponding serological specificities. Therefore, results will be displayed as serological equivalents. If no serological equivalent is found, or if the corresponding alleles are null alleles, the field remains blank. HLA-DQ2 to -DQ9 will be the corresponding serological specificities for HLA-DQB1 alleles and those corresponding to HLA-DRB1*01:01 to –DRB1*10:01 alleles will be HLA-DR1 to –DR18.

2.4. Transfusion History Data Review

Transfusion history from patients' date of first RBC transfusion to December 2013 was extracted from the Children's National Blood Bank database (Sunquest Information

Systems, Tuscon, AZ). Transfusion history includes the number and specificity of RBC alloantibodies and the total number of transfused RBC units either from the first RBC transfusion to December 2013 for non-alloimmunized patients or to the detection of the first alloantibody for alloimmunized patients. Collaborating institutions provided related transfusion data.

2.5. Statistical Analysis

Demographic and patient characteristics were compared according to alloimmunization status. Patient age was compared using a Wilcoxon rank sum test and the number of RBC transfusions using Poisson regression. The number of RBC transfusions was defined as the number of units of RBC transfused before the development of alloantibodies in patients who became alloimmunized, and the total number of RBC units transfused by December 2013 in those who did not. A Chi-squared test was used to evaluate the gender distribution between the alloimmunized and non-alloimmunized groups. The distribution of HLA-DRB1 and HLA-DQB1 alleles among the two patient subsets was analyzed using logistic regression models with individual alleles, compound heterozygous and homozygous pairs. Statistical analysis was performed only if there was an adequate number of patient bearing a specific allele or genotype allowing further viable analysis. Therefore, this number varied for the HLA-DRB1 and HLA-DQB1 loci. Alloimmunization status was compared according to the distribution of HLA-DRB1 and –DQB1 alleles and pairs of alleles with individual logistic regression models to produce odds ratios (OR), p-values and 95% confidence intervals (CI). Additionally, individual alleles were combined into a multiple logistic regression yielding a global *p-value* describing their overall contribution to the immunization status.

Logistic regression models included all patients whose alloimmunization status and genotype were known. Due to the alloantibody-positive patients receiving significantly less RBC transfusion before alloimmunization than the alloantibody-negative patients ($p \leq 0.001$), RBC transfusion load was not considered a confounding factor and was not included in the modeling. Furthermore, exact logistic regression was used where individual sample sizes were smaller than 10. Alloimmunization status was compared between all alleles or allele pairs considered common enough for analysis initially by a single logistic regression model. Here, no adjustment for multiple testing was performed. Those allele or allele pairs were then combined into a single multiple logistic regression to determine how well they, in combination, can predict alloimmunization status. This model yields a *p-value* for each individual allele or allele pair describing its contribution to the prediction of alloimmunization status and a global *p-value* describing the predictive ability of all alleles or allele pairs combined. All analyses were performed using State V13 (College Station, TX).

3. Results

3.1. Patient Demographics

A total of 204 patients were included in this study. All were successfully genotyped for the HLA-DRB1 locus and 203 for the HLA-DQB1 gene. All were homozygous for the sickle cell mutation ($Hb\beta^{S\text{-}S}$). Demographic data on gender and age were missing for 5 and 16 patients respectively. The mean age was 16.4 ± 5.2 years (range, 1.6 to 25.2 years). There

were 83 (41.7%) alloantibody-positive and 116 (58.3%) alloantibody-negative patients, with no differences in age or sex between these two groups (Table 1). Among antibody-positive patients 34 (41%) had developed one RBC alloantibody, while 49 (59%) had developed multiple alloantibodies. The most commonly identified alloantibodies were to anti Rhesus-E (54.2%), -C (51.8%), -V (10.8%), and -C^w (4.8%), -e and -D (3.6%); anti-Kell: -K50.6%) and -Js^a (7.2%). Less frequently observed were antibodies anti-Kidd: -Jk^b (6%), -Jk^a (4.8%); anti-Duffy: -Fy^a, (10.8%); anti-S (18%), anti-M (8.4%), and anti-Dombrock (Do^b) and anti-Lutheran (Lu^a) (1.2%) antigens.

3.2. Analyses of HLA-DR specificities

Two hundred four patients were successfully genotyped for HLA-DRB1 locus. The low/medium HLA typing allowed the classification of HLA-DRB1 alleles into their corresponding serological specificities.

The nine most frequently observed HLA-DR specificities among those tested were as following (The corresponding HLA-DRB1 alleles recognized by the primers of the HLA-DRB1 typing kit are indicated between parentheses): HLA-DR1(HLA-DRB1*01:01, 01:02, 01:03,), -DR3 (HLA-DRB1*03:08, 03:17) -DR4 (HLA-DRB1*04:01 to 04:08, 04:14, 04:10, 04:13, 04:16, 04:36) DR7 (HLA-DRB1*07:01, 07:02,07:04), -DR8 (HLA-DRB1*08:01 to 08:09), DR11 (HLA-DRB1*11:01 to 11:04, 11:08,11:09, 11:11, 11:13, 11:14, 11:19, 11:30) -DR12 (HLA-DRB1*12:01 to 12:03), -DR13 (HLA-DRB1*13:01 to 13:07, 13:09, 13:10, 13:12, 13:17, 13:20, 13:23, 13:25, 13:27) -DR15 (HLA-DRB1*15:01, 15:03) and DR17 (HLA-DRB1*03:01, 03:04, 03:05) (observed in 43, 26, 26, 43, 30, 51, 24, 59 and 19 patients respectively). Conversely, the HLA-DR2 (HLA-DRB1*15:02,16:03, 16:05), DR5 (HLA-DRB1*12:04,12:05), DR6 (HLA-DRB1*13:19 to 14:16, 14:18), -DR9 (HLA-DRB1*09:01), -DR10 (HLA-DRB1*10:01), - DR14 (HLA-DRB*14:03 to 14:10, 14:12, 14:13, 14:15, 14:17 to 14:19, 14:21 and 14:43) and - DR18 (HLA-DRB1*03:02, 03:03) were the less frequently observed specificities (observed in 2, 1, 2, 7, 6, and 8 pts)

The less frequent alleles (those observed in less than 19 times in the total patient population) were excluded from further analysis. The distribution of each of the most observed nine alleles were compared to the status of alloimmunization both individually and in conjunction using logistic regression to determine if the presence or absence of a given allele/specificity was statistically different between alloimmunized and non-alloimmunized groups. The HLA-DR3 and HLA-DR4 alleles were both found to be significantly associated with alloimmunization status at individual level. Six alloantibody positive patients (23.1%) were bearing HLA-DR3 while 20 patients (76.9%) did not. In contrast, HLA-DR4 was observed in 17 alloantibody positive (65.4%) vs. 9 (34.6%) alloantibody negatives patients. Therefore, patients bearing the HLA-DR3 allele were less likely to be alloimmunized (OR=0.38; $p = 0.04$), while those having the HLA-DR04 allele were more likely at risk to be alloimmunized (OR=2.871; $p = 0.02$). However, neither of these two alleles remained significant when multiple logistic regression was performed (Global *p-value* 0.23) (Table 2). Seventy-four HLA-DR heterozygote alleles, were observed, with four pairs found in more than 7 patients: HLA-DR1/13 (11 pts), DR3/15 (9 pts), DR11/13 (12 pts) and DR11/15 (8 pts). None of the four allele pairs was found to be differentially distributed between

alloimmunized and non-alloimmunized groups using single or multiple logistic regression model (Global *p-value* ≤ 0.55) (Data not shown).

3.3. Analyses of HLA-DQ specificities

Two hundred and three patients were successfully genotyped for the HLA-DQB1 locus. All eight specificities of HLA-DQ (HLA-DQB1*02 to -DQB1*09) were observed Six of 8 alleles were found in more than twenty patients: HLA-DQ2 (HLA-DQB1*02:01, 02:02, 02:03 (85 pts)), -DQ3 (HLADQB1*03:09 (37 pts)), -DQ4 (HLA-DQB1*04:01, 04:02; (24 pts)), -DQ5 (HLA-DQB1*05:01to 05:04; (94 pts)), -DQ6 (HLA-DQB1*06:01 to 06:09, 06:11, 06:13, 06:15; (102 pts)), and -DQ7 (HLA-DQB1*03:01, 03:04, 03:19 (45 pts)). In contrast, HLA-DQ8 (HLA-DQB1*03:02, 03:05), HLA-DQ9 (HLA-03:03), were found in 17 and 4 patients respectively and were excluded from further analysis. The distribution of each of the most observed six alleles were compared according to the status of alloimmunization, both individually and in conjunction, using logistic regression. When analyzed individually, HLA-DQ7; a “split antigen” of the broad antigen group of HLA-DQB1*03 was found in 19 (42.2%) alloantibody positive vs. 26 (57.8%) of alloantibody negative patients was significantly associated with alloimmunization (OR=1.97; p = 0.03) (data not shown). When all 6 alleles were analyzed in conjunction, using the multiple logistic regression analysis, no significant association between HLA-DQ7 and alloimmunization were noted. In contrast, 3 other alleles: HLA-DQ2 (OR=0.30; p=0.02), -DQ3 (OR=0.27; p=0.02) and DQ5 (OR=0.28; p=0.01) showed to be all significant predictors of alloimmunization (Global p value = 0.023) (Table 3).

3.4. Analysis of heterozygosity

Four HLA-DRB1 allele pair combinations (HLA-DR1/12 (11 pts), -DR3/15 (9 pts), -DR11/13 (12 pts) and –DR11/15 (8 pts) were found in more than eight patients and were included for the statistical analysis. Both single and multiple logistic regression model of these four alleles did not show any significant association with alloimmunization (Global p value = 0.55) (data not shown).

Eight pairs of HLA-DQ alleles were observed in more than nine patients (HLA-DQ2/5 (16 pts), -DQ2/6 (29 pts), -DQ2/7 (10 pts), -DQ3/6 (11 pts), -DQ5/5 (18 pts), -DQ5/6 (16 pts), -DQ5/7 (10 pts), and -DQ6/7 (14 pts). Only HLA-DQ2/6 was found to be differentially distributed between alloantibody positive and alloantibody negative groups 7 (24.1%) vs. 22 (75.9%); (OR=0.37; p=0.03). Multiple logistic regression analysis using the combination of these eight, confirmed the above observation (OR=0.28; p=0.01) and furthermore, showed that the homozygotes HLA-DQ5/5 can be an additional significant predictor (OR=0.25; p=0.03). Global *p-value* of 0.056 indicates a trend towards significance of these eight allele pairs in predicting the odds of alloimmunization (Table 4).

3.5. Analysis of HLA-DR and HLA-DQ association

The distribution of individual HLA-DR/HLA-DQ alleles was studied in 203 patients. Sixty-five combinations of alleles were observed, of which 6 (HLA-DR1/HLA-DQ5 (18 pts); HLA-DR12/HLA-DQ5 (22 pts); HLA-DR13/HLA-DQ6 (29 pts); HLA-DR15/HLA- DQ6 (54 pts); HLA-DR3/HLA-DQ2 (16 pts); HLA-DR7/HLA-DQ2 (27 pts) were found more

than 15 times. Single or multiple logistic regression analysis did not detect any association of HLA-DR/HLA-DQ alleles with alloimmunization (Global *p-value* 0.29) (data not shown).

4. Discussion

RBC alloimmunization is a common complication of transfusion occurring in up to 40% of SCD patients [6, 17]. Despite prophylactic measures including leuko-reduction/depletion and limited antigen matching (Rh-C, -E, -K antigens-match), its prevalence remains high in such patients [30]. Given the fact that the adaptive arm of the immune system controls humoral responses, including production of specific RBC allo-antibodies, alloimmunization depends on the presentation of the donor's RBC-related peptide antigens by antigen presenting cells (APCs) to the recipient's T-cell receptors expressed on CD4+ T cells. Therefore, HLA-class II molecules are central to the alloimmunization process.

Previous studies have suggested the potential association of HLA class I and class II alleles with alloimmunization in multi-transfused SCD patients [20, 22, 31]. More recently, in non-SCD settings, it was suggested that RBC alloimmunization may be driven specifically by the HLA-DR molecules [20, 23, 26]. In order to substantiate or refute this data, in a large cohort of SCD patients, we analyzed the potential impact of HLA-DRB1 and HLA-DQB1 allelic diversity on alloimmunization.

We found that the frequency of the HLA-DR3 was lower in non-alloimmunized patients compared to alloimmunized counterpart (*p-value: 0.04*), suggesting a potential protective effect of such variant. In contrast, the frequency of HLA-DR4 allele was significantly higher among the alloimmunized patient group as compared to its non-alloimmunized counterpart (p value: 0.02). The susceptibility status conferred by the HLA-DRB1*04 allele might be explained by the previously demonstrated efficient capacity of this allelic variant in presenting antigenic peptides to $CD4^+$ T-cells with consequent activation and polarization of CD4 T-cells [32]. Moreover, even outside the scope of our study, it is important to note that the HLA-DR4 allele represent the most important genetic risk factor for common inflammatory/auto-immune disorders such as rheumatoid arthritis [33-35]. In our context, the HLA-DR4 molecule was demonstrated, in Caucasian population-groups, to act as a restriction molecule for the production of anti-Fy^a antibodies [23] evoking a possible involvement in alloimmunization. Only one subject in our cohort of African-American patients was found to be homozygous for the HLA-DR4 allele. This patient interestingly presented with both anti-E and anti-Jk^b antibodies. Previously, Hoppe et al. suggested that HLA-DRB1*15:03 and HLA-DRB1*09:01 were implicated in susceptibility to/protection against the development of RBC transfusion-related alloantibodies production. In the present study, HLA-DR15 was observed in 56 patients: 22 alloantibody positive vs. 34 alloantibody negative (OR=0.85, p=0.58) and HLA-DR9 was found in only 7 patients: 4 alloantibody positive and 3 alloantibody negative and none of the alleles showed any significant association with alloimmunization. Our data did not confirm Hoppe's observations. One possible explanation of such discrepancy could be related to the significantly higher number of patients involved in our study. The analysis of the potential effect for the different HLA-DRB1 allelic combinations using multiple logistic regression model did not result in any

statistical significance either at a single allele or at compound heterozygous level and therefore no further investigation was undertaken to elucidate the role of different subtypes of HLA-DR3 in this study.

We identified three HLA-DQ alleles that were found more frequently in alloantibody-negative groups: HLA-DQ2, - DQ3 and - DQ5. Odds of developing alloimmunization was found about 3 times less among patients carrying these alleles as compared to the alloantibody-positive group.

The involvement of HLA-DQ molecules has already been demonstrated in various immune-related settings [36-38]. It is well known that some HLA-DQB1 alleles are inherited in close linkage disequilibrium with specific HLA-DRB1 alleles. This is well exemplified by the *cis* association of the HLA-DQ2 and the HLA-DR3 allele, representing part of the so-called "8.1 AH auto-immune haplotype [39] which was widely associated with immune/auto-immune pathological conditions [40-43]. Our data also show a trend towards a cumulative protective effect of the HLA-DR3/DQ2 combination (p=0.06). Concerning the involvement of HLA-DQ5 in immune mediated diseases, few data exist [44, 45]. To the best of our knowledge, this is the first study implicating the HLA-DQ5 allele in RBC-alloimmunization.

Current understanding of the mechanisms of HLA-class II-mediated protection against immune response to RBC antigens is limited but such protection could be hypothesized under the framework of epitope competition. For example, in our case, HLA-DQ2, HLA-DQ3 and HLA-DQ5 binding grooves may present a non-polymorphic portion of an RBC antigen that partially overlaps with an epitope presented by another MHC class II molecule. However, given the plethora of potential RBC antigens, it is difficult to envisage epitope overlap in all of the antigens. A more likely possibility, therefore, is the development of dominant tolerance. Interestingly, HLA-DQ molecules have been implicated in promoting CD8+ T cell mediated suppression in a number of antigen models [46]. However, these studies performed in 1990's have not been replicated. Other possible mediators of suppression may be $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells (Tregs). Although the role of these cells in alloimmunization is not well understood [47, 48], it is known that such cellular component is able to recognize peptide/MHC complexes with greater avidity than the $CD4^{+}CD25^{-}FoxP3^{-}$T cells [49-51]. Thus, the assumption would be that HLA-DQ molecules present RBC epitopes recognized by T cells with relatively high avidity. Therefore, it is possible to envisage that one of these mechanisms is involved in the induction of tolerance during the TCR interaction with RBC peptide–HLA-DQB1 ligand. Although the role of HLA-DQ during RBC antigen presentation to TCRs and generation of a protective status against alloimmunization is not clear, our data will help the understanding of the immunological processes underlying alloimmunization.

In conclusion, our data suggests that HLA-DQB1 genetic diversity influence the clinical course of RBC transfusion in patients with sickle cell disease. If confirmed by additional large and trans-populations studies, such knowledge may pave the way towards personalized transfusion procedures. Moreover, studies regarding the mechanism of antigen presentation by HLA-DQB and T-cell activation are warranted to understand the mechanism of tolerance induction.

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Abbreviation

HLA	Human Leukocyte Antigen
pts	patients
SCD	Sickle Cell Disease
RBC	Red Blood Cells

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Table 1

Demographic characteristics of SCD patient population

	Alloimmunization status	Number of Patients	Mean ± SD	95% CI	*P-value*
Age (Years)†	Alloimmunized	83	17.2 ± 4.6	16.2 – 18.2	***0.13****
	Non-alloimmunized	116	15.9 ± 5.5	14.9 – 16.9	
Number of units of RBC transfusions	Alloimmunized	88	23 ± 38	22 – 24	<***0.001*****
	Non-alloimmunized	116	65 ± 83	64 – 67	
Gender‡					
Female	Alloimmunized	37			***0.62***^
	Non-alloimmunized	45			
Male	Alloimmunized	45			
	Non-alloimmunized	61			

† Data available from 188 patients;

‡ Data available from 199 patients

* *p value* calculated by Wilcoxon rank sum test;

** *p value* calculated by Poisson regression;

^ p value calculated by χ^2 test.

SCD, sickle cell disease; SD, Standard deviation; CI, confidence interval

Table 2
Multiple Logistic Regression analysis of HLA-DR specificities

HLA-DR	OR	*p-value*	95% CI	*Global p-value*
DR1	1.00	0.99	0.39 – 2.54	
DR3	0.42	0.14	0.13 – 1.32	
DR4	2.62	0.08	0.89 – 7.68	
DR7	1.00	0.99	0.39 – 2.54	
DR8	1.06	0.91	0.38 – 2.92	
DR11	1.05	0.92	0.43 – 2.59	***0.23***
DR12	0.83	0.74	0.28 – 2.48	
DR13	1.43	0.42	0.60 – 3.44	
DR15	0.90	0.81	0.37 – 2.19	
DR17	0.64	0.47	0.19 – 2.12	

SCD, sickle cell disease; OR, odds ratio; CI, confidence interval

Table 3

Multiple logistic regression analysis of HLA-DQ specificities

HLA-DQ	OR	*p-value*	95% CI	Global p-value
HLA-DQ2	0.30	0.02	0.11 – 0.83	***0.023***
HLA-DQ3	0.27	0.02	0.09 – 0.84	
HLA-DQ4	0.46	0.21	0.14 – 1.56	
HLA-DQ5	0.28	0.01	0.10 – 0.77	
HLA-DQ6	0.39	0.06	0.14 – 1.04	
HLA-DQ7	0.68	0.49	0.23 – 2.02	

SCD, sickle cell disease; OR, odds ratio; CI, confidence interval

Table 4
Multiple logistic Regression analysis of HLA-DQ heterozygosity

HLA-DQ Pairs	OR	*p-value*	95% CI	*Global p-value*
2 / 5	0.40	0.12	0.13 – 1.26	
2 / 6	0.28	0.009	0.11 – 0.73	
2 / 7	0.88	0.85	0.24 – 3.28	
3 / 6	0.33	0.12	0.08 – 1.34	
5 / 5	0.25	0.024	0.08 – 0.83	***0.056***
5 / 6	0.88	0.82	0.30 – 2.58	
6 / 6	1.11	0.88	0.28 – 4.50	
7 / 6	1.17	0.78	0.37 – 3.70	

OR, odds ratio; CI, confidence interval