Title
HLA class I and II alleles in susceptibility to ankylosing spondylitis

Journal
Annals of the Rheumatic Diseases, 78(1)

ISSN
0003-4967

Authors
Reveille, John D
Zhou, Xiaodong
Lee, MinJae
et al.

Publication Date
2019

DOI
10.1136/annrheumdis-2018-213779

Peer reviewed

Published in final edited form as:

Ann Rheum Dis. 2019 January ; 78(1): 66–73. doi:10.1136/annrheumdis-2018-213779.

HLA Class I and II Alleles in Susceptibility to Ankylosing Spondylitis

John D. Reveille[1], **Xiaodong Zhou**[1], **MinJae Lee**[2], **Michael H. Weisman**[3], **Lin Yi**[4], **Lianne S. Gensler**[5], **Hejian Zou**[6], **Michael M. Ward**[7], **Mariko Ishimori**[3], **Thomas J. Learch**[3], **Dongyi He**[8], **Mohammad H. Rahbar**[2], **Jiucun Wang**[6], **Matthew A. Brown**[9]

[1]Division of Rheumatology and Clinical Immunogenetics, McGovern Medical School at The University of Texas Health Science Center, Houston, TX

[2]Division of Clinical and Translational Sciences; McGovern Medical School at The University of Texas Health Science Center Houston

[3]Cedars-Sinai Medical Center, Los Angeles, CA

[4]Gansu College of Traditional Chinese Medicine, Lanzhou, Gansu, China

[5]The University of California, San Francisco

For correspondence, please contact: John D. Reveille, M.D., The University of Texas-Houston Health Science Center, MSB 5.270, 6431 Fannin, Houston, TX, 77030, Phone 713-500-6900, FAX 713-500-0523, john.d.reveille@uth.tmc.edu.

Contributorship:

Dr. John D. Reveille contributed a large number of patients, carried out the HLA typing, entered the data, interpreted the results, did many of the statistical analyses, wrote the manuscript and provided the funding for the study.

Dr. Xiaodong Zhou established the Chinese collaboration and oversaw its completion, reviewed the manuscript and provided some of the funding for this study.

Dr. MinJae Lee carried out the multivariable and regression statistical analyses and participated in the writing and drafting the manuscript.

Dr. Michael H. Weisman contributed a large amount of the patients for this study as well as some of the controls. He also reviewed the manuscript.

Dr. Lin Yi contributed a large amount of the Chinese patients for this study as well as some of the controls and qc'd the Chinese HLA genotyping and reviewed the manuscript.

Dr. Lianne S. Gensler contributed a number of the U.S. patients for this study. She also reviewed the manuscript.

Dr. Hejian Zou contributed a large amount of the Chinese patients for this study as well as some of the controls. He also reviewed the manuscript.

Dr. Michael M. Ward contributed a number of the U.S. patients for this study. He also reviewed the manuscript and obtained funding from the NIH Clinical Center to carry on the project there.

Dr. Mariko Ishimori contributed number of the U.S. patients for this study as well as some of the controls. She also reviewed the manuscript

Dr. Thomas J. Learch read all the X-rays in the American patients for the study to determine who qualified for inclusion. He also reviewed the manuscript.

Dr. Dongyi He, contributed a large amount of the Chinese patients for this study as well as some of the controls. He also reviewed the manuscript.

Dr. Mohammad H. Rahbar oversaw the qc process of the datasets and worked with Dr. Lee in the statistical analyses.

Dr. Jiucun Wang oversaw the Chinese segment of this study, carrying out the HLA typing and coordinating the participating centers, as well as providing funding for the Chinese portion of this project.

Dr. Matthew A. Brown provided the Australian patients in this study. He also qc'd the HLA typing with the imputed data from our GWAS, confirmed the statistical analyses, and assisted Dr. Reveille in manuscript preparation and review.

Competing interests:

None

Ethical approval information: The Internal Review Boards at The University of Texas-Health McGovern Medical School, The University of California-San Francisco, Cedars-Sinai Medical Center, The NIH-NIAMS Clinical Center, The Princess Alexandra Hospital (Brisbane), and Fudan University reviewed and approved this study

Data sharing statement:

We would be happy to share the data published in this manuscript. There are no unpublished data referable to the work included here.

[6]Huashan Hospital, Fudan University, Shanghai, China

[7]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD

[8]Shanghai Guanghua Hospital, Shanghai, China

[9]Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Princess Alexandra Hospital, Queensland, Australia.

Abstract

Objective: To examine associations of HLA class I and class II alleles with ankylosing spondylitis (AS) in three cohorts of patients of European, Asian and African ancestry.

Methods: HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 alleles were genotyped in 1948 unrelated white and 67 African-American AS patients from the Prospective Study of Outcomes in Ankylosing Spondylitis (PSOAS) cohort, the North American Spondylitis Consortium (NASC) and Australo-Anglo-American Spondyloarthritis Consortium (TASC), 990 white and 245 African American Controls and HLA-B alleles in 442 Han Chinese AS patients and 346 controls from Shanghai and Gansu, China. In addition to the case:control analyses, *HLA-B*27* negative AS patients were analyzed separately, and logistic regression and "relative predispositional effects" (RPE) analyses were carried out to control for the major effect of *HLA-B*27*on disease susceptibility.

Results: Although numerous associations were seen between HLA alleles and AS in whites, among HLA-B*27 negative AS patients, positive associations were seen with *HLA-A*29*, *B*38*, *B*49*, *B*52*, *DRB1*11* and *DPB1*03:01* and negative associations with *HLA-B*07*, *-B*57*, *-DRB1*15:01*, *-DQB1*02:01* and *-DQB1*06:02*. Additional associations with *HLA-B*14* and *B*40* (B60) were observed via RPE analysis, which excludes the *HLA-B*27* alleles. The increased frequency of *HLA-B*40:01* and decreased frequency of *HLA-B*07* was also seen in Han Chinese and African-Americans with AS. *HLA-B*08* was decreased in whites with acute anterior uveitis.

Conclusions: These data, analyzing the largest number of AS patients examined to date in three ethnic groups, confirm that other HLA class I and II alleles other than HLA-B*27 to be operative in AS predisposition.

Introduction

Studies of the contribution of MHC-encoded variants to the heritability of AS suggest that it is responsible for 20.44% of the genetic risk for the disease, with over 114 non-MHC variants identified to date contributing another 7.38% (1). Other MHC region genes have also been implicated in addition to *HLA-B*27*, including *HLA-B*14* (2,3), *HLA-B*60* (4-8), *HLA-DRB1* (9-11), *HLA-DPB1* (11,12), *MICA* (13,14), *TNF-alpha1* (15), *TAP1* (16) and *LMP2* (11,17), although the level of association of these findings has not been definitive and with the exception of *HLA-B*60* replication has not been universal.

HLA class I genes (HLA-A, -B and –C) and HLA class-II genes (HLA-DRA1, -DRB1, -DQA1, -DQB1, -DPA1, and -DPB1) encode cell-surface molecules that play an essential role in the immune defense against intracellular infections and in initiating an immune

response to invading pathogens, respectively. Linkage disequilibrium (LD) with HLA-B*27 makes it difficult to resolve whether they play an independent role themselves, which would provide additional clues to AS pathogenesis, and potentially serve as biomarkers of diagnosis. Studies of direct HLA typing to date have been small and underpowered, providing inconsistent results.

Recently, we genotyped 7,264 MHC single-nucleotide polymorphisms (SNPs) in 9,069 AS cases and 13,578 population controls of European descent using the Illumina Immunochip microarray and controlling for the effects of *HLA-B*27:02* and *B*27:05*, identified several other *HLA-B* allelic associations with AS, including significantly increased frequencies of *HLA-B*13:02*, *B*40:01*, *B*40:02*, *B*47:01*, *B*51:01* and negative associations with *HLA-B*07:02* and *B*57:01*, and association of *HLA-A*02:01*, *HLA-DRB1*0:103* and *HLA-DPB1* (18,19). In a Korean study, imputation was used to additionally show that *HLA-C*15:02* is associated with AS susceptibility (19).

The purpose of this study was, in the largest reported series of patients to date for directly genotyped HLA alleles, to examine the relative contributions of HLA-class I and class II alleles, analyzing overall disease associations and conditioning on the presence of HLA-B*27 in three ethnic groups: 1948 whites of European ancestry, 442 Han Chinese, and 67 African Americans, the latter never having been examined for MHC associations (other than *HLA-B*27 per se*) previously.

Methods

Patient enrollment

White AS patients in this study came mostly from the Prospective Study of Outcomes in Ankylosing Spondylitis (PSOAS) cohort (20), a multi-ethnic study conducted at four U.S. academic institutions (The University of Texas Health-McGovern Medical School [UTH-H], Cedars-Sinai Medical Center, the University of California at San Francisco, and the National Institutes of Health Clinical Center) and from The North American Spondylitis Consortium (NASC) (n=408) (21). The black patients included 53 from the PSOAS study, seven from NASC, and seven other either from patients followed from the outpatient clinics at UTH or referred by Dr. Joel Taurog from the University of Texas Southwestern Medical School. All cases met modified New York criteria for AS (22). Controls were self-identified whites from the U.S. with no history of rheumatic disease obtained from unaffected nonconsanguinous spouses or household or friend controls the Scleroderma Family Registry and DNA repository (23) or the North American Spondylitis Consortium (21) (unrelated spouses or friends-75%) or from University of Texas-Houston Division Controls from Texas (25%). It should be noted that both the NASC and Scleroderma Family Registry controls and families came from all over the U.S., although were enriched from Texas and California (NASC) and from Texas, Maryland, Pennsylvania and Michigan (Scleroderma Family Registry) (23). Approximately one third of the US ASpatients were probands in the NASC study and 2/3 came from the PSOAS study (from which approximately half came from California, 30% from Texas, and 20% from the Mid-Atlantic region. All controls were screened by questionnaire for the presence of autoimmune disease or spondyloarthritis and were excluded if having such. For the HLA-B locus analyses, an additional 555 white British and

Australian AS patients from the Australo-Anglo-American Spondylitis Consortium (TASC) were also analyzed. HLA-B locus typing from 442 Han Chinese AS patients and 346 unrelated Chinese controls were analyzed by the same statistical approaches. Though some of the HLA-typing was reported previously by us (24), additional statistical analyses were carried out for the present study on a significantly larger AS cohort heretofore unreported. The AS patients came from the clinics and hospitals in Shanghai and Jiangsu Province of China and the Chinese controls (who were free of any history of rheumatic disease) were obtained from a study project of Chinese population genetics at Fudan University in Shanghai. All subjects provided written informed consent. The study was approved by the Institutional Review Boards of all participating medical centers.

HLA Genotyping

Single Stranded Conformational Polymorphism (SSCP) typing, of HLA-A, -B and –C alleles, was performed using commercially available kits (Dynal, Inc.) on genomic DNA extracted from peripheral blood. HLA-DRB1, -DQA1, - DQB1 and -DPB1 typing was performed by standard oligotyping techniques with high resolution HLA-DRB1 typing further achieved by sequence analysis of PCR-amplified HLA-DRB1 exon 2, except for 18 of the African American AS patients, where the MHC class II alleles were determined by SSCP typing using commercially available kits (One Lambda, Inc). Genomic DNA from the Chinese AS patients and controls underwent the allele-specific polymerase chain reactions (PCR) using primers supplied in the SeCore kits, and then were followed by sequencing exon 2 and 3 of the HLA-B gene. The HLA SBT uTYPE 6.0 program (Life Technologies) was used in sequencing analysis and assigning HLA-B alleles.

The HLA-B genotyping in HLA-B*40 carriers was confirmed with sequence-based typing using SeCore Kits (Life Technologies, USA). The HLA SBT uTYPE 6.0 program (Life Technologies) was used in sequencing analysis.

Statistical Analysis

We constructed 2×2 tables and tested the proportion of alleles in cases vs. controls with adjusted chi square test using EPI-INFO (cdc.gov/epiinfo/index.html). Another method of "adjusting" for the effect of *HLA-B*27* at the *HLA-B* locus is to mask the *HLA-B*27* alleles and analyze the remaining alleles for association with phenotype by comparing their frequencies (relative predispositional effects analysis-RPE) (25, 26). Typically, RPEs are assessed by sequentially removing the alleles with the largest effect among those remaining (25). Such a procedure has no impact on loci other than HLA-B, as we are not removing individuals, but rather alleles. In addition to the univariable associations of each allele with AS, interaction effects between each allele and *HLA-B*27* in relation to AS were examined using logistic regression models where the effects of each allele on AS were estimated for HLA-B*27 negative and positive patients separately. For the HLA-B analyses, this study has 94% power to detect an association with an additive odds ratio of 1.5 for an allele with minor allele frequency of 0.1 at nominal significance.

Results

The white patients in the PSOAS and NASC cohorts were strikingly similar in their clinical features, with psoriasis occurring in 10.4%, Crohn's disease in 5.9%, ulcerative colitis in 3.5%, and reactive arthritis in 5.0%. Minor differences between the two cohorts included a slightly lower frequency of *HLA-B*27* (85.4%) and uveitis (37%) in the PSOAS cohort compared to 98.5% and 44%, respectively, in the probands from the NASC cohort (the latter enriched for multicase families). The prevalence of *HLA-B*27* and uveitis is higher in familial AS (27). Among the 61 blacks with AS studied who had clinical information available, uveitis occurred in 37.7%, psoriasis in 10.2%, and inflammatory bowel disease in 11.5%.

Examination of HLA-A, -B, and –C allele frequencies in all white AS patients and controls showed a number of highly significant associations at each locus (Table 1). *HLA-B*27* occurred in 87.8% of the white AS patients compared to 7.6% of controls ($p<1\times10^{-9}$, odds ratio (OR)=87.7, 95% confidence interval (CI)=66.8 – 115.0), while *HLA-B*27* homozygosity occurred in 3.5% of patients and 0.1% of controls ($p<1\times10^{-9}$, OR=35.9, 95% CI.=4.96 - 258.0) (data not shown-Table 1 shows *allele* frequencies). In addition to *HLA-B*27*, several other MHC class I alleles, including *HLA-A*02*, *C*01*, and *C*02* were increased in frequency, whereas *HLA-A*01*, *A*03*, *B*07*, *B*08*, *B*13*, *B*35*, *B*40*, *B*44*, *B*51*, *B*57*, *C*03*, *C*06* and *C*07* were decreased in frequency (Table 1). Examination of *HLA-DRB1* and *-DQB1* allele frequencies in 790 patients and 704 controls demonstrated positive associations with *HLA-DRB1*01:01*, *DRB1*01:03*, and *DRB1*04:04*, as well as with *DQB1*03:01* (in linkage disequilibrium (LD) with *DRB1*01:03*), *DQB1*03:02* (in LD with *DRB1*04:04*) and *DQB1*05:01* (in LD with *DRB1*01:01*). Examination of *HLA-DPB1* alleles in 635 patients and 385 controls revealed an association with *HLA-DPB1*03:01*. *HLA-DRB1*15:01* and *DRB1*03:01* and their respective linked alleles *DQB1*06:02* and *DQB1*02:01* were deceased in frequency in the white AS patients.

Examining *HLA-B*27*-negative patients and controls, positive associations were seen with *HLA-A*29*, *B*38*, *B*49* and *B*52*, as well as with *HLA-DRB1*11* (Table 2). On the other hand, *HLA-B*07*, *B*57*, *DRB1*15:01*, *DQB1*02:01* and *DQB1*06:02* were significantly decreased in frequency. The association were seen with *HLA-DPB1*03:01* in the overall group persisted in the HLA-B*27-negative white AS patients.

Removing the effect of the presence of *HLA-B*27* using RPE analysis, new associations emerged with *HLA-B*14* and *B*40:01* (Table 3) and the positive associations with *HLA-B*38* and *B*52* persisted, as did the negative associations with *HLA-B*07* and *B*57*. Based on findings from *HLA-B*27* stratified models for each allele, evidence for a significant interaction effect was observed for *B*44* and *B*49* (Table 3).

Amongst the Han Chinese AS patients, *HLA-B*27* carriage was observed in 93.0% of the AS patients and 7.5% of the controls ($p< \times 10^{-9}$, OR=163) (data not shown). Ten (2.3%) of the 442 Chinese patients were homozygous for *HLA-B*27* compared to none of the 346 controls (p=0.003). Looking at allele frequencies (Table 4), a higher odds ratio was observed for *HLA-B*27:04* than *HLA-B*27:05*, and negative associations were observed for several

other HLA-B alleles, including *HLA-B*07*, *B*13*, *B*15*, *B*35*, *B*46* and *B*51*. A significant interaction effect between *B*58* and *HLA-B*27* in relation to AS was found (OR=1.24 among *HLA-B*27* (−) patients; OR=0.32 among *HLA-B*27* (+) patients). (Table 4).

Among black subjects, *HLA-B*27* occurred in 40 of 67 AS patients (59.7%) compared to 5 of 245 controls (2.0%) ($p< \times 10^{-9}$, OR=71.1) (Table 5). *HLA-B*40:01* occurred in 4 of the 67 AS patients compared to 2 of 245 controls (p=0.03, OR=7.7)(Fisher's exact). *HLA-B*35* and *B*42* were decreased in frequency. *HLA-B*07* was also decreased in frequency, as seen in whites and Han Chinese, though the results were not significant (p=0.15) unless one applies a one tailed p value by Fisher's exact testing (p=0.048, OR=0.46). Weak associations encountered with *HLA-DRB1*08* and *DQB1*03:01*. The frequencies of IBD, psoriasis and reactive arthritis were too low for meaningful statistical analyses.

AAU occurred in 380 of 1389 with clinical data available (27.4%)(Table 6). *HLA-B*27* occurred in 92.9% compared to 85.0% of those without AAU ($p=1.4 \times 10^{-4}$, OR=2.3). Neither of the two most common *HLA-B*27* subtypes seen in whites (*B*27:02* and *B*27:05*) were selectively associated with AAU, independent of their association with AS. Among blacks with AAU, 78.3% were *HLA-B*27* positive compared to 42.1% without AAU (p=0.013, OR=4.95). The frequencies of IBD, psoriasis and reactive arthritis were too low for meaningful statistical analyses.

Discussion

*HLA-B*27* is the major genetic association with AS in all major ethnic groups. Association of other MHC Class I and II loci with either AS specifically (3-8, 10-12) or spondyloarthritis in general (2,9) have been suggested, but have been confounded due to LD between HLA-B and the MHC class II loci. In this study, by conducting stratified analyses of *HLA-B*27*, we have shown associations with other MHC alleles that cannot be attributed to LD with *HLA-B*27* in a moderately large number of AS patients from three ethnic groups. This study also analyzes these alleles in the largest collection of *HLA-B*27* negative AS patients reported to date. We confirm by direct HLA typing some of the associations we previously reported by imputation (19).

HLA-B60 is a serologically defined specificity that correlates at the DNA level with *HLA-B*40:01* (23). We were able to confirm the association with *HLA-B*40:01* with AS in three ethnic groups. In this study, the largest to date employing direct HLA-typing, we were able to confirm the positive association with HLA-B60 (*B*40*), especially *B*40:01*, seen in other studies of whites by direct typing (4-7) and by imputed alleles (19) and of Taiwanese Chinese (8). We were also able to confirm the negative association with *HLA-B*07* and *B*57* that we reported previously in whites by imputation (19) and in the case of *B*07* in Han Chinese by DNA sequencing (24). The association seen with HLA-B*14, albeit only by RPE analysis, is compatible with what has been observed in French SpA families (2) as well as in African blacks (3). The association with *HLA-B*38* seen most strikingly in *HLA-B*27* negative patients is interesting, given its known association with psoriatic arthritis (29), although this was not seen in the Chinese cohort, where a weak association was described

with *HLA-B*39* (*HLA-B*38* and *B*39* being "splits" of the parent specificity HLA-B*16 (http://hla.alleles.org/antigens/broads_splits.html).

We observed an association with *HLA-A*02* in the overall cohort that was not seen in the smaller *HLA-B*27* negative cohort. This is compatible with the independent association with *HLA-A*02:01* that we observed in a much larger patient cohort by imputation (18), although in the current study we were not able to resolve the association to the 4 digit level. Associations with *HLA-DRB1*01* and *HLA-DRB1*04:04* seen here have previously been described in UK AS patients and from French spondyloarthritis families (2,10), although the lack of confirmation in *HLA-B*27* negatives does not allow us to rule out that this may reflect LD with *HLA-B*27*. Similarly, the association with *HLA-DRB1*01:03* that we observed by imputation (19) was again seen here, but was not seen in the *HLA-B*27* negatives. *HLA-DRB1*01:03* is also strongly associated with inflammatory bowel disease, (30) in particular where associated with peripheral spondyloarthritis (31). On the other hand, the decreased frequency of *HLA-DRB1*15:01* and its linked allele *DQB1*06:02* overall and in the *HLA-B*27* negatives confirms what we have observed by imputation analysis in a larger cohort (19), now seen by direct HLA typing. An increased frequency of *HLA-B*14* has been observed in French SpA families (2) and in African AS patients (3) in other studies.

We were not able to confirm all the associations by direct HLA typing we previously described by imputation (19).In some cases, even with the rather large number of patients studied (1948 whites), given the low odds ratios (<1.5) there may have not have been adequate power. Alternatively, although the patients and controls were from all over the U.S., ancestry informative markers were not examined in the controls and we cannot rule out potential stratification issues. We previously described an association with HLA-B*51 by imputation, seen only in a conditional regression analysis, which we could not confirm here, even by RPE analysis. However, we did find an association with *HLA-B*52* in whites, both in the *HLA-B*27* negatives and in the overall cohort. *HLA-B*51* and *B*52* are well-recognized "splits" of the parent specificity HLA-B5 (http://hla.alleles.org/antigens/broads_splits.html), and, although *HLA-B*51* was decreased overall, it was increased in frequency in the *HLA-B*27* negatives and by RPE analysis, albeit not significantly. *HLA-B*51* was significantly reduced in the Chinese cohort overall, but having controlled for the presence of *HLA-B*27* by RPE, no association was observed. We were unable to establish an independent association with *HLA-B*13*, an allele long associated with psoriasis, described previously by imputation in whites (19). However, as with *HLA-B*51*, *HLA-B*13* was reduced in the overall Han Chinese dataset, but having controlled for *HLA-B*27* no association was observed. *HLA-B*47*, observed by imputation as AS-associated (19), only occurred in five AS patients, and hence was too uncommon to establish as an AS association (data not shown).

The association of *HLA-DPB1*03:01* with AS seen in whites, further extends what was demonstrated previously in smaller cohorts (11,12). This is compatible with the association of SNPs around the *HLA-DPB1* locus recently established by imputation (19). We did not examine HLA-DPA1 alleles, and so could not confirm the associations with HLA-

DPA1 01:02* and *DPA1* 01:03* reported by Díaz-Peña et al (12). However, we did not observe any association with *HLA-DPB1* 13:01* that was seen in their cohort..

We could not confirm some other associations described in smaller AS or SpA cohorts elsewhere (32-34) perhaps due to clinical heterogeneity. One small recent study of HLA-A, -B, -C and DRB1 alleles in 75 Moroccan AS patients reported an allele frequency of *HLA-B* 27* of 32%, with associations also seen with *B* 57*, *C* 02* and *DRB1* 15* and negative associations with *HLA-B* 35* and *B* 49* (32). The reasons for the discrepancies of these data and ours are hard to interpret given the small size of the cohort, which precluded examination of *HLA-B* 27* negatives. Another recent study of 189 Colombian patients with SpA (including 87 with AS, but also reactive arthritis and undifferentiated SpA) found associations with HLA-B*15 as well as with *HLA-DRB1* 01* and *HLA-DRB1* 04*, as well as with *HLA-B* 27*, which occurred at an allele frequency of 26.2% (33). Again, the small size of the cohort of AS patients did not allow examination of *HLA-B* 27* negatives.

The size of the black AS cohort was small (n=67 patients), which would have restricted the statistical power of our observations. Nevertheless, we were able to confirm the positive association with *HLA-B* 40:01* and the negative association with *B* 07*, for the first time in this ethnic group. The finding of *HLA-B* 27* in 60% of the black AS patients is compatible with what was reported by Khan et al. in a smaller cohort several years ago (35).

We were able to confirm the association with *HLA-DRB1* 01:03* as observed by Cortes et al by imputation (19), although not an association of *HLA-DRB1* 08* with either AS or the uveitis phenotype, as has been reported elsewhere (10, 11), in fact *HLA-DRB1* 08* was actually decreased in those with AAU. We did observe a significant association with the presence of HLA-B*27 and the occurrence of AAU in our patients, as also seen by imputation (34) but not the higher frequency of HLA-B*27 homozygosity seen there. Otherwise the most robust association, albeit negative, was the decreased frequency of *HLA-B* 08* in those with AAU, which contrasts what was observed by imputation, where an increased frequency of a single nucleotide polymorphism (SNP) associated with *HLA-B* 08*, namely rs115937001, was observed (36). The reasons for this was unclear, as the odds ratio of 1.8 in that larger group of 1,711 patients with uveitis would suggest that there should have been sufficient power in this cohort of 380 patients with AAU to confirm this.

Thus, in the largest study of direct HLA typing of AS patients and controls from three ethnic groups (and the only one examining African-American blacks other than HLA-B*27), these data show that the impact of the MHC on AS susceptibility extends beyond HLA-B*27. Many (though not all) of the observations made in a prior larger study of imputed HLA alleles (19) are seen here. That positive and negative associations of certain of these alleles cross ethnic boundaries suggests an independent role of both MHC class I and II alleles in influencing susceptibility to AS and subsets thereof.

Acknowledgements:

These data previously presented at the American College of Rheumatology meeting in 2014.and published as a conference abstract

Funding info:

This study was supported by the National Institutes of Health-National Institute of Allergy and Infectious Diseases (NIH-NIAIS) grant UO1 AI09090 (Drs. Zhou, Reveille), National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIH-NIAMS) grants R01 AR-46208 and 2P01AR052915-06A1 (Dr. Reveille), and by University Clinical Research Grants M01-RR-02558 (The University of Texas Health Science Center at Houston), and M01-RR-000425 (Cedars-Sinai Medical Center) and by a grant from the Spondylitis Association of America. Matthew A. Brown was funded by a National Health and Medical Research Council (Australia) Senior Principal Research Fellowship. Lianne Gensler is supported by the Rosalind Engelman research center at the University of California, San Francisco. Michael Ward is funded by the Intramural Research Program, NIAMS/NIH.

References

1. Ellinghaus D, Jostins L, Spain SL, Cortes A, Bethune J, Han B, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. Nat Genet. 2016 5;48(5):510–8 [PubMed: 26974007]
2. Breban M Genetic studies of spondylarthropathies. French Spondylarthropathy Genetic Study Group.Ann Med Interne (Paris) 1998 4;149(3):142–4. [PubMed: 11499406]
3. López-Larrea C, Mijiyawa M, González S, Fernández-Morera JL, Blanco-Gelaz MA, Martínez-Borra J, López-Vázquez A. Association of ankylosing spondylitis with HLA-B*1403 in a West African population. Arthritis Rheum 2002 11;46 (11):2968–71 [PubMed: 12428239]
4. Robinson WP, van der Linden SM, Khan MA, Rentsch HU, Cats A, Russell A, Thomson G. HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B*27+ patients. Arthritis Rheum 1989 9; 32(9): 1135–41 [PubMed: 2789045]
5. Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J, et al. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis 1996 4;55(4): 268–270 [PubMed: 8733445]
6. van Gaalen FA, Verduijn W, Roelen DL, Böhringer S, Huizinga TW, van der Heijde DM, Toes RE. Epistasis between two HLA antigens defines a subset of individuals at a very high risk for ankylosing spondylitis. Ann Rheum Dis 2013 6;72(6):974–8. [PubMed: 22887649]
7. Díaz-Peña R, Vidal-Castiñeira JR, López-Vázquez A, López-Larrea C. HLA-B*40:01 Is Associated with Ankylosing Spondylitis in HLA-B*27-positive Populations. J Rheumatol. 2016 6;43(6):1255–6.
8. Wei JC, Sung-Ching HW, Hsu YW, Wen YF, Wang WC, Wong RH, et al. Interaction between HLA-B60 and HLA-B*27 as a Better Predictor of Ankylosing Spondylitis in a Taiwanese Population. PLoS One. 2015 10 15;10(10):e0137189. doi: 10.1371/journal.pone.0137189.eCollection 2015 [PubMed: 26469786]
9. Said-Nahal R, Miceli-Richard C, Gautreau C, Tamouza R, Borot N, Porcher R,et al. The role of HLA genes in familial spondyloarthropathy: a comprehensive study of 70 multiplex families Ann Rheum Dis 2002 3 ;61(3):201–6. [PubMed: 11830423]
10. Brown MA, Kennedy LG, Darke C, Gibson K, Pile KD, Shatford JL, Taylor A, Calin A, Wordsworth BP. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. Arthritis Rheum. 1998 3;41(3):460–5 [PubMed: 9506574]
11. Ploski R, Flato B, Vinje O, Maksymowych W, Førre O, Thorsby E. Association to HLA-DRB1*08, HLA-DPB1*0301 and homozygosity for an HLA-linked proteasome gene in juvenile ankylosing spondylitis. Human Immunology 1995 10; 44 (2), 88–96. [PubMed: 8847233]
12. Díaz-Peña R, Aransay AM, Bruges-Armas J, Lñpez-Vázquez A, Rodríguez-Ezpeleta N, Mendibil I, et al. Fine mapping of a major histocompatibility complex in ankylosing spondylitis: association of the HLA-DPA1 and HLA-DPB1 regions. Arthritis Rheum 2011 11;63 (11):3305–12. [PubMed: 21769851]
13. Ricci-Vitiani L, Vacca A, Potolicchio I, Scarpa R, Bitti P, Sebastiani G, et al. MICA gene triplet repeat polymorphism in patients with HLA-B*27 positive and negative ankylosing spondylitis from Sardinia. J Rheumatol 2000 9;27 (9):2193–7 [PubMed: 10990233]
14. Zhou X, Wang J, Zou H, Ward MM, Weisman MH, Espitia MGG, et al. MICA, a gene contributing strong susceptibility to ankylosing spondylitis.Ann Rheum Dis 2014 8;73(8):1552–7 [PubMed: 23727634]
15. Hohler T, Schaper T, Schneider PM, Meyer zum Büschenfelde KH Märker-Hermann E. Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing

spondylitis in HLA-B*27 positive individuals. Arthritis Rheum 1998; 8; 41(8):1489–92. [PubMed: 9704649]

16. Fraile A, Collado MD, Mataran L, Martin J, Nieto A. TAP1 and TAP2 polymorphism in Spanish patients with ankylosing spondylitis. Exp Clin Immunogenet 2000; 17(4):199–204 [PubMed: 11096258]
17. Maksymowych WP, Wessler A, Schmitt-Egenolf M, Suarez-Almazor M, Ritzel G, Von Borstel RC, et al. Polymorphism in an HLA linked proteasome gene influences phenotypic expression of disease in HLA-B*27 positive individuals. J Rheumatol 1994 4; 21(4): 665–9 [PubMed: 8035391]
18. Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, et al. Multiple novel loci harbouring common and rare variants implicated in ankylosing spondylitis. Nature Genetics 2013 7; 45(7):730–8. [PubMed: 23749187]
19. Cortes A, Pulit SL, Leo PJ, Pointon JJ, Robinson PC, Weisman MH, et al. Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. Nat Commun 2015 5 21;6:7146. doi: 10.1038/ncomms8146. [PubMed: 25994336]
20. Ward MM, Weisman MH, Davis JC, Reveille JD. Risk factors for functional limitations in patients with long-standing ankylosing spondylitis. Arthritis Rheum 2005 10 15; 53(5): 710–717. [PubMed: 16208654]
21. Zhang G, Luo J, Bruckel J, Weisman MA, Schumacher HR, Khan MA, et al. Genetic studies in familial ankylosing spondylitis susceptibility. Arthritis Rheum. 2004 7; 50(7): 2246–54 [PubMed: 15248224]
22. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum. 1984 4; 27(4): 361–8 [PubMed: 6231933]
23. Assassi S, Arnett FC, Reveille JD, Gourh P, Mayes MD. Clinical, immunologic, and genetic features of familial systemic sclerosis. Arthritis Rheum. 2007 6;56(6):2031–7. [PubMed: 17530643]
24. Lin Y, Wang J, Guo X, Espitia MG, Chen E, Assassi S, et al. Profiling of HLA-B Alleles for Association Studies with Ankylosing Spondylitis in the Chinese Population. Open Rheumatol J. 2013 8 19; 7:51–4 [PubMed: 24062861]
25. Payami H, Joe S, Farid NR, Stenszky V, Chan SH, Yeo PP, et al. Relative predispositional effects (RPEs) of marker alleles with disease: HLA-DR alleles and Graves disease. Am J Hum Genet. 1989 10;45(4):541–6. [PubMed: 2491013]
26. Mu H, King MC, Criswell LA. Relative predispositional effects and mode of inheritance of HLA-DRB1 alleles among community-based Caucasian females with rheumatoid arthritis. Genet Epidemiol. 1998;15(2):123–34 [PubMed: 9554551]
27. Joshi R, Reveille JD, Brown MA, Weisman MH, Ward MM, Gensler LS et al. Is there a higher genetic load of susceptibility loci in familial ankylosing spondylitis? Arthritis Care Res (Hoboken) 2012 5;64(5):780–4. [PubMed: 22231927]
28. Kawaguchi G, Kato N, Kashiwase K, Karaki S, Kohsaka T, Akaza et al. Structural analysis of HLA-B40 epitopes. Hum Immunol 1993 3;36(3):193–8 [PubMed: 7686546]
29. Espinoza LR, Vasey FB, Oh JH, Wilkinson R, Osterland CK. Association between HLA-BW38 and peripheral psoriatic arthritis. Arthritis Rheum 1978 1-2;21(1):72–5. [PubMed: 623697]
30. Goyette P, Boucher G Mallon D, Ellinghaus E, Jostins L, Huang H, et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet. 2015 2;47(2):172–9. [PubMed: 25559196]
31. Orchard TR, Thiyagaraja S, Welsh KI, Wordsworth BP, Hill Gaston JS, Jewell DP, et al. Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease. Gastroenterology. 2000 2;118(2):274–8. [PubMed: 10648455]
32. El Mouraghi I, Ouarour A, Ghozlani I, Collantes E, Solana R, El Maghraoui A. Polymorphisms of HLA-A, -B, -Cw and DRB1 antigens in Moroccan patients with ankylosing spondylitis and a comparison of clinical features with frequencies of HLA-B*27. Tissue Antigens. 2015 2;85(2): 108–16. [PubMed: 25626601]

33. Santos AM, Peña P, Avila M, Briceño I, Jaramillo C, Vargas-Alarcon G, et al. Association of human leukocyte A, B, and DR antigens in Colombian patients with diagnosis of spondyloarthritis. Clin Rheumatol. 2017 4;36(4):953–958 [PubMed: 28013432]

34. Londono J, Santos AM, Peña P, Calvo E, Espinosa LR, Reveille JD, et al. Analysis of HLA-B15 and HLA-B*27 in spondyloarthritis with peripheral and axial clinical patterns BMJ Open. 2015 11 11;5(11):e009092. doi: 10.1136/bmjopen-2015-009092.

35. Khan MA, Kushner I, Braun WE (1976) Letter: Low incidence of HLA-B*27 in American Blacks with spondyloarthropathies.Lancet 1976; 1 (7957):483

36. Robinson PC, Claushuis TA, Cortes A, Martin TM, Evans DM, Leo P, et al. Genetic dissection of acute anterior uveitis reveals similarities and differences in associations observed with ankylosing spondylitis. Arthritis Rheumatol.2015 1;67(1):140–51 [PubMed: 25200001]

What is already known about this subject?

Although the association of HLA-B27 with ankylosing spondylitis (AS) is well established, as is the association with B60 (B*40), those of other HLA alleles that have been reported in smaller studies have been inconsistent or unconfirmed.

The role of HLA alleles other than HLA-B27 have not been studied in African Americans

In a large recent study imputing HLA alleles in whites from our group, a number of new HLA associations have been reported.

What does this study add?

In this study, the largest to date in whites and Asians and the first to date in African Americans (other than HLA-B27) to employ direct HLA typing, a number of the HLA-associations we described by imputing HLA alleles in whites are confirmed not only in whites but also in Chinese and African American AS patients. A number of other HLA association not seen in the imputation analysis, including some seen in previous small studies, especially in the largest cohort of HLA-B27 negative AS patients reported to date, are seen with other HLA-B, DRB1, DQB1 and DPB1 alleles, some reported in earlier smaller studies and other novel associations (though the latter because of possible power or stratification issues will need confirmation). This study shows a role for not only HLA-class I alleles other that HLA-B27 but also class II HLA genes in predisposition to AS and subsets thereof, independent of linkage with HLA-B27.

How might this impact on clinical practice?

This study gives the clinician not only a better understanding of the relative inputs of HLA alleles other than HLA-B27 in predisposition to AS, but also better informs how to interpret HLA-B27 typing results in blacks.

Table 1.

Selected HLA Class I and II Associations with AS in Whites overall

Allele	Cases freq.%	Control freq.%	# Cases	# Controls	Odds Ratio	P value
A*01	8.6	13.9	1326	1134	0.58	3.44×10^{-5}
A*02	35.1	27.9	1326	1134	1.40	1.56×10^{-4}
A*03	8.2	16.3	1326	1134	0.50	$<1 \times 10^{-8}$
B*07:00	5.9	15.0	3896	1980	0.36	$<1 \times 10^{-8}$
B*08:00	11.5	20.9	3896	1980	0.49	$<1 \times 10^{-8}$
B*13:00	1.5	2.5	3896	1980	0.60	0.01
B* 14:00	2.9	2.9	3896	1980	1.00	1.00
B*15:00	3.3	7.1	3896	1980	0.45	$<1 \times 10^{-8}$
B*27:00	46.1	3.8	3896	1980	21.4	$<1 \times 10^{-8}$
B*35:00	4.2	8.7	3896	1980	0.46	$<1 \times 10^{-8}$
B*38:00	1.3	1.5	3896	1980	0.91	0.78
B*40 all	5.4	7.6	3896	1980	0.69	0.001
B*40:01	4.4	5.7	3896	1980	0.76	0.03
B*40:02	0.7	1.2	3896	1980	0.59	0.09
B*44:00	8.2	15.0	3896	1980	0.51	$<1 \times 10^{-8}$
B*51:00	2.7	3.8	3896	1980	0.70	0.03
B*52:00	0.9	0.9	3896	1980	1.02	0.95
B*57:00	1.3	3.7	3896	1980	0.34	$<1 \times 10^{-8}$
C*01:00	19.2	3.0	1302	1290	7.62	$<1 \times 10^{-8}$
C*06:00	4.5	9.8	1302	1290	0.43	2.1×10^{-7}
C*07:00	15.9	31.2	1302	1290	0.42	$<1 \times 10^{-8}$
DRB1*01:01	16.5	9.0	1580	1416	2.00	$<1 \times 10^{-8}$
DRB1*01:03	4.6	2.0	1580	1416	2.34	0.0001
DRB1*03:01	6.0	12.0	1580	1416	0.46	$<1 \times 10^{-8}$
DRB1*04:04	6.5	2.5	1580	1416	2.72	3.0×10^{-7}
DRB1*15:01	8.7	14.7	1580	1416	0.56	4.8×10^{-7}
DQB1*02:01	6.3	12.7	1580	1416	0.46	$<1 \times 10^{-9}$
DQB1*03:01	26.4	19.2	1580	1416	1.29	0.006
DQB1*03:02	14.3	9.2	1580	1416	1.65	2.0×10^{-5}
DQB1*05:01	19.9	12.6	1580	1416	1.71	1.2×10^{-7}
DQB1*06:02	9.2	15.1	1580	1416	0.57	$<1 \times 10^{-9}$
DPB1*02:01	12.8	10.3	1264	768	1.28	0.10
DPB1*03:01	11.1	8.1	1264	768	1.42	0.03
DPB1*04:01	42.5	44.3	1264	768	0.93	0.46
DPB1*04:02	12.7	14.2	1264	768	0.88	0.38

Table 2.

Associations in White HLA-B*27 Negative AS Patients

Allele	Case freq.%	Control freq.%	No. case	No. control	Odds Ratio	P value
A*01	14.5	14.2	110	1030	1.03	1.00
A*02	24.5	27.7	110	1030	0.85	0.56
A*03	10.0	16.6	110	1030	0.56	0.10
A*11	7.3	3.5	110	1030	2.17	0.09
A*24	12.7	8.8	110	1030	1.50	0.24
A*29	**9.1**	**3.5**	**110**	**1030**	**2.76**	**0.01**
B*07:00	**10.2**	**15.4**	**472**	1828	**0.62**	**0.005**
B*08:00	9.3	21.9	472	1828	0.78	0.17
B*13:00	2.5	2.7	472	1828	0.95	0.99
B*14:00	4.4	3.1	472	1828	1.45	0.20
B*38:00	**3.2**	**2.8**	**472**	1828	**2.04**	**0.04**
B*40 all	7.4	15.3	472	1828	0.90	0.70
B*40:01	5.3	8.1	472	1828	0.87	0.60
B*49:00	**2.5**	**1.1**	**472**	**1828**	**2.36**	**0.03**
B*51:00	4.7	3.8	472	1828	1.23	0.49
B*52:00	**2.8**	**1.0**	**472**	**1828**	**2.85**	**0.006**
B*57:00	**1.7**	**3.7**	**472**	**1828**	**0.45**	**0.04**
C*01:00	2.7	1.3	110	1146	2.11	0.43
C*02:00	0.9	2.2	110	1146	0.41	0.59
C*03:00	11.8	14.6	110	1146	0.79	0.52
C*04:00	12.7	11.9	110	1146	1.08	0.91
C*05:00	5.5	9.6	110	1146	0.54	0.21
C*06:00	7.3	10.5	110	1146	0.67	0.37
C*07:00	30.0	32.9	110	1146	0.87	0.61
DRB1*01:01	4.7	7.9	148	948	0.58	0.24
DRB1*01:03	1.4	1.8	148	948	0.75	0.97
DRB1*03:01	6.8	12.2	148	948	0.52	0.07
DRB1*04:04	4.7	2.3	148	948	2.09	0.16
DRB1*11:00	**17.6**	**9.6**	**148**	**948**	**2.01**	**0.005**
DRB1*15:01	**6.1**	**14.8**	**148**	**948**	**0.37**	**0.006**
DQB1*02:01	**6.8**	**12.7**	**148**	**948**	**0.50**	**0.05**
DQB1*03:01	26.4	19.2	148	948	1.51	0.06
DQB1*03:02	8.8	8.5	148	948	1.59	1.00
DQB1*05:01	10.2	11.4	148	948	0.74	0.40
DQB1*06:02	**6.8**	**15.1**	**148**	**948**	**0.41**	**0.009**
DPB1*01:01	2.9	4.9	102	678	0.59	0.54
DPB1*02:01	12.8	10.3	102	678	1.47	0.25
DPB1*03:01	**11.1**	**8.1**	**102**	**678**	**1.42**	**0.03**
DPB1*04:01	42.5	(44.3	102	678	0.93	0.46

Allele	Case freq.%	Control freq.%	No. case	No. control	Odds Ratio	P value
DPB1*04:02	12.7	14.1	102	678	0.88	0.38

Table 3.
HLA-B Allele Frequency Analysis in White AS Patients and Controls

*The "-co" nomenclature for B*27 and B40 indicates the grouping of all such 4-digit alleles into 2-digit only.

Allele	Univariable Model		Rel Predisp. Effects		Stratified Model			
					B*27(−)		B*27(+)	
	Odds Ratio	P value	Odds Ratio	P value	Odds Ratio	P value	Odds Ratio	P value
***07:00**	**0.35**	**$<1\times10^{-4}$**	**0.70**	**4×10^{-4}**	**0.54**	**$<1\times10^{-2}$**	n/a	n/a
***08:00**	**0.49**	**$<1\times10^{-4}$**	0.92	0.45	**0.69**	**0.05**	n/a	n/a
***13:00**	**0.60**	**0.01**	1.09	0.73	0.90	0.75	n/a	n/a
***14:00**	1.03	0.87	**1.84**	**2×10^{-4}**	1.59	0.08	n/a	n/a
***15:00**	**0.43**	**$<1\times10^{-4}$**	0.82	0.12	0.77	0.23	2.54	0.36
*18:00	0.77	0.11	1.35	0.07	1.56	0.07	n/a	n/a
27all	**21.48**	**4×10^{-287}**	n/a	n/a	n/a	n/a	n/a	n/a
***27:02**	**10.87**	**1×10^{-15}**	n/a	n/a	n/a	n/a	n/a	n/a
***27:04**	**13.62**	**3×10^{-4}**	n/a	n/a	n/a	n/a	n/a	n/a
***27:05**	**36.46**	**2×10^{-275}**	n/a	n/a	n/a	n/a	n/a	n/a
***35:00**	**0.47**	**$<1\times10^{-4}$**	0.86	0.20	1.05	0.80	0.47	0.09
***38:00**	0.92	0.74	**1.73**	**0.02**	**2.65**	**$<1\times10^{-2}$**	0.83	0.86
***39:00**	**0.47**	**$<1\times10^{-3}$**	0.90	0.73	1.23	0.53	0.36	0.17
40all	**0.71**	**0.001**	**1.32**	**0.02**	0.97	0.89	1.09	0.87
***40:01**	1.24	0.16	**1.41**	**0.008**	1.51	0.10	1.17	0.80
*40:02	0.88	0.69	0.98	0.94	1.62	0.32	n/a	n/a
***44:00**§	0.50	$<1\times10^{-4}$	1.03	0.92	1.04	0.82	0.34	**$<1\times10^{-2}$**
***49:00**§	0.61	0.07	1.14	0.75	**2.27**	**0.03**	**0.10**	**$<1\times10^{-4}$**
***50:00**	**0.29**	**$<1\times10^{-4}$**	0.68	0.23	0.86	0.74	n/a	n/a
***51:00**	**0.72**	**0.04**	1.28	0.12	1.27	0.35	1.03	0.97
***52:00**	1.02	0.95	**1.83**	**0.05**	**3.01**	**$<1\times10^{-2}$**	n/a	n/a
***57:00**	**0.34**	**$<1\times10^{-4}$**	**0.62**	**0.01**	**0.45**	**0.03**	0.34	0.08

n/a: odds ratio is not estimable due to frequencies of zero in contingency tables of AS (case/control) and each allele (−/+).

§ significant interaction effect found with B*27 (−/+)

Table 4.

HLA-B Allele Frequency Analysis in 442 Han Chinese AS Patients and 346 Controls

Allele	AS Cases	Controls	Univariable Model		Rel Predisp. Effects		Stratified Model			
	%	%	Odds Ratio	P value	Odds Ratio	P value	B*27(−)		B*27(+)	
							Odds Ratio	P value	Odds Ratio	P value
B*07:00	**0.1**	**1.9**	**0.06**	**6.0×10^{-4}**	**0.11**	**0.02**	**0.79**	**0.82**	n/a	n/a
B*08:00	0.2	0.7	0.31	0.16	0.57	0.78	n/a	n/a	n/a	n/a
B*13:00	4.9	12.1	0.38	**$<1\times10^{-4}$**	0.71	0.10	0.50	0.21	0.80	0.73
B*14:00	0.2	0.1	2.35	0.46	n/a	n/a	n/a	n/a	n/a	n/a
B*15:00	**7.7**	**17.3**	**0.40**	**$<1\times10^{-4}$**	0.78	0.16	0.70	0.49	0.70	0.50
B*18:00	0.2	0.1	1.57	0.71	n/a	n/a	n/a	n/a	n/a	n/a
B*27all	**47.6**	**3.8**	**23.3**	**$<1\times10^{-9}$**	n/a	n/a	n/a	n/a	n/a	n/a
B*27:04	**34.8**	**2.3**	**22.6**	**$<1\times10^{-9}$**	n/a	n/a	n/a	n/a	n/a	n/a
B*27:05	**10.9**	**1.3**	**9.24**	**$<1\times10^{-9}$**	n/a	n/a	n/a	n/a	n/a	n/a
B*27:15	**1.4**	**0**	**n/a**	**0.04**	n/a	n/a	n/a	n/a	n/a	n/a
B*35:00	**1.8**	**4.5**	**0.32**	**$<1\times10^{-3}$**	0.86	0.20	0.26	0.20	n/a	n/a
B*38:00	1.6	2.9	0.53	0.08	1.0	1.0	1.23	0.79	**0.23**	**0.03**
B*39:00	1.8	1.6	1.26	0.57	2.13	0.08	**5.12**	**0.01**	0.75	0.79
B*40all	10.5	12.9	0.86	0.38	**1.63**	**0.003**	1.90	0.10	6.04	0.08
B*40:01	6.5	8.9	0.84	0.4	**1.41**	**0.008**	2.21	0.06	3.23	0.26
B*40:02	1.5	2.5	0.67	0.3	0.98	0.94	1.40	0.66	n/a	n/a
B*44:00	1.2	2.3	0.61	0.22	1.00	1.00	2.53	0.17	0.50	0.52
B*46:00	**4.5**	**9.8**	**0.44**	**$<1\times10^{-3}$**	0.83	0.44	0.45	0.20	1.19	0.82
B*50:00	0.3	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
B*51:00	**4.4**	**8.8**	**0.47**	**$<1\times10^{-3}$**	0.91	0.75	0.89	0.81	2.18	0.45
B*52:00	1.7	2.8	0.61	0.15	1.14	0.84	1.09	0.91	n/a	n/a
B*57:00	0.6	0.6	0.98	0.97	1.81	0.58	2.64	0.39	n/a	n/a
B*58:00[§]	**3.9**	**7.2**	**0.52**	**0.01**	1.0	0.98	1.24	0.68	**0.32**	**0.03**

n/a: odds ratio is not estimable due to frequencies of zero in contingency tables of AS (case/control) and each allele (−/+).

[§]significant interaction effect found with B*27 (−/+)

Table 5.

Selected HLA Allele Frequencies in African American AS patients and Controls*

Allele	AS Patients (%)	2×N++	Controls (%)	2×N	Odds Ratio	P value**
B*07:00	3.7	134	7.8	490	0.46	0.15
B*08:00	2.2	134	6.1	490	0.35	0.12
B*14:00	1.5	134	2.9	490	0.52	0.56
B*15:00	10.5	134	11.0	490	0.94	0.97
B*27:00	**29.9**	**134**	**1.0**	**490**	**41.3**	**$<1 \times 10^{-8}$**
B*35:00	2.2	134	8.6	490	**0.24**	**0.02**
B*40:00	3.0	134	1.4	490	2.12	0.40
B*40:01	**3.0**	**134**	**0.4**	**490**	**7.51**	**0.03**
B*42:00	**3.7**	**134**	**10.3**	**490**	**0.34**	**0.03**
B*44:00	3.0	134	6.9	490	0.41	0.14
B*51:00	0.8	134	3.5	490	0.21	0.17
B*57:00	3.0	134	4.9	490	0.60	0.48
B*58:00	4.5	134	7.8	490	0.56	0.26
DRB1*01:01	7.7	130	4.6	482	1.74	0.23
DRBl*15:01/03	13.1	130	11.6	482	1.14	0.76
DRB1*03:01	3.1	130	6.2	482	0.48	0.24
DRB1*03:02	2.3	130	6.2	482	0.36	0.13
DRB1*04	10.0	130	6.0	482	1.74	0.16
DRB1*07:00	3.9	130	7.3	482	0.51	0.23
DRB1*08:00	**10.0**	**130**	**5.0**	**482**	**2.12**	**0.05**
DRB1*11	12.3	130	12.0	482	1.02	1.00
DRB1*13	21.5	130	19.1	482	1.16	0.61
DQB1*02:01	3.1	130	6.6)	482	0.45	0.19
DQB1*03:01	**27.7**	**130**	**19.5**	**482**	**1.58**	**0.05**
DQB1*03:02	7.7	130	4.8	482	1.66	0.28
DQB1*04:02	4.6	130	7.9	482	0.56	0.28
DQB1*05:01	17.7	130	11.6	482	1.64	0.09
DQB1*06:02	16.9	130	13.9	482	1.26	0.47
DPB1*01:01	16.2	130	26.5	132	0.53	0.06
DPB1*02:01	15.4	130	12.1	132	1.32	0.56
DPB1*03:01	9.2	130	7.6	132	1.24	0.80
DPB1*04:01	14.6	130	9.1	132	1.71	0.23
DPB1*04:02	7.7	130	8.3	132	0.92	1.00
DPB1*13:01	6.9	130	3.0	132	2.42	0.23
DPB1*17:01	6.9	130	11.2	132	0.59	0.32

** Unless stated, the p-values shown were multiplied by the number of alleles examined at each locus. Alleles with frequencies of <5% were not examined unless potential biologic relevance was perceived.

*** After removing the effect of B*27, HLA-B*40:01 occurred in 4 of 94 genotypes in patients compared to 7 of 450 genotypes in controls, p=0.157, OR=3.03

[+] These represent uncorrected p-values. These were no longer significant after applying Bonferroni's correction

[++] Two patients previously typed for HLA-B did not have DNA available for MHC class II typing

Table 6.

HLA-Class I and II Associations with AS in Uveitis in Whites AS Cases

Allele	AS with Uveitis %	AS w/o Uveitis %	No. AS w AAU	No. AS w/o AAU	Odds Ratio	P value
A*01	**5.7**	**9.6**	**334**	**984**	**0.57**	**0.04**
A*02	34.4	35.5	334	984	0.96	0.78
A*03	10.0	11.3	334	984	0.86	0.54
B*07:00	5.5	5.5	760	2018	1.01	1.00
B*08:00	**5.8**	**12.0**	**760**	**2018**	**0.43**	**5.1×10^{-4}**
B*13:00	1.8	1.3	760	2018	1.44	0.36
B*14:00	2.4	3.5	760	2018	0.68	0.18
B*27 all	48.6	44.9	760	2018	1.16	0.08
B*27:02	4.6	3.6	760	2018	1.29	0.28
B*27:05	42.8	39.6	760	2018	1.14	0.15
B*35:00	4.9	4.5	760	2018	1.10	0.72
B*38:00	1.3	1.9	760	2018	0.68	0.35
B*40 all	5.8	5.2	760	2018	1.12	0.61
B*40:01	4.3	4.3	760	2018	1.01	1.00
B*40:02	1.1	0.5	760	2018	2.03	0.20
B*44:00	6.7	7.6	760	2018	0.83	0.31
B*51:00	2.6	1.1	760	2018	1.12	0.61
B*57:00	1.1	1.4	760	2018	0.76	0.61
C*01:00	19.4	18.9	324	972	1.03	0.90
C*02:00	27.2	24.3	324	972	1.62	0.33
C*06:00	5.3	4.2	324	972	1.26	0.50
C*07:00	14.5	16.5	324	972	0.86	0.46
DRB1*01:01	17.2	16.1	430	1150	1.08	0.64
DRB1*01:03	4.2	4.5	430	1150	0.92	0.87
DRB1*03:01	4.9	6.4	430	1150	0.76	0.30
DRB1*04:04	13.1	12.0	430	1150	1.10	0.77
DRB1*08:01	12.3	5.9	430	1150	1.64	0.12
DRB1*15:01	**10.9**	**7.7**	**430**	**1150**	**1.48**	**0.05**
DQB1*02:01	4.9	6.9	430	1150	0.70	0.19
DQB1*03:02	15.1	13.8	430	1150	1.11	0.57
DQB1*05:01	19.8	19.7	430	1150	1.01	1.00
DQB1*06:02	10.9	8.4	430	1150	1.35	0.14
DPB1*01:01	2.8	3.3	322	940	0.84	0.79
DPB1*02:01	12.4	13.0	322	940	0.95	0.87
DPB1*03:01	11.5	11.0	322	940	1.06	0.87
DPB1*04:01	41.3	43.2	322	940	0.93	0.60
DPB1*04:02	12.4	12.9	322	940	0.96	0.91