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Genetic Dissection of Acute Anterior Uveitis Reveals Similarities and Differences in Associations Observed With Ankylosing Spondylitis

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Objective. **To use high-density genotyping to investigate the genetic associations of acute anterior uveitis (AAU) in patients with and those without ankylosing spondylitis (AS).**

Methods. **We genotyped samples from 1,711 patients with AAU (either primary or combined with AS), 2,339 AS patients without AAU, and 10,000 control subjects on an Illumina Immunochip Infinium microarray. We also used data for AS patients from previous genome-wide association studies to investigate the AS risk locus *ANTXR2* for its putative effect in AAU. *ANTXR2* expression in mouse eyes was investigated by**

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real-time quantitative reverse transcription–polymerase chain reaction.

***Results*. A comparison between all patients with AAU and healthy control subjects showed strong association over *HLA–B*, corresponding to the *HLA–B27* tag single-nucleotide polymorphism rs116488202. The association of 3 non–major histocompatibility complex loci, *IL23R*, the intergenic region 2p15, and *ERAP1*, reached genome-wide significance ($P < 5 \times 10^{-8}$). Five loci harboring the immune-related genes *IL10–IL19*, *IL18R1–IL1R1*, *IL6R*, the chromosome 1q32 locus harboring *KIF21B*, as well as the eye-related gene *EYS*, were also associated, reaching a suggestive level of significance ($P < 5 \times 10^{-6}$). Several previously confirmed AS associations demonstrated significant differences in effect size between AS patients with AAU and AS patients without AAU. *ANTXR2* expression varied across eye compartments.**

***Conclusion*. These findings of both novel AAU-specific associations and associations shared with AS demonstrate overlapping but also distinct genetic susceptibility loci for AAU and AS. The associations in *IL10* and *IL18R1* are shared with inflammatory bowel disease, suggesting common etiologic pathways.**

Acute anterior uveitis (AAU) has a cumulative incidence rate in the Caucasian general population of 0.2%; however, among those who are *HLA–B27* positive (8–10% of the Caucasian population), the cumulative incidence rate is 1% (1). Recurrent AAU may lead to glaucoma, cataract development, and significant visual loss. Uveitis is a major cause of eye disease, affecting an estimated 2 million Americans, and accounts for up to 10% of blindness (2,3).

Evidence from both humans and animal models suggests a large genetic component to uveitis, and strong familiality has been demonstrated (4). The risk of recurrence in first-degree relatives is 6% compared with a population prevalence of only 0.038–0.38% (5). AAU occurs in 30–40% of patients with ankylosing spondylitis (AS), suggesting a shared etiology (6). AAU is strongly associated with *HLA–B27*, both in those with AS and those without AS, and >50% of patients with primary AAU are *HLA–B27* positive (7). A previous study demonstrated evidence that genes other than *HLA–B* influence the risk of developing AAU. The prevalence of AAU in the *HLA–B27*–positive first-degree relatives of probands with AAU (13%) is much higher than that in the normal *HLA–B27*–positive (Dutch) population (1%), indicating that genetic factors other than *HLA–B27* are involved (5). In the same study, 11% of *HLA–B27*–positive first-degree relatives older than age 45 years had AS compared with an expected frequency of AS in *HLA–B27* carriers of ~1%, highlighting the strong cofamiliality of AS and AAU.

Other genetic associations described for AAU include *HLA–A*02* (8), *HLA–DRB1*08:03* (9), *HLA–B*58* (10), *MICA* (11), *LMP2* (12), *CYP27B1* (13), *IL10* (14), the complement components *CFB*, *CFH*, and *C2* (15,16), *TNF* (17,18), and the killer cell immunoglobulin-like receptor region (19), and suggestive linkage to the chromosome 9p region has been reported (20). No findings have achieved genome-wide significance ($P < 5 \times 10^{-8}$), and few associations have been replicated (6). Few of these studies were adequately powered to reliably identify genes involved in AAU; therefore, we sought to investigate its association in the largest data set assembled for this purpose to date.

PATIENTS AND METHODS

To identify AAU genetic associations, 2 main analyses were performed. AS patients with AAU (cases) were first compared with AS patients without AAU (controls). Although this analysis studied AAU genetic associations while controlling for AS comorbidity, potential issues such as delayed onset of uveitis and subclinical disease affected it. Therefore, a second analysis compared all AAU patients with healthy controls, and a subsequent heterogeneity test was performed to assess whether associated single-nucleotide polymorphisms (SNPs) had differing effect sizes in AS patients with uveitis and those without uveitis. Genetic associations that were identified by comparing AS patients with AAU and healthy control subjects that were not identified by the larger and better-

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powered International Genetics of Ankylosing Spondylitis Consortium (IGAS) Immunochip study in patients with AS (21) are likely to be AAU-specific associations.

Collection of samples for Immunochip analysis and phenotyping. Patients with AS (as defined by the modified New York criteria [22]) who were of European descent, either with AAU (n = 1,422) or without AAU (n = 2,339), were recruited. Ophthalmologists also collected samples from 289 patients with AAU (in whom the AS status was unknown). The patients with AS had either self-reported or ophthalmologist-diagnosed AAU (see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). Patients were positively selected on the basis of their phenotype, and no exclusions were applied. Historical genotypes from 10,000 Caucasian control subjects from the 1958 British Birth Cohort and the UK National Blood Transfusion Service were used as common controls. All patients gave informed consent, and ethics approval was obtained from all relevant institutional ethics committees.

After quality control procedures were performed (see Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract), 9,564 control subjects, 1,199 AS patients with AAU, 238 patients with AAU alone, and 1,731 AS patients without AAU remained for analysis.

Samples were genotyped on the Illumina Immunochip microarray. Intensity data were processed and normalized using Illumina GenomeStudio software and subsequently clustered using optiCall (23).

Quality control. The thresholds used were a genotyping missingness rate of 0.03, a between-center missingness threshold of 1×10^{-7}, an individual missingness rate of 0.03, and a Hardy-Weinberg equilibrium threshold in control subjects of 1×10^{-7}. Heterozygosity versus missingness outliers of >3 SD were excluded. An identity by descent (IBD) threshold of PI_HAT (proportion [ibd = 2] + 0.5 [ibd = 1]) 0.20 was used. Principal components were then computed using shellfish software (http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php) including the HapMap populations. Individuals identified as non-European by model-based unsupervised clustering implemented in R by the mclust package were excluded. Details of the excluded SNPs are shown in Supplementary Table 3 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract).

Association analysis. A case–control analysis was performed with SNPTEST version 2.5 beta, using the "expected" method and including 10 eigenvectors. Scree plots are shown in Supplementary Figure 1 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). Genomic inflation factor (λ) 1,000 values for the Immunochip control SNPs were 1.058 for the analysis of AAU patients versus healthy controls and 1.035 for the analysis of AS patients with AAU versus AS patients without AAU. The Q-Q plots for the studies are shown in Supplementary Figure 2 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). *P* values less than 5×10^{-8} were considered significant (genome-wide significance), and *P* values greater than 5×10^{-8} but less than 5×10^{-6} were considered suggestive. After conditional analyses, analyses with significant results ($P < 1 \times 10^{-4}$) were reported.

Imputation. Data were phased with SHAPEIT version 2 software (24) and imputed with IMPUTE2 (25) using the 1,000 Genomes phase 1 integrated variant reference set. A post-imputation quality control threshold of 0.8 "info" score from IMPUTE2 was used. Classic major histocompatibility complex (MHC) alleles were imputed from the genotype data with the computational strategy SNP2HLA (26) against the supplied type 1 diabetes reference set. The carriage rate of *HLA–B27* was calculated using SNP2HLA-imputed doses and a dose threshold of 0.6.

Tag SNP calculation. The imputed genotypes of each individual and their classic alleles imputed by SNP2HLA were used to calculate the sensitivity and specificity of the SNPs for tagging classic alleles.

Interaction analysis. Interaction analysis was performed using the formula:

$$\log it(\psi) = \beta_0 + \beta_{SNP}SNP + \beta_{B27}B27 + \sum_{i=1}^{10}\beta_i \times PC_i + \beta_{int}SNP \times B27$$

where the uveitis phenotype ψ and the imputed SNP dose of rs2032890 ($\beta_{SNP}SNP$, lead *ERAP1*-associated SNP) and imputed SNP2HLA dose of the *HLA–B27* ($\beta_{B27}B27$) and 10 eigenvectors ($\Sigma\beta_i \times PC_i$) were regressed with an interaction term between the SNP and HLA–B27 ($\beta_{int}SNP \times B27$). β_0 is the intercept.

Comparison of SNP effects between AS patients with and those without AAU. The healthy control subjects were categorized into 2 groups by random sampling and allocated as controls to either the group of AS patients with AAU or the group of AS patients without AAU, to ensure the independence of the 2 regressions. These 2 sets of patients and controls were then allocated a dummy variable of 1 or 0, coded as Z. The following regression model was then used:

$$\log it(\psi) = \beta_0 + \beta_{snp}SNP + \sum_{i=1}^{10}\beta_i \times PC_i + \beta_Z Z + \beta_{Z2}SNP \times Z$$

where ψ is the uveitis phenotype (either 1 = uveitis in the "AS with AAU" cases or 1 = no uveitis in the "AS without AAU" cases; controls in both sets were coded as 0). β_{snp} is the regression coefficient for the SNP dose, and SNP is the dose of the SNP genotype. $\sum_{i=1}^{10}\beta_i$ are the regression coefficients of the 10 principal components, PC_i from 1 to 10 are the 10 principal components. β_Z is the regression coefficient for the dummy variable Z. β_{Z2} is the regression coefficient for the interaction term between the dummy variable and the SNP dose: SNP × Z. β_0 is the intercept. We also analyzed the model with *HLA–B27* as a component, as follows:

$$\log it(\psi) = \beta_0 + \beta_{snp}SNP + \beta_{B27}B27 + \sum_{i=1}^{10}\beta_i \times PC_i + \beta_Z Z + \beta_{Z2}SNP \times Z$$

In this model, $\beta_{B27}B27$ is the SNP2HLA-imputed dose of *HLA–B27*.

AS genome-wide association study (GWAS) data for *ANTXR2* analysis. The post–quality control data from the Australo-Anglo-American Spondylitis Consortium (TASC) and Wellcome Trust Case Control Consortium 2 (WTCCC2) studies and an unpublished Canadian AS GWAS were subjected to the same quality control process as that used for the Immunochip data in this study. The Canadian study used the Illumina OmniExpress microarray and included 189 patients with AS. Briefly, the WTCCC2–TASC study genotyped 3,023 patients and 8,779 controls (27). The TASC study examined 2,951 AS patients and 6,658 healthy controls; 439 patients were removed during the quality control process (28).

ANTXR2 locus imputation was completed with SHAPEIT and IMPUTE2, identical to the Immunochip data. The MHC SNPs were used to impute classic alleles, using SNP2HLA to determine the *HLA–B27* dose (range 0–2).

Previously reported AAU genetic associations. When the exact SNP was not present on the Immunochip microarray, the SNP with the highest linkage disequilibrium (LD) represented on the Immunochip was determined, based on 1,000 Genomes data.

Concordance of the directions of effect. To assess whether shared associations had the same or opposite directions of effect, the in-phase alleles were calculated using Haploxt (http://genome.sph.umich.edu/wiki/Haploxt) and 1,000 Genomes reference data.

HLA–B27 heterozygosity and homozygosity calculations. To calculate the odds for AAU based on homozygosity and heterozygosity for *HLA–B27*, the SNP2HLA-imputed doses of *HLA–B27* in the AAU group versus healthy controls were used. Contingency table analysis and the cross-products ratios were used.

RNA isolation for the assessment of *ANTXR2* expression. For each experiment, total RNA was isolated from a 25–50-mg piece of mouse lung or the dissected components pooled from 5 mouse eyes, using a PureLink RNA kit (Ambion). The eyes were dissected to isolate the following components: 1) cornea, 2) iris including ciliary body, 3) choroid and sclera, and 4) retina. Briefly, tissue samples were collected, placed directly into lysis buffer containing β-mercaptoethanol, and homogenized using a TissueLyser II (Qiagen). The lysates were then put through PureLink RNA columns, treated with DNase I, and eluted in RNase-free water. The purity and quantity of RNA were assessed with an Eppendorf BioPhotometer.

Determination of *ANTXR2* expression by real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR). The relative expression of *ANTXR1* and *ANTXR2* messenger RNA (mRNA) was determined using a 2-step real-time PCR assay; the expression of *ANTXR1* and *ANTXR2* messenger RNA was compared with that of ribosomal protein L32 mRNA. First, complementary DNA (cDNA) was generated from 2 μg of total RNA, using random primers and Moloney murine leukemia virus reverse transcriptase (Promega). Second, cDNA samples were used in the RT-PCR using Bio-Rad SsoAdvanced Universal SYBR Green Supermix and run on a Bio-Rad CFX Connect system. The primer sequences used for *ANTXR1* and *ANTXR2* were obtained from Harvard PrimerBank (for *ANTXR1*, PrimerBank ID 32189436a1; for *ANTXR2*, PrimerBank ID 13278124a1). The primer sequences for L32 were as follows: forward 5′-CATCGGTTATGGGAGCAAC-3′ and reverse 5′-GCACACAAGCCATCTACTCAT-3′. Samples were run in triplicate, and the assay was repeated 3 times. The amounts of *ANTXR1* and *ANTXR2* mRNA in the lung and various eye compartments were normalized relative to L32 mRNA, because lung has been shown to express both *ANTXR1* and *ANTXR2* (29).

RESULTS

To examine the validity of the self-reported diagnosis of AAU, we calculated *HLA–B27* carriage rates. The frequency of *HLA–B27*, as inferred from SNP2HLA imputation, was 81.8% (613 of 749 patients) in the group with ophthalmologist-diagnosed AAU and 92.0% (633 of 688 patients) in the group with self-reported AAU. Of note, the group with self-reported AAU was composed entirely of AAU patients with AS, whereas the group with ophthalmologist-diagnosed AAU included AAU patients with AS and AAU patients without AS.

Associations with the MHC. Because AAU has previously been linked with classic alleles, we examined the MHC for association with AAU. In the comparison of AS patients with AAU versus AS patients without AAU, SNPs in the MHC class I region harboring *HLA–B* were strongly associated with AAU at rs115879499 ($P = 4.9 \times 10^{-18}$, odds ratio [OR] 1.4 [95% confidence interval (95% CI) 1.2–1.5]) (see Supplementary Figure 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). Conditioning on this SNP showed association at rs9274411 in *HLA–DQB1* ($P = 6.4 \times 10^{-5}$, OR 1.2 [95% CI 1.1–1.3]). Conditioning on these top 2 associations showed no further association ($P > 1 \times 10^{-4}$). When *HLA–B27*–negative subjects (as determined by SNP2HLA classic MHC allele imputation) were analyzed separately, there was moderate association at rs114199502 ($P = 2.6 \times 10^{-5}$, OR 2.9 [95% CI 1.3–6.6]) between *HLA–DRA* and *HLA–DRB5*. After controlling for the effect of rs114199502, there was moderate association at the intronic variant rs71542449 in *HLA–DQB2* ($P = 4.7 \times 10^{-5}$, OR 0.57 [95% CI 0.39–0.84]).

In the analysis of all patients with AAU versus healthy controls, strong association was also seen in the MHC, and the lead SNP was the previously described (21) *HLA–B27* tag SNP rs116488202 ($P < 1 \times 10^{-300}$, OR 16.8 [95% CI 15.0–18.7]). After conditioning on this SNP, the next most-associated SNP was rs114102658, between *HLA–B* and *MICA* (see Figure 1) ($P = 1.2 \times$

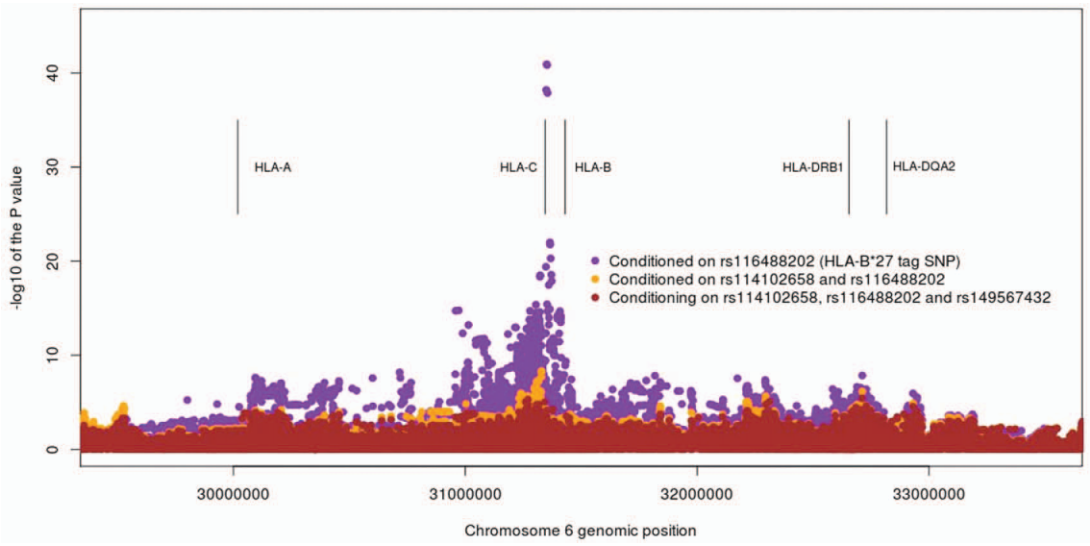

Figure 1. Associations between acute anterior uveitis (AAU) and the major histocompatibility complex in the analysis of all patients with AAU versus healthy control subjects. SNP = single-nucleotide polymorphism.

10^{-41}, OR 17.4 [95% CI 16.7–19.4]). After conditioning on both rs114102658 and rs116488202, lead association was seen with rs149567432, just centromeric to *HLA–B* ($P = 4.9 \times 10^{-9}$, OR 9.0 [95% CI 8.2–9.9]). None of these SNPs tagged any classic class I allele accurately (all sensitivities and specificities <70%). Conditioning on these top 3 signals showed association with rs114977878 in the MHC class II locus in *HLA–DQA2* ($P = 3.8 \times 10^{-6}$, OR 1.4 [95% CI 1.3–1.6]). Conditioning on these 4 SNPs revealed association at rs115711695, an intronic variant in *HLA–DRB5* ($P = 6.2 \times 10^{-6}$, OR 1.7 [95% CI 1.5–1.9]). After conditioning on these top 5 SNPs, association in the *HLA–B* locus at rs114560492 was still observed ($P = 3.1 \times 10^{-5}$, OR 1.9 [95% CI 1.7–2.0]). Conditioning on these top 6 associations left no further residual signals ($P > 1 \times 10^{-4}$).

Because some patients with AS do not carry *HLA–B27*, we examined MHC associations in this group by analyzing *HLA–B27*–negative patients, as assessed by any imputed dose of SNP2HLA-imputed *HLA–B27*. In the analysis of all patients with AAU versus healthy controls, there was association at the *HLA–B* locus at rs115937001 ($P = 2.0 \times 10^{-5}$, OR 1.8 [95% CI 1.5–2.3]). This SNP tagged *HLA–B0801* with 76% specificity and 94% sensitivity. Conditioning on this SNP left no residual association ($P > 10^{-4}$).

Allelic diversity at the classic HLA loci is extensive and is challenging to impute with single SNPs. Therefore, imputation with multiple SNPs was performed with SNP2HLA, to provide a potentially more accurate assessment of the associations between classic MHC alleles and disease (26). In the analysis of AS patients with AAU versus AS patients without AAU, the classic allele *HLA–B27* was again strongly associated ($P = 1.4 \times 10^{-16}$, OR 2.1 [95% CI 1.8–2.5]). Controlling for *HLA–B27* effect showed residual association with *HLA–DQB1:05* ($P = 2.1 \times 10^{-5}$, OR 0.78 [95% CI 0.70–0.87]). In the analysis of all patients with AAU versus healthy controls, strong association with *HLA–B27* was evident ($P < 1 \times 10^{-300}$, OR 59.7 [95% CI 51.4–69.5]). After controlling for the *HLA–B27* effect, *HLA–DRB1:0103* was associated ($P = 2.0 \times 10^{-5}$, OR 1.9 [95% CI 1.4–2.5]).

The question of whether *HLA–B27* exerts its influence through a dominant or additive genetic model was assessed. In the analysis of all patients with AAU versus healthy control subjects, heterozygosity for *HLA–B27* (as determined by SNP2HLA imputation) conferred an OR for AAU of 66.8 (95% CI 66.7–67.0), homozygosity for *HLA–B27* conferred an OR of 130.6 (95% CI 130.1–131.1), and the risk of 2 *HLA–B27* alleles over 1 *HLA–B27* allele conferred an OR of 2.0 (95% CI 1.5–2.4). Thus, homozygosity for *HLA–B27* does confer an additional risk of AAU compared with heterozygosity (see Supplementary Table 4, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract).

The carriage rates for *HLA–B27* (as determined by SNP2HLA imputation) were 91.2% in AS patients with AAU, 80.6% in AS patients without AAU, and only

63.9% in the small cohort of AAU patients with unknown AS status (recruited by ophthalmologists).

Non-MHC associations. Because several non-MHC associations with AAU were previously described, we sought to examine non-MHC areas for association. In the comparison of AS patients with AAU versus AS patients without AAU, association was observed with variants within *ERAP1* (rs2032890; $P = 9.0 \times 10^{-6}$, OR 1.3 [95% CI 1.2–1.5]) (see Supplementary Table 5 and Supplementary Figure 4, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). In AS, there is an interaction between *ERAP1* and *HLA–B27* (27). After controlling for *HLA–B27* using SNP2HLA doses (21) as a covariate in the analysis, association at rs2032890 was essentially unchanged ($P = 2.9 \times 10^{-5}$, OR 1.3 [95% CI 1.2–1.5]). A regression analysis with an interaction term between *HLA–B27* (as determined by SNP2HLA) and rs2032890 was negative ($P = 0.28$). However, when subjects were split into *HLA–B27*–negative and *HLA–B27*–positive groups and the rs2032890 SNP was assessed by logistic regression in each group, association was observed in the *HLA–B27*–positive group but not in the *HLA–B27*–negative group ($P = 1.6 \times 10^{-5}$, OR 1.26 [95% CI 1.13–1.40] and $P = 0.76$, OR 1.05 [95% CI 0.78–1.42], respectively).

In the analysis of all AAU patients versus healthy controls, genome-wide significant associations were observed at *ERAP1*, the intergenic region chromosome 2p15, and *IL23R*. The observed associations were concordant with associations with AS, involving the same haplotypes with the same direction of effect (Table 1) (see also Supplementary Figures 5–12, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). In this study, the logistic regression model examining the interaction between the *ERAP1* SNP rs2032890 and *HLA–B27* was strongly significant ($P < 2 \times 10^{-16}$). In *HLA–B27*–negative participants (as determined by SNP2HLA imputation), there was association over *ERAP2* (lead SNP rs4869314; $P = 8.8 \times 10^{-5}$), consistent with that previously observed in *HLA–B27*–negative patients with AS. After conditioning on this SNP, no residual association was seen in *ERAP2* ($P > 10^{-4}$).

In the analysis of all AAU patients and healthy controls, 5 loci had suggestive levels of association ($P < 5 \times 10^{-6}$). These loci included both of those known to be associated with AS (*IL6R*, chromosome 1q32) and novel loci not previously reported to be AS-associated, including *IL10–IL19*, the gene encoding the α chain of interleukin-18 receptor (IL-18R) (*IL18R1–IL1R1*), and *EYS* (eyes shut Drosophila homolog, a gene associated with retinitis pigmentosa [30,31]). The *IL6R* and chromosome 1q32 AAU associations were concordant with the AS associations (alleles are in phase; $r^2 = 0.98$ and $r^2 = 1.0$, respectively). After conditioning on the 1q32 association (rs12132349), a secondary association nearby at rs10920074 became apparent ($P = 1.7 \times 10^{-7}$, OR 1.3 [95% CI 1.2–1.4]); both of these SNPs were found to be in linkage disequilibrium (LD) ($D' = 0.87$, $r^2 = 0.48$ [source: 1,000 Genomes]). No further association was apparent after conditioning on both rs10920074 and rs12132349 ($P > 1 \times 10^{-4}$). At the *IL18R1–IL1R1* association, conditioning on rs10197284 showed a secondary signal at rs6750020 ($P = 1.2 \times 10^{-4}$, OR 1.2 [95% CI 1.1–1.4]); these SNPs were in LD ($D' = 0.92$, $r^2 = 0.85$). Conditioning on rs6750020 in addition to rs10197284 removed all association ($P > 10^{-4}$). The association between *IL18R1–IL1R1* SNP rs10197284 and AAU was not in LD with the previously reported suggestive AS association at rs4851529 ($D' = 0.07$, $r^2 =$

Table 1. Results of the association analyses of all patients with AAU versus healthy controls*

rs ID no.	Chr.	Position†	*P*	Risk allele	Protective allele	RAF, patients/ controls	OR	95% CI	Nearby genes	Also associated with AS
rs2032890	5q15	96,121,152	2.11×10^{-16}	A	C	0.77/0.69	1.51	1.37–1.66	*ERAP1/ERAP2/LNPEP*	Yes
rs4672507	2p15	62,570,573	2.05×10^{-12}	T	A	0.43/0.36	1.38	1.27–1.50	Intergenic	Yes
rs79755370	1p31	67,699,915	1.27×10^{-8}	C	A	0.96/0.94	1.80	1.45–2.23	*IL23R*	Yes
rs12132349	1q32	200,875,242	1.57×10^{-7}	T	A	0.76/0.71	1.31	1.19–1.44	*KIF21B–C1orf106*	Yes
rs6690230	1q21	154,432,877	1.09×10^{-6}	C	G	0.41/0.36	1.23	1.13–1.34	*IL6R*	Yes
rs17351243	1q32	206,959,527	1.46×10^{-6}	A	G	0.51/0.45	1.24	1.14–1.35	*IL10–IL19*	No
rs10197284	2q12	102,982,703	1.67×10^{-6}	G	A	0.26/0.22	1.25	1.14–1.38	*IL18R1–IL1R1*	No
rs665873	6q12	65,298,551	4.99×10^{-6}	A	G	0.98/0.97	2.03	1.48–2.79	*EYS*	No

* AAU = acute anterior uveitis; Chr. = chromosome; RAF = risk allele frequency; OR = odds ratio; 95% CI = 95% confidence interval; AS = ankylosing spondylitis.
† UCSC human genome build 19.

Table 2. Results of heterogeneity analyses in AS patients with AAU versus healthy controls and in AS patients without AAU versus healthy controls*

SNP	Gene/ region	SNP effects, model 1, SNP + principle components				SNP effects, model 2, SNP + HLA–B27 + principle components				*P* for interaction	
		AS with AAU		AS without AAU		AS with AAU		AS without AAU			
		P	OR	*P*	OR	*P*	OR	*P*	OR	Model 1	Model 2
rs2283790	*UBE2LE*	8.70×10^{-1}	1.001	7.00×10^{-3}	0.98	8.90×10^{-1}	0.999	1.20×10^{-1}	0.992	4.90×10^{-2}	2.90×10^{-1}
rs2836883	21q22	5.90×10^{-3}	1.02	3.00×10^{-2}	1.03	2.60×10^{-1}	1.005	1.70×10^{-4}	1.019	1.90×10^{-1}	4.00×10^{-2}
rs2032890	*ERAP1*	1.80×10^{-17}	1.06	3.30×10^{-4}	1.03	2.00×10^{-2}	1.02	2.00×10^{-2}	1.01	1.00×10^{-3}	8.00×10^{-2}
rs30187	*ERAP1*	4.80×10^{-19}	1.06	1.04×10^{-7}	1.04	2.00×10^{-2}	1.02	3.00×10^{-3}	1.02	2.00×10^{-2}	2.40×10^{-1}
rs10045403	*ERAP1*	1.10×10^{-14}	1.05	5.30×10^{-4}	1.02	3.60×10^{-4}	1.02	3.00×10^{-2}	0.01	5.00×10^{-3}	5.00×10^{-1}
rs2910686	*ERAP2*	1.80×10^{-1}	1.01	3.00×10^{-2}	0.99	7.10×10^{-1}	1.00	1.40×10^{-1}	0.99	1.00×10^{-2}	4.30×10^{-1}
rs6759298	2p15	8.50×10^{-16}	1.06	1.70×10^{-22}	1.07	2.00×10^{-6}	1.02	9.70×10^{-12}	1.04	1.30×10^{-1}	5.00×10^{-2}
rs4333130	*ANTXR2*	4.80×10^{-2}	0.98	3.00×10^{-4}	0.97	7.70×10^{-1}	1.002	5.60×10^{-3}	0.98	2.80×10^{-1}	4.00×10^{-2}
rs7282490†	*ICOSLG*	9.80×10^{-1}	1.00	7.00×10^{-3}	1.02	4.10×10^{-1}	1.004	1.00×10^{-2}	1.013	5.40×10^{-2}	1.70×10^{-1}
rs665873†	*EYS*	6.10×10^{-5}	0.92	1.40×10^{-1}	0.97	2.00×10^{-3}	0.96	2.00×10^{-1}	0.98	9.00×10^{-2}	3.00×10^{-1}
rs4672507†	2p15	4.00×10^{-16}	1.06	2.90×10^{-22}	1.07	2.60×10^{-7}	1.02	1.10×10^{-12}	1.04	1.70×10^{-1}	6.00×10^{-2}

* AS = ankylosing spondylitis; AAU = acute anterior uveitis; SNP =single-nucleotide polymorphism; OR = odds ratio.
† Suggestive.

0.00) (21). After conditioning on the top SNP, the other loci had no secondary signals ($P > 1 \times 10^{-4}$).

The *IL10* SNP rs17351243 is in LD ($D' = 0.65$, $r^2 = 0.52$ [source: 1,000 Genomes) with the inflammatory bowel disease (IBD)–associated SNP rs3024505 (32), and the direction of association is the same. The *IL18R1–IL1R1* SNP rs10197284 is in strong LD with SNPs previously reported to be associated with celiac disease (33) and IBD (32) and in lesser LD with those associated with asthma (34) and eosinophil counts (35) (see Supplementary Table 6, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). The IBD and celiac disease associations share the same direction of effect, but the SNPs associated with asthma and eosinophil counts have opposite directions of effect. The *EYS* SNP rs665873 has low frequency (HapMap CEU minor allele frequency [MAF] = 0.051) but is in tight LD ($D' = 0.91$, $r^2 = 0.01$; source 1,000 Genomes) with the common (MAF = 0.43) SNP associated with statin-induced myopathy, rs3857532 (36), and with 6 SNPs associated with retinitis pigmentosa (30) ($D' = 1$, $r^2 = 0.001$) (see Supplementary Table 7, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract). The SNPs associated with AAU, statin-induced myopathy, and retinitis pigmentosa all had the same direction of effect.

Comparisons of odds ratios (ORs). We performed heterogeneity testing to assess whether the shared associations influence AS and AAU with differing magnitudes. All SNPs associated with AAU ($P < 5 \times 10^{-6}$) in the analysis of all AAU patients versus healthy controls and all SNPs associated with AS from the recently published IGAS Immunochip study (21) were assessed, using 2 models. The first (model 1) included the SNP and principal components; the second (model 2) included *HLA–B27* dose as a model covariate, reflecting the previously demonstrated interaction between *ERAP1* SNPs and *HLA–B27* in AS.

Both of the AS-associated intergenic loci, chromosomes 21q22 and 2p15, had significantly different effect sizes in the analysis of AS patients with AAU versus healthy controls compared with AS patients without AAU versus healthy controls in model 2 but not model 1 (Table 2). At chromosome 2p15, both models showed a larger effect size in AS patients without AAU versus healthy controls compared with AS patients with AAU versus healthy controls, but this reached statistical significance only in model 2 (for SNP rs4672507 and SNP rs6759298, $P = 0.17$ and $P = 0.13$, respectively, in model 1, and $P = 0.06$ and $P = 0.05$, respectively, in model 2). The lead AAU-associated *ERAP1* SNP had a significantly stronger effect size in the analysis of AS patients with AAU versus healthy controls compared with that in the analysis of AS patients without AAU versus healthy controls, even after taking into account *HLA–B27* (model 2). The *EYS* SNP rs665873 had a much greater effect size (OR 0.92 [95% CI 0.88–0.97], $P = 6 \times 10^{-5}$) in AS patients with AAU compared with AS patients without AAU (OR 0.97 [95% CI 0.93–1.01], $P = 0.14$).

The effect size of AS-associated *ANTXR2* SNP rs4389526 was significantly different in AS patients with AAU compared with AS patients without AAU in

model 2 ($P = 0.04$). SNP rs4389526 had an effect in AS patients without AAU ($P = 5.6 \times 10^{-3}$, OR 0.98 [95% CI 0.97–0.99]) but no effect in AS patients with AAU ($P = 0.77$, OR 1.00 [95% CI 0.99–1.02]).

Analysis of *ANTXR2* expression in the eye using real-time qRT-PCR. Given the negative association between *ANTXR2* SNP rs4389526 and AS patients with AAU, we examined the expression of *ANTXR2* and the related *ANTXR1* (for comparison) in the eye using a murine model. *ANTXR1* and *ANTXR2* mRNA was detected in all of the tested eye compartments, as well the lung (Figure 2). *ANTXR1* expression was equivalent in the cornea, iris/ciliary body, and choroid/sclera and slightly lower in the retina (Figure 2A). In contrast, *ANTXR2* was expressed most abundantly in the iris/ciliary body, retina, and choroid/sclera compared with the cornea, where expression of *ANTXR2* was minimal (Figure 2B).

Previously identified AAU associations. Several non-MHC associations with AAU have been reported previously. Therefore, we sought to examine whether there was evidence to support these reported associations. Although not all previously reported AAU genetic associations were on the Immunochip microarray, we were able to investigate a number of regions previously reported to be associated with AAU. Of the previously reported AAU associations within the MHC, all were strongly associated in the analysis of all patients with AAU versus healthy controls (Table 3). After controlling for the lead MHC SNP (rs116488202), no residual association was observed for the *TNF* SNPs previously reported to be associated with AAU, although nominal association was seen for *TNF-238* (rs1800629; $P = 0.0029$). This association disappeared after conditioning on the next 2 associated HLA class I SNPs rs114102658 and rs149567432 ($P = 0.08$). Outside the MHC, nominally significant associations were observed with SNPs previously reported to be associated with AAU in *IL10* (rs6703630; $P = 0.038$) and *CYP27B1* (rs703842; $P = 0.0027$). As mentioned above, the association with *IL10* SNP rs17351243 was much stronger in the current study; this SNP was in moderately strong LD with *IL10* SNP rs6703630 ($D' = 0.87$, $r^2 = 0.24$ versus $D' = 0.71$, $r^2 = 0.09$ according to 1,000 Genomes) (14). This suggests that rs17351243 and rs6703630 may tag the same association, because conditioning on rs17351243 means rs6703630 becomes nonsignificant ($P = 0.7$).

Associations with AS. Given the extensive comorbidity between AS and AAU, we examined all SNPs previously reported to be associated with AS in studies of AS patients with AAU versus AS patients without AAU, and all AAU patients versus healthy controls. The strength of association was significantly weaker than that reported for AS, and this is likely explained by

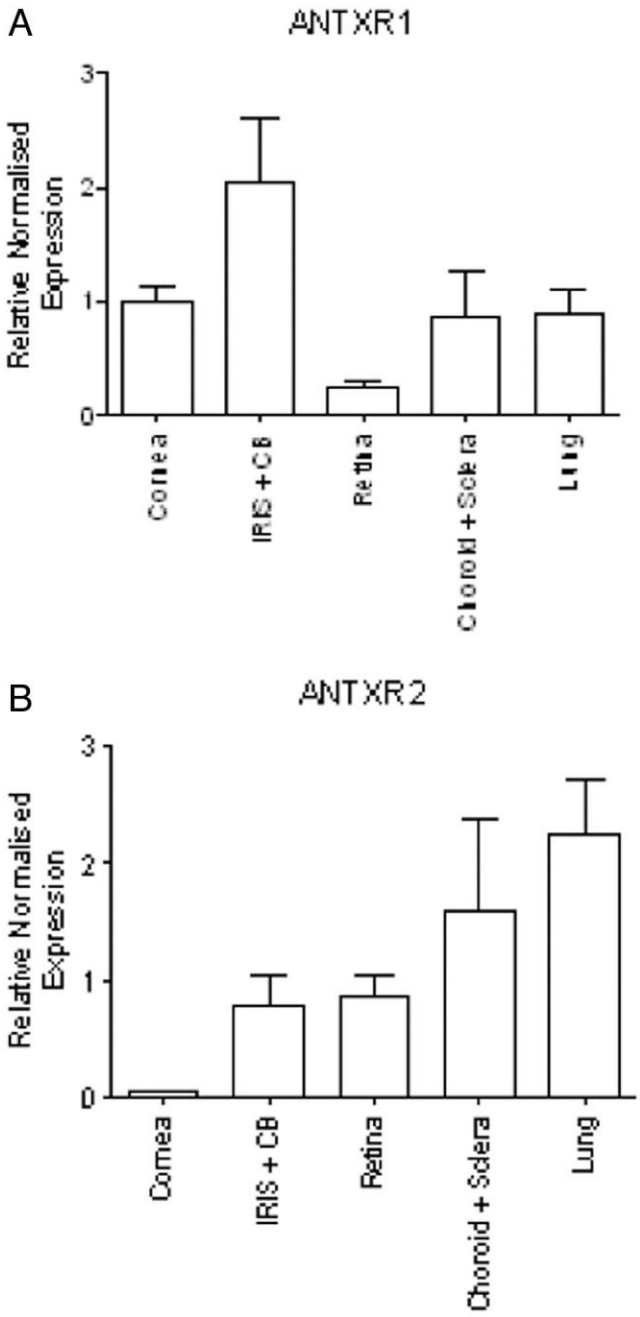

Figure 2. Relative expression of *ANTXR1* (**A**) and *ANTXR2* (**B**) mRNA in eye compartments. Total RNA was isolated from different compartments of the eye, as well as the lung, and real-time reverse transcription–polymerase chain reaction was performed to determine the relative abundance of *ANTXR1* and *ANTXR2* mRNA compared with that of L32 mRNA. Values are the mean ± SEM and are representative of 3 independent experiments, each including triplicate samples. CB = ciliary body.

Table 3. Previously identified associations with acute anterior uveitis

Chromosome	Position*	rs ID no.	Gene	*P*, unconditioned	*P*, after conditioning on B27†	*P*, after conditioning on top 2 associations‡	*P*, after conditioning on top 3 associations§	Ref.
1	206,948,639	rs6703630	*IL10*	3.81×10^{-3}	–	–	–	14
1	206,945,381	rs2222202	*IL10*	5.50×10^{-1}	–	–	–	14
1	206,945,311	rs3024490	*IL10*	1.23×10^{-1}	–	–	–	14
6	31,542,482	rs1799724	*TNF-857*	7.04×10^{-40}	1.78×10^{-1}	1.67×10^{-2}	7.18×10^{-3}	17,18
6	31,543,101	rs361525	*TNF-308*	2.74×10^{-11}	6.80×10^{-2}	2.44×10^{-1}	3.61×10^{-1}	17,18
6	31,543,031	rs1800629	*TNF-238*	7.79×10^{-32}	2.96×10^{-3}	1.58×10^{-2}	7.60×10^{-2}	17,18
6	31,542,308	rs1799964	*TNF-103*	2.67×10^{-18}	6.45×10^{-2}	3.37×10^{-1}	3.03×10^{-1}	17
6	31,542,476	rs1800630	*TNF-863*	3.58×10^{-9}	2.44×10^{-1}	5.80×10^{-1}	4.40×10^{-1}	17
6	31,914,935	rs1048709	*CFB*	4.11×10^{-9}	2.02×10^{-1}	7.49×10^{-1}	4.04×10^{-1}	16
12	58,162,739	rs703842	*CYP27B1*	2.79×10^{-3}	–	–	–	13

* UCSC human genome build 19.
† Conditioned on HLA–B27 dose as imputed by SNP2HLA.
‡ The top 2 associations were rs116488202 and rs114102658.
§ The top 3 associations were rs116488202, rs114102658, and rs149567432.

small numbers of participants and hence greatly reduced power. The results of this assessment are shown in Supplementary Tables 8 and 9 (available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract).

DISCUSSION

This study demonstrates genetic associations specific to AAU as well as shared associations with AS that have both similar and significantly different effect sizes and directions of association in the 2 related diseases.

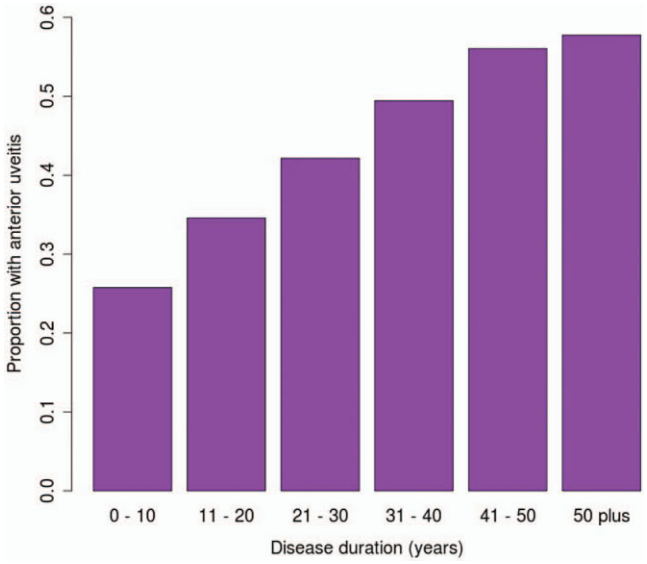

Figure 3. Proportions of ankylosing spondylitis (AS) patients with acute anterior uveitis according to the duration of AS. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.38873/abstract.

We observed evidence suggesting that MHC class I or class II alleles other than *HLA–B27* contribute to AAU susceptibility. *HLA–B08,* which we observed to be tagged by rs115937001 in *HLA–B27*–negative patients, has been associated previously with AAU and also with sarcoidosis, a disease in which AAU also occurs (37–39). In conditional analyses, *HLA–DQB1:05* and *HLA–DRB1:0103* have been implicated, and SNPs in or around *HLA–DRA*, *HLA–DRB5*, and *HLA–DQA2* have also shown association with AAU. This suggests that additional non–*HLA–B27* MHC factors affect the etiology of AAU. In our study, *HLA–B27* does show association even when comparing AAU patients with and those without AS, confirming the long-held view that *HLA–B27* is an AAU risk gene regardless of whether or not AS is present.

We observed several genetic associations shared with IBD (40,41). *IL10* is associated with IBD, and IL-10 was shown to abrogate disease in an animal model of experimentally induced uveitis (42). It was also shown recently that IL-10 is important in IL-35–induced regulatory B cell suppression of EAU (43). In patients with AAU, peripheral blood mononuclear cells show up-regulation of both IL-10 and IL-19 (44). Patients with AS who also have IBD have much lower levels of *HLA–B27* expression, offering relative protection against AAU. Uveitis develops in up to 3.8% of patients with IBD; this rate is substantially lower than that in patients with AS (45).

The suggestive association of the IL-18 receptor highlights the importance of the innate immune recog-

nition of foreign microorganisms and the triggering of an appropriate adaptive immune response. Research has linked infection with organisms such as chlamydia with AAU, and the mechanism of this association may be related to inappropriate or abnormal activation of a cell-mediated immune response by IL-18 (46,47). In sarcoidosis, a condition in which AAU occurs, enhanced expression of IL-18Rα in CD4 T cells has been observed (48).

In considering the shared associations between AS and AAU, the intergenic region chromosome 21q22 and *ANTXR2* have an effect only in AS patients without AAU and not in AS patients with AAU. Furthermore, the chromosome 2p15 intergenic region shows significantly greater association in AS patients without AAU. These findings suggest that there may be genetic subgroups among patients with AS, with heterogeneity in the genetic profiles of these patients, and that genetic factors influence which AS patients develop AAU and which patients do not develop AAU. A suggestive association between SNPs 17 kb upstream of *ANTXR2* and myopia has been described (49). However, because no effect was observed in the cohort of AS patients with AAU, it appears that *ANTXR2* is potentially an AS risk locus alone. The difference in *ANTXR2* expression across the different components of the eye is, however, of interest in view of the positive genetic associations. The mechanisms for disease association have not been identified at either chromosome 2p15 or chromosome 21q22; long noncoding mRNA transcripts have been identified, but their function is currently unknown (50).

These results should be interpreted in light of several limitations. The study is underpowered, and the inclusion of additional cases is likely to result in new associations. In addition, the suggestive associations require replication to be considered robust. The use of self-reported AAU is a potential limitation, although self-report has been shown to differ little from an ophthalmologist diagnosis in patients with spondyloarthritides (51). The high incidence of AAU in AS, and the progressive increase in AAU penetrance with disease duration, means that many patients with AS currently classified as AAU-negative may ultimately develop AAU (Figure 3). Furthermore, AAU may have been underdiagnosed because of subclinical disease in some patients, AS treatment may have suppressed the manifestations of AAU, or the diagnosis of AAU may have been missed even when it was clinically apparent. Accurately quantifying the power of the study is not possible, because the size of these effects is completely unknown. Finally, the etiology of AAU includes environmental factors; therefore, there is the potential issue of incomplete penetrance of AAU susceptibility loci.

Because of these problems, we took advantage of the availability of a large study of AS susceptibility loci, reasoning that any novel loci identified in the small samples of patients with AAU and healthy control subjects were highly likely to be associated with AAU. We were able to identify several novel immune-related loci associated with AAU. The 2 shared associations with IBD are of particular interest, because these are not associations shared with AS. The pathways identified will help ascertain novel treatment strategies for this common and important disease and highlight further pathways shared between AS, IBD, and AAU.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Brown had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Robinson, Claushuis, Cortes, Leo, Mukhopadhyay, Maksymowych, Inman, van der Horst-Bruinsma, Weisman, Wordsworth, Reveille, Brown.

Acquisition of data. Robinson, Claushuis, Martin, Leo, Mukhopadhyay, Bradbury, Cremin, Harris, Maksymowych, Inman, Haroon, Gensler, van der Horst-Bruinsma, Hewitt, Craig, Lim, Wakefield, McCluskey, Voigt, Fleming, Pointon, Weisman, Wordsworth, Reveille, Rosenbaum, Brown.

Analysis and interpretation of data. Robinson, Claushuis, Evans, Leo, Mukhopadhyay, Harris, Maksymowych, Rahman, Powell, Degli-Esposti, Weisman, Wordsworth, Reveille, Brown.

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