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Article *in* Tissue Antigens · April 2009

DOI: 10.1111/j.1399-0039.2008.01189.x · Source: PubMed

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BRIEF COMMUNICATION

HLA DRB1*130101-DQB1*060101 haplotype is associated with acute chest syndrome in sickle cell anemia patients

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Key words
acute chest syndrome; Bahrain; HLA class II; PCR-SSP; sickle cell anemia

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Received 15 July 2008; revised 4 September 2008, 2 October 2008, 21 October 2008; accepted 11 November 2008

doi: 10.1111/j.1399-0039.2008.01189.x

Abstract

We investigated the association of human leukocyte antigens (HLA) class II alleles and haplotypes with the pathogenesis of acute chest syndrome (ACS) in 186 sickle cell anemia (SCA) patients, of whom 58 had documented ACS (new pulmonary infiltrate, fever, and other associated clinical events) and 128 with a negative history of ACS, serving as controls. HLA DRB1* and -DQB1* genotyping was performed by polymerase chain reaction–sequence-specific priming. Of the DRB1* and DQB1* alleles analyzed, only DRB1*130101 ($Pc < 0.001$) was positively associated with ACS. DRB1*130101-DQB1*060101 haplotype was more prevalent among ACS patients ($P = 0.018$), thus conferring disease susceptibility. Specific HLA alleles and haplotypes may influence ACS risk in SCA patients, and specific HLA genotypes may be useful markers for identifying high-risk SCA ACS patients.

Acute chest syndrome (ACS) is a major complication of sickle cell anemia (SCA); and a significant cause of morbidity and mortality (1, 2) and a leading cause of hospitalization among SCA patients (3, 4). While several etiologies have been proposed for the pathogenesis of ACS, the underlying cause remains unknown in most (50%) cases (1, 3). It has been suggested that infectious (*Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) (4–6) and noninfectious (pulmonary infarction and fat embolism) (4, 6, 7) causes contribute to ACS development. ACS is a combination of respiratory symptoms and new pulmonary infiltrates (2, 4) and is accompanied by fever, cough, sputum production, or new-onset hypoxia, with repeated ACS episodes progressing to chronic lung disease and even death (2, 8). A number of risk factors have been documented in association with ACS, including younger age, low hemoglobin (Hb) levels, vaso-occlusive crisis (VOC; 3, 9), and high steady-state Hb levels, and leukocyte count (4, 7, 10).

Human leukocyte antigens (HLA) alleles and haplotypes were previously shown to influence the pathogenesis of SCA complications, including stroke (11, 12), VOC (13), osteomyelitis (14), infection (15, 16), and avascular necrosis of the femoral head (17). Despite the strong immunological facet of ACS, no study has examined the potential association of HLA markers with ACS. This study investigated the association of HLA class II alleles and haplotypes with ACS in a population of Bahraini SCA patients.

From April 2006 to March 2007, 186 SCA (HbSS or S/beta 0 thal) patients, diagnosed according to Hb profile (HbA, HbS, HbA_2, and HbF), were enrolled. Patients were assigned to one of two groups: ACS ($n = 58$) and no-ACS SCA controls ($n = 105$), according to clinical presentation and laboratory findings. While the majority of ACS cases included were first attacks, all cases (new and recurrent) were definite ACS cases according to the international criteria used. ACS was defined as the development of a new

pulmonary infiltrate on chest X-ray, combined with fever, coughs, and related respiratory symptoms, or chest pain. Associated clinical events (event that occurred in the 2 weeks before or on the day of ACS presentation) were documented and included pain crises (leg, chest, and backache), febrile events, hypoxia, dizziness, transient ischemic attacks, and anemia.

Data on daily physical exams and laboratory results (complete blood counts; chest X-ray; and blood, sputum, and pleural fluid cultures) were collected following ACS diagnosis. ACS management included hydration, antibiotics (parenteral cefuroxime and oral erythromycin), and pain medication (narcotics and/or nonsteroidal antiinflammatory drugs), with transfusion used only if needed based on the patient's clinical course and at the attending physician's discretion. Control patients included SCA patients with no past history of ACS. The Arabian Gulf University Research and Ethics Committee approved the study protocol, and all participants (or guardians in the pediatric cases) gave written consent.

HLA DRB1 and -DQB1 gene alleles were analyzed using the polymerase chain reaction (PCR)–sequence-specific priming (SSP) technique, using the Micro SSP™ Generic HLA Class II (DRB/DQB) DNA Typing kit (Lot #05A), according to manufacturer's specifications (One Lambda, Thousand Oaks, CA). PCR products were analyzed on ethidium-bromide-stained agarose gel. HLA alleles nomenclature was as previously reported (18). In total, 15 DRB1 and 7 DQB1 alleles were tested. Allele frequencies were determined by the gene counting method, using the HLA-STAT 2000 software, which also computed the *P* values (Fisher's exact probability test) and odds ratios (OR). The frequencies of the most frequent haplotypes were determined by the maximum likelihood method, using the ARLEQUIN (v. 2.000) population genetics data analysis software. *P* values were corrected for the number of different alleles tested (*Pc*) using the Bonferroni inequality method and for the different haplotypes analyzed using the Sidak correction factor; significance was determined at $P < 0.05$. Additional statistical was performed with SPSS VERSION 14.0 for Windows statistical package.

The demographic and clinical characteristics of SCA ACS patients and SCA controls are shown in Table 1. Controls were matched with patients with respect to age ($P = 0.969$) and gender ($P = 0.860$). Except for platelet count, which was higher ($P = 0.027$), and HbA2, which was lower ($P = 0.029$) among ACS patients, biochemical, and hematologic indices were comparable between both SCA patient groups. The relatively high baseline HbF level in patients and controls was most likely because of HU therapy, which, while not yet routinely administered to all patients, was associated with a significant rise in HbF levels in treated ACS patients (24.0% ± 8.0% *vs* 17.6% ± 5.2%) and control subjects (24.7% ± 6.9% *vs* 15.1% ± 5.0%).

Table 1 Demographics and clinical characteristics of SCA patients

Characteristics	ACS patients ($n = 58$)	SCA control ($n = 128$)	*P*
Male:female (%)	33:25 (56.9:43.1)	72:56 (56.3:43.8)	0.860
Mean age ± SD (years)	13.6 ± 8.3	13.5 ± 9.0	0.969
Hb profile (%)			
HbS	71.3 ± 10.7	71.4 ± 9.3	0.942
HbF	20.7 ± 8.7	19.9 ± 8.3	0.568
HbA2	2.9 ± 1.2	3.5 ± 1.4	0.029
HbA	2.0 ± 0.7	2.6 ± 0.7	0.324
Laboratory values			
Total Hb[a]	9.4 ± 1.1	9.6 ± 1.4	0.516
Hematocrit (%)	27.6 ± 3.3	30.5 ± 2.4	0.505
MCH[b]	26.4 ± 4.2	25.7 ± 3.9	0.378
MCHC[a]	33.9 ± 1.7	33.6 ± 2.0	0.502
Reticulocytes (%)	6.4 ± 3.7	5.2 ± 2.0	0.133
WBC ($\times 10^3/\mu l$)	10.7 ± 6.2	10.0 ± 5.5	0.665
Platelets	416.1 ± 207.6	324.4 ± 205.3	0.027
Fibrinogen	351.7 ± 106.8	380.3 ± 130.3	0.783
LDH (U/l)	455.3 ± 176.2	462.0 ± 230.1	0.943
Bilirubin[c]	37.5 ± 27.0	36.8 ± 24.3	0.894

ACS, acute chest syndrome; SD, standard deviation; Hb, hemoglobin; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SCA, sickle cell anemia; WBC, white blood cells.

[a] Concentration in g/dl.

[b] MCH (Hb content) in pg.

[c] Concentration in μmol/l.

Significant DRB1 allelic differences were seen between ACS patients and controls subjects; 3 of 15 loci being significantly different. These comprised DRB1*110101 ($P = 0.006$) and DRB1*150101 ($P = 0.004$), which were present at lower, and DRB1*130101 ($P < 0.001$), which was found at higher frequencies among ACS patients (Table 2). When the Bonferroni correction was applied, differences were significant for only DRB1*130101 ($Pc < 0.001$), which was present at higher frequency among the ACS case patients (Table 2). No significant allelic differences were seen at the DQB1 locus, even before applying the Bonferroni correction (Table 2).

The haplotype frequencies of DRB1*010101-DQB1*050101 ($P = 0.019$; OR = 5.42) and DRB1*130101-DQB1*060101 ($P = 0.002$; OR = 5.26) were higher, while the haplotype frequency of DRB1*150101-DQB1*060101 ($P = 0.039$; OR = 0.21) were lower in ACS SCA patients (Table 3). Of these, only DRB1*130101-DQB1*060101 ($P = 0.018$) remained positively associated with ACS after applying the Sidak correction factor (Table 3).

SCA is a monogenic hematological disease (19), and its clinical manifestations are heterogeneous and influenced by nearby and distant genes. An increasing body of evidence implicates the involvement of inflammatory events and HLA genes in diseases characterized by endothelial injury, including SCA (11, 13, 14, 16, 17). This case–control study is the first to identify unique class II susceptible haplotype

Table 2 HLA DRB1* allele distribution among SCA patients

Locus	Allele	ACS patients (58)		SCA controls (128)				
		Allele frequency	SE	Allele frequency	SE	P^a	Pc^b	OR
DRB1	010101	0.0776	0.0248	0.0273	0.0102	0.053	0.558	2.766
	030101	0.1466	0.0328	0.1289	0.0209	0.396	1.000	1.361
	030201	0.0431	0.0189	0.0234	0.0095	0.536	1.000	1.506
	040101	0.1034	0.0283	0.0898	0.0179	0.668	1.000	1.193
	070101	0.0948	0.0272	0.0938	0.0182	0.777	1.000	0.885
	080101	0.0086	0.0086	0.0313	0.0109	0.183	0.951	0.263
	090102	0.0086	0.0086	0.0117	0.0067	0.787	1.000	0.731
	100101	0.1379	0.0320	0.0977	0.0186	0.270	0.991	1.512
	110101	0.0862	0.0261	0.1836	0.0242	0.006	0.090	0.339
	120101	0.0086	0.0086	0.0156	0.0078	0.584	1.000	0.544
	130101	0.1724	0.0351	0.0469	0.0132	2.6×10^{-5}	2.3×10^{-4}	5.088
	1327	0.0086	0.0086	0.0078	0.0055	0.935	1.000	1.105
	140101	0.0086	0.0086	0.0313	0.0109	0.244	0.985	0.303
	150101	0.0172	0.0121	0.1094	0.0195	0.004	0.057	0.147
	160101	0.0776	0.0248	0.1016	0.0189	0.777	1.000	0.885
DQB1	0201	0.2931	0.0423	0.2148	0.0257	0.204	0.798	1.501
	030101	0.1207	0.0302	0.1797	0.0240	0.112	0.566	0.554
	030201	0.0690	0.0235	0.0742	0.0164	0.712	1.000	0.839
	030302	0.0862	0.0261	0.0586	0.0147	0.576	0.998	1.303
	0401	0.0431	0.0189	0.0430	0.0127	0.851	1.000	1.113
	050101	0.2586	0.0407	0.2852	0.0282	0.285	0.904	0.712
	060101	0.1293	0.0312	0.1445	0.0220	0.811	1.000	0.916

ACS, acute chest syndrome; SE, standard error; Hb, hemoglobin; SCA, sickle cell anemia; OR, odds ratio.

[a] Determined by Fisher's exact test.

[b] Pc = corrected P for the number of allele tested, determined by Bonferonni method.

associated with ACS and thus extends the role that HLA genes may play in the pathogenesis of SCA complications. DRB1*130101 was positively associated with ACS, while at the haplotype level, DRB1*130101-DQB1*060101 conferred ACS susceptibility.

Varied distribution of DRB1*130101 was shown among healthy Arab populations, ranging from 2.1% among healthy Bahraini Arabs (20) to 3.4% in Tunisians (21), 3.7% in Lebanese (20), and 9.0% among Kuwaiti (17). While the increased frequency of DRB1*130101 among ACS controls (4.7%) and ACS patients (17.2%) may suggest enrichment for this allele among unselected Bahraini SCA patients, the frequency of DRB1*130101-DQB1*060101 haplotype among SCA controls (2.1%) was similar to its frequency among non-SCA healthy Bahrainis (2.1%) and Lebanese (3.2%) established earlier (20), thereby showing enrichment for this haplotype only in ACS patients, suggesting a role for it in ACS development. Previous studies suggested a functional role for the DRB1*1301 allele in the pathogenesis of several pulmonary disorders, including pulmonary inflammation associated with coccidioidomycosis (22), in presentation of specific lung allergens (23), and in primary pulmonary hypertension (24). Accordingly, the association of DRB1*130101 with ACS indicates a broader role for this allele in the pathogenesis of pulmonary diseases.

Despite the presence of the milder beta-globin gene haplotype form (Saudi Arabian–Indian) in our population, the emergence of severe SCA-related complications such as ACS, noteworthy in our (pediatric) population, with the major cause of ACS being infection. The HLA association with ACS found in the current study differed from those found in our previous studies, where DRB1*100101-DQB1*050101, which was a neutral haplotype in ACS patients, was positively associated with both VOC (13) and osteomyelitis (14). These differences in HLA associations were reminiscent of previous findings on stroke in which the contribution of a specific HLA (DPB) allele to stroke pathogenesis was dependent on the specific stroke subtype (11, 12). It is likely that involvement of DRB1*130101-DQB1*060101 haplotype in ACS development may reside in its affinity to specific antigenic fragments presented by it, which in turn confers disease susceptibility (25). This reflects distinct genetic etiologies for SCA complications, further confirming the distinct genetic and phenotypic nature of SCA complications.

HLA system regulates immune responses by facilitating foreign antigen processing and presentation of to specific T cells (25). Unlike previous earlier studies that focused on

Table 3 HLA DRB1*-DQB1* haplotype frequencies

DRB-DQB haplotype[a]	ACS patients[b]	ACS controls[b]	P^c	Pc^d	OR (95% CI)[e]
*DRB1*010101-DQB1*050101*	0.063	0.011	0.019	0.159	5.42 (2.46–8.96)
*DRB1*030101-DQB1*0201*	0.152	0.090	0.092	0.581	1.86 (0.90–3.39)
*DRB1*040101-DQB1*030201*	0.054	0.043	0.915	1.000	1.21 (0.47–3.37)
*DRB1*070101-DQB1*0201*	0.063	0.051	0.896	1.000	1.20 (0.49–3.10)
*DRB1*100101-DQB1*050101*	0.117	0.068	0.121	0.687	1.93 (0.93–4.03)
*DRB1*110101-DQB1*030101*	0.082	0.107	0.618	1.000	0.77 (0.39–1.74)
*DRB1*130101-DQB1*060101*	0.094	0.021	0.002	0.018	5.26 (1.76–14.12)
*DRB1*150101-DQB1*060101*	0.017	0.077	0.039	0.301	0.21 (0.07–0.95)
*DRB1*160101-DQB1*050101*	0.036	0.049	0.787	1.000	0.73 (0.24–2.15)

ACS, acute chest syndrome; SE, standard error; Hb, hemoglobin; SCA, sickle cell anemia; OR, odds ratio.

[a] DRB1* and DQB1* alleles were assessed by PCR-SSP, and haplotype frequencies determined by the maximum likelihood method.

[b] SCA patients with (ACS patients, $n = 58$) or without (SCA controls, $n = 128$) ACS.

[c] Determined by Fisher's exact test.

[d] Pc = Sidak-adjusted P values.

[e] ORs determined according to Woolf method.

the association of HLA genes with diseases that have an immune component, it is becoming increasingly evident that other HLA associations with diseases that do not have a classic immune basis do exist. The association of DRB1*130101 and DRB1*130101-DQB1*060101 with ACS suggests that unique antigen-dependent mechanisms contribute to ACS pathogenesis, without ruling out the possibility that HLA may serve as marker for other gene(s) in linkage disequilibrium, which in turn mediate ACS susceptibility (or protection).

The role played by HLA genes in ACS development is plausible when viewed in the context of endothelial injury accompanying SCA, which is precipitated by physical (increased shear stress), biochemical (increased thrombin production), and cellular (adherence of sickle red blood cells to the endothelium) events (19). There is emerging consensus that a proinflammatory condition contributes to SCA complications (26, 27), and a role for cytokines in the pathogenesis of SCA complications was proposed (26, 28, 29). SCA-induced endothelial injury may augment the activity of professional antigen-presenting cells or upregulate the expression of HLA class II molecules on endothelial cells which, while do not act as professional antigen-presenting cells, can be induced to express class II HLA molecules in response to cytokines and other stimuli (30, 31). This would be followed by a wave of cytokine release, leading to leukocyte activation and adhesion to the vasculature (32, 33), which exacerbates and propagates inflammatory reactions, likely increasing the adherence of sickle erythrocytes to the endothelium (30, 32, 33).

Despite the strength of the association observed, our study is limited to a specific ethnic group (Bahraini Arabs) and age population (pediatric patients) and that it examined only the HLA DRB and -DQB regions. Larger confirmatory studies will be needed to confirm the association the HLA system, or other genes in linkage disequilibrium with HLA alleles, with ACS risk in SCA patients. While the implications of our findings remain speculative at this stage, identification of ACS-prone SCA patients would be instrumental in the design of prophylactic therapies.

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