



HLA-DQB1*06 is a risk marker for chlamydia reinfection in African American women

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Abstract

Associations between human leukocyte antigen (HLA) variants and chlamydia-related outcomes have been inconsistent. We previously identified HLA-DQB1*06 as a risk marker for chlamydia reinfection in a cohort of predominately HIV-infected adolescents. As chlamydia reinfection can lead to reproductive complications, validation of this finding in HIV-seronegative women may help reveal the underlying biology. We performed *HLA-DQB1* genotyping in HIV-seronegative, chlamydia-infected African American women who were evaluated for reinfection at 3- and 6-month visits after treatment. Of 185 evaluable women for whom *HLA-DQB1* genotyping was performed, only HLA-DQB1*06 was associated with chlamydia reinfection ($P = 0.009$), with no evidence of a dose–response effect for this allele. African American women with HLA-DQB1*06 may warrant more frequent chlamydia screening. More comprehensive genotyping of HLA class II and neighboring genes is needed to establish whether HLA-DQB1*06 is a causal variant for chlamydia reinfection or a surrogate for other causal variants in the major histocompatibility complex.

Introduction

Chlamydia is the most prevalent sexually transmitted bacterial infection in the United States [1]. There is a major disparity in chlamydia rates by race, with African Americans having an almost sixfold higher rate than Caucasians. Limited studies suggest that untreated chlamydial infection may persist for a year or longer in up to 50% of chlamydia-infected women [2]. Recommended treatments for chlamydia are highly efficacious [3], but up to 20% of treated patients may experience reinfection within months of treatment [4]. Even in the absence of symptoms, both persistent chlamydial infection and reinfection may lead to severe sequelae, including pelvic inflammatory disease (PID) and tubal factor infertility (TFI) [5].

The underlying mechanism through which some women are more susceptible to chlamydia reinfection than others has not been fully elucidated. In gene knockout studies in mice, CD4+ T-helper type-1 responses have been shown to

be necessary and sufficient for the clearance of chlamydia infection from the genital tract, whereas CD8+ T cells appear to have a lesser role in the development of protective immunity to chlamydia [6]. As such, human leukocyte antigen (HLA) association studies of chlamydia outcomes have predominately focused on HLA class II genes, although the results have in general been inconsistent [7–12]. In a few of those studies, HLA-DQB1*06 has been implicated as a risk marker for specific chlamydia outcomes [10–12]. In our previous studies of *HLA-DQB1* alleles and chlamydia outcomes in a longitudinal cohort of mostly HIV-infected African American adolescents, we found that HLA-DQB1*06 was associated with chlamydia reinfection and possibly PID [11, 12]. The same allele group has also been reported by Kinnunen et al. [10] as a risk marker for chlamydia-related TFI.

As our previous study cohort was a predominately HIV-infected population, it was unknown whether the findings on HLA associations with chlamydia outcomes from that cohort could be generalized to HIV-seronegative African American women, the population with the most reported cases of chlamydia [1]. Therefore, we conducted a follow-up study in a cohort of HIV-seronegative African American women with uncomplicated chlamydia to confirm the association of HLA-DQB1*06 with chlamydia reinfection.

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Table 1 Baseline characteristics of chlamydia-infected African American participants ($n = 185$)

Characteristic	Reinfection ($n = 38$) n (%)	No reinfection ($n = 147$) n (%)	P -value ^a
Age, years, median (range)	22 (16–32)	22 (16–38)	0.284 ^b
Number of sexual partners last 3 months, median (range)	1.5 (0–5)	1 (0–9)	0.167 ^b
Hormonal contraceptive use, n (%)	20 (52.6)	67 (45.6)	0.414 ^c
Prior chlamydia, n (%)	24 (63.2)	70 (47.6)	0.095 ^c
Concomitant infections at baseline, n (%)			
Trichomoniasis	2 (5.3)	7 (4.8)	1.000 ^d
Candidiasis	3 (7.9)	21 (14.3)	0.419 ^d
Bacterial vaginosis	11 (29.0)	37 (25.2)	0.636 ^c

^aSignificance set at the $\alpha = 0.05$ level

^bWilcoxon's rank-sum test

^cPearson's χ^2 -test for independence

^dFisher's exact test

Results and discussion

We performed high-resolution *HLA-DQB1* genotyping (to four-digit specificity) using genomic DNA extracted from 185 HIV-seronegative African American women with uncomplicated chlamydia, who were evaluated for chlamydia reinfection at 3- and 6-month follow-up visits after treatment as part of a prospective study. Select participant characteristics based on demographical and clinical data collected at baseline (i.e., enrollment) are shown in Table 1. The median age was 22 years, 51% were asymptomatic, 26% had concomitant bacterial vaginosis, and 51% had prior chlamydia based on self-report and medical record review of laboratory test results. Chlamydia reinfection, defined as a positive *Chlamydia trachomatis* nucleic acid amplification test (NAAT) at the 3- and/or 6-month follow-up visit, occurred in 38 (20%) participants. There was a trend ($P = 0.095$) toward a lower chlamydia reinfection rate in participants with prior chlamydia, but no other demographic or clinical characteristics were associated with chlamydia reinfection.

Of *HLA-DQB1* allele groups analyzed at two-digit specificities, only DQB1*06 was associated with chlamydia reinfection (Table 2), with the odds of reinfection 2.71 times greater in those with HLA-DQB1*06 (95% confidence interval (CI) = 1.31–5.86, $P = 0.009$). At least one copy of DQB1*06 was present in 46% ($n = 86$) of the study cohort. The association remained consistent after controlling for prior chlamydia in a multivariable regression model (adjusted $P = 0.010$). At the four-digit resolution level, the most common alleles, DQB1*0602 and DQB1*0604, accounted for most of the DQB1*06 association with reinfection (Table 3). At least one copy of either DQB1*0602 or DQB1*0604 was present in 39% ($n = 72$) of the study cohort. These allele associations were not

statistically significant; however, the analysis was limited by the small sample size at the four-digit resolution level. Moreover, the finding of an unfavorable chlamydia outcome associated with DQB1*0602 was consistent with our previous finding from our predominately HIV-infected cohort [11, 12]. We did not find a dose-response effect when evaluating for an association of number of HLA-DQB1*06 allele copies with chlamydia reinfection (Table 4).

The consistent association of HLA-DQB1*06 with chlamydia reinfection in women regardless of HIV infection status suggests that this allele group could serve as a risk marker for reinfection. Based on known associations with PID and TFI [10], DQB1*06 could also serve as a risk marker for reproductive complications after chlamydia infection. The implication of this risk association is that the HLA-DQB1*06 allele may impair protective immune responses that influence susceptibility to chlamydial infection and/or the ability to clear infection. HLA-DQB1*06 allele variants and/or associated haplotypes have been found to be associated with autoimmune diseases (multiple sclerosis and pemphigoid) and other infections (HIV and streptococcal infections) [13]. One of most widely reported is the HLA-DRB1*1501-DQB1*0602 haplotype association with multiple sclerosis. The next step in our investigation of immunogenetic determinants of CT reinfection is to comprehensively sequence the major histocompatibility complex (MHC) region in our study cohort, in order to assess for haplotype associations and to evaluate variants that are in linkage disequilibrium with the HLA-DQB1*06 allele.

Despite a limited sample size, this candidate gene approach was expected to be informative based our previously published results of an association between HLA-DQB1*06 and chlamydia reinfection, and based on inclusion of a study population with similar HLA-DQB1*06

Table 2 Analyses of *HLA-DQB1* allele groups (two-digit specificities) and chlamydia reinfection

DQB1 allele	Participant frequency ^a (n = 185) n (%)	Reinfection (n = 38) n (%)	No reinfection (n = 147) n (%)	OR (95% CI)	P-value ^b
<i>DQB1</i> *02	56 (30%)	8 (21%)	48 (33%)	0.55 (0.22–1.24)	0.169
<i>DQB1</i> *03	72 (39%)	12 (32%)	60 (41%)	0.67 (0.30–1.41)	0.300
<i>DQB1</i> *04	24 (13%)	7 (18%)	17 (12%)	1.73 (0.62–4.39)	0.267
<i>DQB1</i> *05	75 (41%)	13 (34%)	62 (42%)	0.71 (0.33–1.48)	0.374
<i>DQB1</i> *06	86 (46%)	25 (66%)	61 (42%)	2.71 (1.31–5.86)	0.009

^aFrequency refers to individuals who had at least one allele

^bAfter Bonferroni correction, threshold significance is set at $\alpha_{\text{adjusted}} = (0.05/5) = 0.01$ level, logistic regression models. CI, confidence interval; OR, odds ratio for chlamydia reinfection

frequency and chlamydia reinfection rates [12]. An additional limitation is that our study focused on African Americans, which reflects the population routinely evaluated at our study site, and did not evaluate haplotypes. It remains to be seen whether the risk conferred by *DQB1**06 applies to other races/ethnicities. Nonetheless, a more comprehensive analysis of genes and single-nucleotide polymorphisms in the MHC class II region should help with fine-mapping efforts and will facilitate a haplotype analysis.

The potential clinical translation of our study findings is that African American women with *HLA-DQB1**06 may need more frequent chlamydia screening to detect initial chlamydia and reinfection after treatment, in order to facilitate timely treatment and prevention of reproductive complications. Conversely, analyses of *C. trachomatis* epitopes restricted by non-*DQB1**06 alleles could identify correlates of immune protection and advance chlamydia vaccine development.

Materials and methods

Study cohort and clinical procedures

Women ≥ 16 years of age presenting to the Jefferson County Department of Health Sexually Transmitted Diseases Clinic in Birmingham, Alabama, for treatment of a recent positive screening *C. trachomatis* NAAT were enrolled in a chlamydia immunogenetics study after providing written informed consent. Those who were pregnant, had a prior hysterectomy, were co-infected with HIV, syphilis, or gonorrhea, or had received antibiotics with anti-chlamydial activity in the prior 30 days were excluded. At enrollment, participants were interviewed regarding their demographics, symptoms, sexual history, and hormonal contraception use, and provided blood for genomic DNA. A pelvic examination was performed to obtain a vaginal swab specimen for wet mount testing to diagnose trichomoniasis, bacterial vaginosis, and candidiasis, and an endocervical swab specimen for chlamydia and gonorrhea testing by

NAAT (Aptima Combo 2 [AC2]; Hologic, Marlborough, MA) as per the manufacturer's instructions. Participants received azithromycin 1 g orally for chlamydia treatment and then returned for 3- and 6-month follow-up visits for repeat chlamydia testing by NAAT (AC2) to evaluate for chlamydia reinfection. Our analyses focused on women with confirmed chlamydia at enrollment. Women were classified as having chlamydia reinfection if they had a positive NAAT result for *C. trachomatis* at the 3- and/or 6-month follow-up visit, as well as for any positive *C. trachomatis* NAAT result during any non-study visit within the 6-month study period (and at least 28 days since treatment). The study was approved by the University of Alabama at Birmingham Institutional Review Board and Jefferson County Department of Health.

HLA genotyping

Using genomic DNA extracted from peripheral blood (Genra Puregen Kit, Qiagen Inc., Germantown, MD), we performed high-resolution *HLA-DQB1* genotyping. Briefly, the *HLA-DQB1* locus was amplified by gene-specific primers using polymerase chain reaction (PCR), then resolved by Sanger sequencing (Olerup, Inc., West Chester, PA) designed for capillary electrophoresis. Alleles were assigned using the ABI DNA Analyzer (Applied Biosystems, Foster City, CA). Individual alleles were resolved to four-digit specificities against a recent HLA database (Assign SBT v4.7, Olerup Inc.). Data analyses evaluated allele groups (i.e., 2-digit specificity) and four-digit alleles corresponding to distinct protein sequences.

Statistical analysis

Analyses of baseline participant characteristics and chlamydia reinfection were performed using the Wilcoxon's rank-sum, Pearson's χ^2 , or Fisher's exact tests as appropriate. Analyses of chlamydia reinfection by HLA allele frequency were performed using logistic regression or exact logistic regression as appropriate. Any participant characteristics or HLA alleles associated with chlamydia

Table 3 Analyses of four-digit alleles in the *DQB1*06* group and chlamydia reinfection

Allelic variants	Participant frequency ^a (<i>n</i> = 185) <i>n</i> (%)	Reinfection (<i>n</i> = 38) <i>n</i> (%)	No reinfection (<i>n</i> = 147) <i>n</i> (%)	OR (95% CI)	<i>P</i> -value ^b
<i>DQB1*0602</i>	67 (36%)	18 (47%)	49 (33%)	1.80 (0.87–3.71)	0.111 ^c
<i>DQB1*0603</i>	12 (6%)	3 (8%)	9 (6%)	1.31 (0.34–5.11)	0.714 ^d
<i>DQB1*0604</i>	9 (5%)	4 (11%)	5 (3%)	3.34 (0.85–13.11)	0.088 ^d
<i>DQB1*0609</i>	7 (4%)	2 (5%)	5 (3%)	1.58 (0.29–8.47)	0.634 ^d
<i>DQB1*0602</i> and <i>*0604</i>	72 (39%)	20 (53%)	52 (35%)	2.03 (0.99–4.21)	0.054 ^c

^aFrequency refers to individuals who had at least one allele

^bAfter Bonferroni correction, threshold significance set at the $\alpha_{\text{adjusted}} = (0.05/5) = 0.01$ level

^cLogistic regression

^dExact logistic regression. CI, confidence interval; OR, odds ratio for chlamydia reinfection.

Table 4 Analyses of *HLA-DQB1* allele copy numbers and chlamydia reinfection

<i>DQB1*</i> allele	Participant frequency (<i>n</i> = 185)	Reinfection (<i>n</i> = 38)	No reinfection (<i>n</i> = 147)	<i>P</i> -value ^a
<i>DQB1*06</i>				0.066 ^b
0 Copies	100 (54%)	14 (37%)	86 (59%)	
1 Copy	63 (34%)	19 (50%)	44 (30%)	
2 Copies	22 (12%)	5 (13%)	17 (12%)	
<i>DQB1*0602</i> and <i>*0604</i>				0.114 ^b
0 Copies	113 (61%)	18 (47%)	95 (65%)	
1 Copy	55 (30%)	16 (42%)	39 (27%)	
2 Copies	17 (9%)	4 (11%)	13 (9%)	

^aSignificance set at the $\alpha = 0.05$ level; ^bCochran–Armitage test for trend

reinfection on univariate analyses with a nominal *P*-value < 0.1 were further evaluated using a multivariable logistic regression model. Analyses of chlamydia reinfection by HLA allele copy frequency were performed using the Cochran–Armitage test for trend. Statistical significance was set at the $\alpha = 0.05$ level and a Bonferroni correction adjusted α -level was used as appropriate. All statistical analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC).

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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