HLA Class II Haplotypes Distinctly Associated with Vaso-Occlusion in Children with Sickle Cell Disease

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We investigated the association of HLA class II alleles and haplotypes with sickle cell anemia vaso-occlusive crisis (VOC). DRB1*100101 was positively associated, while DRB1*140101, DRB1*150101, and DQB1*060101 were negatively associated, with VOC. Both susceptible (DRB1*100101-DQB1*050101) and protective (DRB1*110101-DQB1*030101 and DRB1*150101-DQB1*060101) haplotypes were identified, indicating that HLA class II haplotypes influence VOC risk.

Vaso-occlusive crisis (VOC), a major cause of morbidity and mortality in children with sickle cell anemia (SCA) (7, 19), is a complex process of periodically recurring painful episodes (7, 10) and a major contributor to other sickle cell disease complications, including infection, acute chest syndrome, and stroke (7, 10, 19). VOC is linked with hypercoagulation and platelet activation (4, 16) and is triggered by factors that enhance the adherence of sickle erythrocytes (RBCs) to endothelial cells (13, 22), as evidenced by intravital microscopy in human sickle hemoglobin (HbS)-expressing mice (22) and because blocking of RBC-leukocyte interactions was protective against VOC (22, 23), thereby demonstrating a direct role for adherent leukocytes in VOC pathogenesis (4, 16, 23).

T cells and antigen-presenting cells were associated with endothelial injury in SCA (5), and human leukocyte antigen (HLA) alleles were identified as modifying risk factors for vascular disease (18) and in the development of SCA complications, including stroke (9, 20), infections (21), RBC alloimmunization (2), and skin ulcers (15). As specific HLA alleles and haplotypes may influence immune-mediated events associated with VOC, this study addressed the association of HLA class II polymorphism with SCA VOC development.

SCA patients (n = 167) diagnosed according to hemoglobin profile (HbA, HbS, HbA2, and HbF) were assigned to VOC (n = 104) and steady-state (n = 63) groups according to hospitalization, blood transfusion, and presentation with painful episodes, defined as pain in the upper or lower extremities, chest, abdomen, or back not related to SCA complication or to SCA-unrelated cause (trauma, cancer). VOC treatment consisted of nonsteroidal anti-inflammatory drugs (44.6%), narcotics (13.3%), or both nonsteroidal anti-inflammatory drugs and narcotics (8.4%). Comparable frequencies of hydroxyurea-treated patients were seen in the two patient groups (P = 0.196). Inclusion criteria for steady-state SCA patients included an afebrile state, no hospitalization or transfusion within the previous 6 months, and no VOC episode within 3 months of specimen collection. The Arabian Gulf University Research and Ethics Committee approved the study protocol, and all participants (or guardians in pediatric cases) gave written informed consent.

HLA-DRB1 and -DQB1 genotyping was performed by the PCR-sequence-specific priming (SSP) technique, using the SSP2L HLA class II (DRB/DQB) genotyping kit according to the manufacturer’s specifications (One Lambda, Thousand Oaks, CA). Allele frequencies were determined by the gene counting method using HLAStat 2000 software, which also computed P values (Fisher’s exact probability test) and odds ratios (ORs) with 95% confidence intervals (95% CIs) (24).

| Characteristic | VOC group (n = 104) | Steady-state group (n = 63) |
|---------------|--------------------|---------------------------|
| No. (%) male:female | 61:43 (58.7:41.3) | 29:34 (46.0:54.0) |
| Age (yr) | 14.1 ± 8.3 | 15.5 ± 10.4 |
| Hemoglobin profile (%) | | |
| HbS | 66.6 ± 18.9 | 66.0 ± 20.0 |
| Hbf | 19.2 ± 13.6 | 27.1 ± 18.1 |
| HbA2 | 3.2 ± 1.7 | 2.7 ± 2.0 |
| Laboratory values | | |
| Total hemoglobin (g/dl) | 9.6 ± 1.4 | 9.8 ± 1.6 |
| Hematocrit (%) | 28.0 ± 4.0 | 30.0 ± 5.0 |
| Mean corpuscular hemoglobin content (pg) | 25.9 ± 4.0 | 24.5 ± 3.4 |
| Mean corpuscular hemoglobin concn (µmol/liter) | 33.5 ± 1.4 | 33.0 ± 0.8 |
| Reticulocytes (%) | 5.3 ± 3.9 | 4.4 ± 3.6 |
| Leukocytes (10³/µl) | 9.3 ± 4.8 | 10.0 ± 4.8 |
| Platelets (10³/µl) | 335.6 ± 191.4 | 330.0 ± 180.0 |
| Lactate dehydrogenase (U/liter) | 517.3 ± 231.3 | 328.7 ± 177.4 |
| Bilirubin (µmol/liter) | 39.2 ± 27.1 | 33.5 ± 24.9 |

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a P < 0.05 versus steady-state group.

b Values are means ± standard deviations unless otherwise stated.

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ratios (OR). Haplotype frequencies were determined by the maximum-likelihood method, using Arlequin (v. 2.000) population genetic data analysis software. Additional statistical analyses were performed with the SPSS version 13.0 for Windows statistical package.

Table 1 summarizes the demographic and clinical characteristics of the SCA VOC patients and steady-state SCA controls. Controls were matched with patients with respect to age and sex. The mean HbF level was lower in VOC than in steady-state patients \((P < 0.05)\). Except for the reticulocyte count, which was higher among VOC patients \((P < 0.05)\), biochemical and hematologic indices were generally comparable.

The \(DRB1*100101\) \((P = 0.005)\) frequency was higher, while the \(DRB1*110101\) \((P = 0.050)\), \(DRB1*140101\) \((P = 0.048)\), and \(DRB1*150101\) \((P = 0.033)\) frequencies were lower, among VOC patients than among steady-state SCA patients (Table 2). A lower prevalence of \(DQB1*060101\) \((P = 0.024)\) was also noted in VOC versus steady-state SCA patients (Table 2). The frequency of \(DRB1*100101-DQB1*050101\) \((P = 0.007)\) was higher, while \(DRB1*110101-DQB1*030101\) \((P = 0.041)\) and \(DRB1*150101-DQB1*060101\) \((P = 0.052)\) frequencies were lower in VOC than in steady-state subjects (Table 3).

This case-control study was the first to identify unique class II susceptible and protective haplotypes associated with SCA VOC. It was of interest to note that the protective \(DRB1*110101-DQB1*030101\) haplotype was also negatively as-

| Table 2. HLA-DRB1* and -DQB1* allele distributiona |
|-----------------------------------------------|
| Locus Allele | VOC patients \((n = 104)\) | SCA controls \((n = 63)\) | \(\chi^2\) | \(p^b\) | OR |
|---------------|-----------------------------|-----------------------------|---------|-------|----|
| DRB1 010101   | 0.0433                      | 0.0141                      | 0.1599  | 0.1111| 1.422 | 0.233 | 2.541 |
| 030101        | 0.1779                      | 0.0265                      | 0.1349  | 0.0304| 2.778 | 0.096 | 1.889 |
| 030201        | 0.0337                      | 0.0125                      | 0.1599  | 0.0111| 0.973 | 0.324 | 2.201 |
| 040101        | 0.0817                      | 0.0190                      | 0.1032  | 0.0271| 2.753 | 0.000 | 0.797 |
| 070101        | 0.1058                      | 0.0213                      | 0.1032  | 0.0271| 1.057 | 0.000 | 1.185 |
| 080101        | 0.0036                      | 0.0018                      | 0.0317  | 0.1556| 2.219 | 0.036 | 2.299 |
| 090101        | 0.0096                      | 0.0068                      | 0.0079  | 0.0079| 0.025 | 0.005 | 1.216 |
| 100101        | 0.1442                      | 0.0244                      | 0.0556  | 0.0204| 7.984 | 0.000 | 3.673 |
| 110101        | 0.1154                      | 0.0222                      | 0.1825  | 0.0344| 3.832 | 0.050 | 0.500 |
| 120101        | 0.0996                      | 0.0068                      | 0.0079  | 0.0079| 0.025 | 0.000 | 1.216 |
| 130101        | 0.0865                      | 0.0195                      | 0.0476  | 0.0190| 1.632 | 0.000 | 1.988 |
| 140101        | 0.0048                      | 0.0048                      | 0.0371  | 0.0156| 3.921 | 0.048 | 0.143 |
| 150101        | 0.0721                      | 0.0179                      | 0.1270  | 0.0297| 4.548 | 0.033 | 0.420 |
| 160101        | 0.1058                      | 0.0213                      | 0.1349  | 0.0304| 0.882 | 0.000 | 0.699 |
| DQB1 0201     | 0.2692                      | 0.0308                      | 0.2619  | 0.0392| 0.809 | 0.369 | 1.340 |
| 030101        | 0.1298                      | 0.0233                      | 0.1667  | 0.0332| 1.348 | 0.026 | 0.658 |
| 0302          | 0.0721                      | 0.0179                      | 0.0635  | 0.0217| 0.397 | 0.000 | 1.244 |
| 03032         | 0.0529                      | 0.0155                      | 0.0556  | 0.0204| 0.001 | 0.000 | 0.894 |
| 0401          | 0.0577                      | 0.0162                      | 0.0317  | 0.0156| 0.858 | 0.000 | 1.244 |
| 050101        | 0.3125                      | 0.0321                      | 0.2381  | 0.0379| 2.617 | 0.010 | 1.681 |
| 060101        | 0.1058                      | 0.0213                      | 0.1825  | 0.0344| 5.131 | 0.024 | 0.165 |

\(a\) Partial listing of alleles occurring in more than five patients and controls. \(DRB1^*\) and \(DQB1^*\) alleles were assessed by PCR-SSP. Boldface indicates a significant difference between VOC and control groups.

\(b\) Determined by Fisher’s exact test.

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Table 3. HLA-DRB1*-DQB1* haplotype frequenciesa

| DRB-DQB haplotype | Frequency in: | \(p^b\) | OR (95% confidence interval)c |
|-------------------|---------------|-------|-----------------------------|
| VOC patients \((n = 104)\) | SCA controls \((n = 63)\) |       |                             |
| \(DRB1*010101-DQB1*030302\) | 0.014 | 0.003 | 0.449 |
| \(DRB1*030101-DQB1*0201\) | 0.152 | 0.105 | 0.250 |
| \(DRB1*030201-DQB1*0401\) | 0.024 | 0.016 | 0.912 |
| \(DRB1*040101-DQB1*0302\) | 0.033 | 0.041 | 0.987 |
| \(DRB1*070101-DQB1*0201\) | 0.053 | 0.061 | 0.871 |
| \(DRB1*110101-DQB1*030101\) | 0.042 | 0.017 | 0.297 |
| \(DRB1*110101-DQB1*030101\) | 0.123 | 0.029 | 0.007 |
| \(DRB1*110101-DQB1*030101\) | 0.063 | 0.133 | 0.041 |
| \(DRB1*110101-DQB1*030101\) | 0.028 | 0.028 | 0.857 |
| \(DRB1*150101-DQB1*060101\) | 0.049 | 0.113 | 0.052 |
| \(DRB1*160101-DQB1*050101\) | 0.071 | 0.103 | 0.430 |

\(a\) \(DRB1^*\) and \(DQB1^*\) alleles were assessed by PCR-SSP, and haplotype frequencies were determined by the maximum-likelihood method.

\(b\) Determined by Fisher’s exact test.

\(c\) Determined by the Woolf method.
associated with type 1 diabetes among Bahraini subjects (3), indicating a broader protective role for this haplotype in vascular endothelial injury diseases. Most Bahraini patients are homozygous for the Indian/Arab (IA) haplotype, with the co-existing α-thal trait and glucose-6-phosphate dehydrogenase deficiency (11, 12). The HbF levels seen in VOC patients and control SCA patients were comparable to those established for neighboring Kuwait (1) but higher than earlier values reported for Bahrain (11, 12), probably due to differences in assay method and sensitivity and to the recent introduction of hydroxyurea treatment. Phenotypic heterogeneity in the clinical manifestations of SCA was evident among Bahraini patients, thus prompting searches for modifying factors.

The increased DRB1*100101 prevalence in VOC patients was reminiscent of a Kuwaiti study where DRB1*10 was described as an “SCA-susceptible” allele (1). The same study failed to note an association of DRB1*10 with avascular necrosis, without investigating possible associations with other SCA complications. Associations of HLA alleles and genotypes have been established for many SCA complications (9, 15, 20, 21). DRB1*0301, DQB1*0302, and HLA DPB1*0401 were positively associated, while DQB1*0201 and DPB1*1701 were negatively associated, with stroke in sickle cell disease (9, 20), and DRB1*03 and DQB1*02 were associated with susceptibility to infection in SCA (21), while increased prevalences of HLA-B35 and Cw4 were noted in SCA patients with chronic leg ulcers (15). The select usage of these alleles may result in altered antigen recognition and presentation and hence in the mounting of an inappropriate immune response.

The genetics of VOC are complex (7). Earlier studies focused on prothrombotic risk factors, including factor V-Leiden (17), prothrombin G20210A (8), MTHFR C677T (8, 14), and human platelet alloantigens (6). The varied prevalences of these polymorphisms coupled with ethnic considerations necessitate searching for other candidate risk factors. Insofar as adhesion of sickled RBCs to vascular endothelium plays a central role in VOC (13, 23), and since T cells and other leukocytes contribute to endothelial injury in SCA (5), this implicates dysregulated immunity in VOC pathogenesis (4, 16, 22, 23). It is tempting to speculate that the contribution of HLA haplotypes to VOC risk is related to altered antigen recognition and presentation, thereby conferring disease susceptibility or protection.

Accordingly, differences in pathogenic capacity between candidate HLA haplotypes are explained by differences in the repertoires of antigenic peptides. In conclusion, our results provide the first evidence for HLA contributions to the development of SCA VOC. Confirmation of these findings using a larger sample size sufficiently powered to decrease the probability of false-positive associations will be needed to elucidate the contribution of HLA haplotypes and genotypes to SCA phenotypic heterogeneity.

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