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## Genetic Dissection of Acute Anterior Uveitis Reveals Similarities and Differences in Associations observed with Ankylosing Spondylitis

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## Abstract

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

**Method**—We genotyped 1,711 patients with AAU (either primary or with AAU and AS), 2,339 AS patients without AAU, and 10,000 controls on the Illumina ImmunoChip Infinium microarray. We also used data on AS patients from previous genomewide association studies to investigate the AS risk locus *ANTXR2* for its putative effect in AAU. *ANTXR2* expression in mouse eyes was investigated by RT-PCR.

**Results**—Comparing all AAU cases with HC, strong association was seen over *HLA-B* corresponding to the *HLA-B27* tag SNP rs116488202. Three non-MHC loci *IL23R*, the intergenic region 2p15 and *ERAP1* were associated at 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 at a suggestive level of significance ( $P < 5 \times 10^{-6}$ ). A number of previously confirmed AS associations demonstrated significant differences in effect size between AS patients with AAU and AS patients without AAU. *ANTXR2* expression was found to vary across eye compartments.

**Conclusion**—These findings, with 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.

## Keywords

Anterior uveitis; uveitis; ankylosing spondylitis; HLA-B27; ANTXR2

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Acute anterior uveitis (AAU) has a cumulative incidence rate in the Caucasian general population of 0.2%, but in 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 with strong familiarity demonstrated[4]. The first-degree recurrence risk of AAU is 6% compared to a population prevalence of only 0.038–0.38%[5]. AAU occurs in 30–40% of individuals with ankylosing spondylitis (AS) suggesting a shared etiology[6]. It is strongly associated with *HLA-B27* both in those with AS, and those without, with more than 50% of those with primary AAU being *HLA-B27* positive[7]. There is 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 in the normal *HLA-B27* positive (Dutch) population (1%), indicating that other genetic factors

besides *HLA-B27* are involved[5]. In the same study, 11% of *HLA-B27* positive first-degree relatives >45 years of age had AS compared with an expected frequency of AS in *HLA-B27* carriers of ~1%, highlighting the strong co-familiality 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], the Killer Immunoglobulin Receptor (KIR) region[19], and suggestive linkage has been reported to the chromosome 9p region[20]. No findings have achieved genomewide significance ( $P < 5 \times 10^{-8}$ ), and few associations have been replicated[6]. Few of these studies were adequately powered to identify genes involved in AAU reliably so 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, two main analyses were performed. AS patients with AAU (as cases) were first compared with AS patients without AAU (as controls). Whilst this analysis studies AAU genetic associations controlling for AS-comorbidity, potential issues such as delayed onset of uveitis and subclinical disease affect it. Therefore a second analysis compared all AAU patients with healthy controls (HC), with a subsequent heterogeneity test performed to assess whether associated SNPs had differing effect sizes in AS patients with and without uveitis. Genetic associations identified comparing AS patients with AAU to HC, that were not identified by the larger and better powered IGAS AS ImmunoChip study[21], are likely to be AAU-specific associations.

### ImmunoChip Sample Collection and Phenotyping

AS cases (defined by the modified New York criteria[22]) of European descent either with (n=1,422) or without AAU (n=2,339) were recruited. Ophthalmologists also collected 289 patients with AAU (in whom the AS status was unknown). AS cases had either self-reported or ophthalmologist diagnosed AAU (Supplementary Table 1). Patients were positively selected on the basis of their phenotype, no exclusions were applied. Historical genotypes from 10,000 Caucasian controls from the 1958 British Birth Cohort and the UK National Blood Transfusion Service were used as common controls. All patients gave informed consent and ethical approval was obtained from all relevant institutional ethics committees.

After quality control (Supplementary Table 2) there remained 9,564 controls, 1,199 AS cases with AAU, 238 AAU cases with alone and 1,731 AS cases without AAU patients.

Samples were genotyped on the Illumina ImmunoChip microarray. Intensity data was processed and normalized in the Illumina GenomeStudio software and subsequently clustered with optiCall[23].

### Quality Control

The thresholds used were a genotyping missingness rate of 0.03, between centre missingness threshold of  $1 \times 10^{-7}$ , individual missingness rate of 0.03, a Hardy Weinberg Equilibrium (HWE) threshold in controls of  $1 \times 10^{-7}$ . Heterozygosity versus missingness outliers beyond 3 SD were excluded. Identity by descent (IBDes) threshold of PI-HAT

(Proportion(IBDes=2) + 0.5(IBDes=1)) 0.20 was used. Principal components were then computed using SHELLFISH (<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 SNPs excluded are shown in Supplementary Table 3.

**Association Analysis**—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. Genomic inflation factor 1000 values for the ImmunoChip control SNPs were 1.058 for the AAU versus HC analysis and 1.035 for the AS with AAU versus AS without AAU analysis. The Q-Q plots for the studies are shown in Supplementary Figure 2. Results were deemed significant if they had  $P < 5 \times 10^{-8}$  (genome-wide significance), and suggestive if they had  $P$  values  $> 5 \times 10^{-8}$  but  $< 5 \times 10^{-6}$ . After conditional analyses, analyses with significance of  $P < 1 \times 10^{-4}$  were reported.

**Imputation**—Data was phased with SHAPEIT version 2[24], and imputed with IMPUTE2[25] using the 1000G phase 1 integrated variant reference set. A post-imputation quality control threshold of 0.8 ‘info’ score from IMPUTE2 was used. Classical Major Histocompatibility Complex (MHC) alleles were imputed from the genotype data with SNP2HLA[26] against the supplied type 1 diabetes reference set. The carriage rate of *HLA-B27* was calculated using imputed SNP2HLA doses and a dosage threshold of 0.6.

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

**Interaction Analysis**—This was completed using the formula:

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

where the uveitis phenotype  $\psi$  and the imputed SNP dosage of rs2032890 ( $\beta_{SNP} SNP$ , lead *ERAP1* associated SNP) and imputed SNP2HLA dosage of the *HLA-B27* ( $\beta_{B27} B27$ ) and ten

eigenvectors ( $\sum_{i=1}^{10} \beta_i \times PC_i$ ) were regressed with an interaction term between the SNP and *HLA-B27* ( $\beta_{int} SNP \times B27$ ).  $\beta_0$  is the intercept.

### Comparison of SNP effects between AS with and without AAU

The HC were split into two by random sampling and allocated as controls to either the AS with AAU cases or the AS without AAU cases to ensure the independence of the two regressions. These two sets of cases and controls were then allocated a dummy variable of 1 or 0 coded as Z. The following regression model was then used:

$$\text{logit}(\psi) = \beta_0 + \beta_{\text{snp}} \text{SNP} + \sum_{i=1}^{10} \beta_i \times PC_i + \beta_z Z + \beta_{z2} \text{SNP} \times Z$$

where  $\psi$  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=0).  $\beta_{\text{snp}}$  is the

regression co-efficient for the SNP dose, SNP is the dosage of the SNP genotype.  $\sum_{i=1}^{10} \beta_i$  are the regression coefficients of the ten principal components,  $PC_i$  from 1–10 are the ten principal components.  $\beta_z$  is the regression co-efficient for the dummy variable Z.  $\beta_{z2}$  is the regression co-efficient for the interaction term between the dummy variable and the SNP dosage: SNP×Z.  $\beta_0$  is the intercept. We also analysed the model with the *HLA-B27* as a component:

$$\text{logit}(\psi) = \beta_0 + \beta_{\text{snp}} \text{SNP} + \beta_{B27} B27 + \sum_{i=1}^{10} \beta_i \times PC_i + \beta_z Z + \beta_{z2} \text{SNP} \times Z$$

In this model ( $\beta_{B27} B27$ ) is the SNP2HLA imputed dosage of *HLA-B27*.

**Ankylosing spondylitis 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 genome wide association study (GWAS) were taken through the same quality control process as the ImmunoChip data in this study. The Canadian study using the Illumina OmniExpress microarray and included 189 AS patients. Briefly, the WTCCC2-TASC study genotyped 3,023 cases and 8,779 controls[27]. The TASC study examined 2,951 AS cases and 6,658 HC, with 439 cases removed in QC[28].

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

**Previously Reported AAU Genetic Associations**—Where the exact SNP was not present on the ImmunoChip microarray, the SNP with the highest LD represented on the ImmunoChip was determined from 1000 genomes data.

**Concordance of Effect Directions**—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 1000 genomes reference data.

**HLA-B27 Heterozygosity and Homozygosity Calculations**—To calculate the odds of the disease for homozygosity and heterozygosity of *HLA-B27* the SNP2HLA imputed doses of *HLA-B27* in the AAU group versus HC were used. Contingency table analysis and the cross-products ratio was used.

**ANTXR2 Expression RNA isolation**—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 the PureLink RNA kit (Ambion). Eyes were dissected to isolate the following components: (i) cornea, (ii) iris including ciliary body, (iii) choroid and sclera, (iv) retina. Briefly, tissues were collected, placed directly into lysis buffer containing  $\beta$ -mercaptoethanol and homogenised using a Tissue Lyzer 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 was assessed with a BioPhotometer (Eppendorf).

**ANTXR2 Expression Real-time quantitative PCR**—The relative expression of ANTXR1 and ANTXR2 mRNA was determined using a two-step RT-PCR assay by comparison to L32 mRNA. First, cDNA was generated from 2  $\mu$ g of total RNA using random primers and M-MLV Reverse Transcriptase (Promega). Second, cDNA samples were then used in the RT-PCR using the 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 from the Harvard Primer Bank (ANTXR1; ID# 32189436a1 and ANTXR2; ID# 13278124a1). The primer sequences for L32 were L32-F (5'-CATCGGTTATGGGAGCAAC-3') and L32-R (5'-GCACACAAGCCATCTACTCAT-3'). Samples were run in triplicate and the assay repeated 3 times. The amounts of ANTXR1 and 2 mRNA in the lung and various eye compartments were normalized relative to L32 mRNA as lung has been shown to express both ANTXR1 and ANTXR2 genes[29].

## RESULTS

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

### Major Histocompatibility Complex Associations

AAU has been linked with classical MHC alleles previously so we examined the MHC for association. In the comparison of AS with AAU versus AS without AAU, single nucleotide polymorphisms (SNPs) in the MHC Class I region harboring *HLA-B* were strongly AAU-associated, see Supplementary Figure 3 (rs115879499,  $P=4.9 \times 10^{-18}$ , OR=1.4, 95% CI 1.2–1.5). 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 classical MHC allele imputation) were analysed 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*. 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 AAU versus HC, strong association was also seen in the MHC, the lead SNP being the previously described[21] *HLA-B27* tag SNP rs116488202 ( $P < 1 \times 10^{-300}$ , OR=16.8, 95% CI 15.0–18.7). On conditioning on this SNP the next associated SNP was



rs114102658, between *HLA-B* and *MICA*, see Figure 1 ( $P=1.2\times 10^{-41}$ , OR=17.4, 95% CI 16.7–19.4). Conditioning on both rs116488202 and rs114102658 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 tag any classical class I allele accurately (all sensitivities and specificities < 70%). Conditioning on these top three signals, association was seen with rs114977878 in the MHC Class II locus in the gene *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). Conditioning on these top 5 SNPs still left association in the *HLA-B* locus at rs114560492 ( $P=3.1\times 10^{-5}$ , OR=1.9, 95% CI 1.7–2.0). Conditioning on these top 6 associations left no further residual signals ( $P > 1\times 10^{-4}$ ).

Some AS patients do not carry *HLA-B27* so to examine for MHC associations in this group we analysed *HLA-B27* negative patients, as assessed by any imputed dose of the SNP2HLA imputed *HLA-B27*. In the all AAU versus HC analysis 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 tags *HLA-B0801* with 76% specificity and 94% sensitivity. Conditioning on this SNP left no residual association ( $P>10^{-4}$ ).

Allelic diversity at classical HLA loci is extensive, and challenging to impute with single SNPs. Therefore imputation with multiple SNPs was performed with SNP2HLA, to provide a potentially more accurate assessment of classical MHC allele-disease associations[26]. In analysis of AS with AAU versus AS without AAU, the classical allele *HLA-B27* was again strongly associated ( $P=1.4\times 10^{-16}$ , OR=2.1, 95% CI 1.8–2.5). Controlling for the *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 all AAU versus HC analysis, strong association with *HLA-B27* was evident ( $P < 1\times 10^{-300}$  OR=59.7, 95% CI 51.4–69.5). 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 all AAU versus HC analysis heterozygosity for *HLA-B27* (as determined by SNP2HLA imputation) confers OR for AAU of 66.8 (95% CI 66.7 – 67.0), homozygosity for *HLA-B27* confers OR of 130.6 (95% CI 130.1 – 131.1), and the risk of two *HLA-B27* alleles over one *HLA-B27* allele confers an OR of 2.0 (95% CI 1.5 – 2.4). Thus homozygosity for *HLA-B27* does confer additional risk of AAU over heterozygosity (see Supplementary Table 4).

The carriage rates for *HLA-B27* (as determined by SNP2HLA imputation) were 91.2% in AS cases with AAU 80.6% in AS cases without AAU, and only 63.9% in the small cohort of AAU patients with unknown AS status (recruited by ophthalmologists).

### Non-MHC Associations

A number of non-MHC associations have been described for AAU previously so we sought to examine non-MHC areas for association. In the comparison of AS cases with AAU versus AS cases without AAU, association was observed with variants within *ERAPI* (rs2032890,  $P=9.0\times 10^{-6}$ , OR=1.3, 95% CI 1.2–1.5), see Supplementary Table 5 and Supplementary



Figure 4. In AS there is an interaction between *ERAP1* and *HLA-B27*[27]. Controlling for *HLA-B27* using SNP2HLA dosages[21] as a covariate in the analysis, association was essentially unchanged at rs2032890 ( $P=2.9\times 10^{-5}$ , OR=1.3, 95% CI 1.2–1.5). Performing 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 with logistic regression in each group it was associated in the *HLA-B27* positive group but not the 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 all AAU cases vs HC analysis, genomewide 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 and Supplementary Figure 5–12). 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 seen in *HLA-B27* negative AS. After conditioning on this SNP no residual association was seen in *ERAP2* ( $P > 10^{-4}$ ).

In the analysis of all AAU cases with HC, five loci had suggestive levels of association ( $P < 5\times 10^{-6}$ ). These loci included both loci known to be associated with AS (*IL6R*, chromosome 1q32) and novel loci not previously reported to be AS-associated including interleukin (*IL*)10 - *IL19*, the gene encoding the alpha chain of the IL-18 receptor (*IL18RI*)-*IL1RI*, and the *EYS* gene (Eyes shut Drosophila homolog, a gene associated with retinitis pigmentosa[30, 31]). The *IL6R* and chromosome 1q32 AAU associations are concordant with the AS associations (alleles are in phase and  $r^2=0.98$  and 1.0, respectively). On 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), LD between these SNPs is  $D'=0.87$  and  $r^2=0.48$  (source: 1000 genomes). No further association was apparent after conditioning on both rs10920074 and rs12132349 ( $P > 1\times 10^{-4}$ ). At the *IL18RI*-*IL1RI* association conditioning on rs10197284 shows a secondary signal at rs6750020 ( $P=1.2\times 10^{-4}$ , OR=1.2, 95% CI 1.1–1.4), linkage disequilibrium (LD) between these SNPs is  $D'=0.92$  and  $r^2=0.85$ . Conditioning on rs6750020 in addition to rs10197284 removes all association ( $P>10^{-4}$ ). The *IL18RI*-*IL1RI* AAU rs10197284 association is not in LD with the previously reported[21] suggestive AS association at rs4851529 ( $r^2=0.00$ ,  $D'=0.07$ ). The other loci had no secondary signals on conditioning on the top SNP ( $P > 1\times 10^{-4}$ ).

The *IL10* SNP rs17351243 has LD ( $D'=0.65$ ,  $r^2=0.52$ , source:1000 genomes) with the inflammatory bowel disease (IBD) associated SNP rs3024505[32], and the direction of association is the same. The *IL18RI*-*IL1RI* SNP rs10197284 is in strong LD with previously reported celiac disease[33] and IBD[32] SNPs, and in lesser LD with asthma[34] and eosinophil count[35] SNPs (Supplementary Table 6). The IBD and celiac disease associations share the same direction of effect, but the asthma and eosinophil count SNPs have opposite directions of effect. The *EYS* SNP rs665873 has low frequency (HapMap CEU MAF=0.051) but is in tight LD with the common (MAF=0.43) statin-myopathy

associated SNP rs3857532[36], ( $D'=0.91$ ,  $r^2=0.01$ , source:1000 genomes), and with six SNPs associated with retinitis pigmentosa[30] ( $D'=1$ ,  $r^2=0.001$ ) (Supplementary Table 7). The AAU, statin-myopathy and retinitis pigmentosa SNPs all have the same direction of effect.

### Odds Ratio Comparisons

We assessed whether the shared associations influence AS and AAU with differing magnitudes using a heterogeneity test. All SNPs associated with AAU ( $P<5\times 10^{-6}$ ) in the all AAU cases versus HC analysis and all SNPs associated with AS from the recently published IGAS Immunochip study[21] were assessed. We used two models, the first (model 1) has the SNP and principal components; the second (model 2) includes *HLA-B27* dose as a model covariate, reflecting the previously demonstrated interaction between *ERAP1* SNPs and *HLA-B27* in AS.

The two AS-associated intergenic loci, chromosomes 2p15 and 21q22, both had significantly different effect sizes in AS patients with AAU versus HC compared to AS patients without AAU versus HC in model 2 but not model 1 (Table 2). At chromosome 2p15 both models showed a larger effect size in AS cases without AAU versus HC compared to AS with AAU versus HC, but this only reached statistical significance in model 2 (model 1: SNP rs4672507  $P=0.17$  and SNP rs6759298  $P=0.13$  and model 2: SNP rs4672507  $P=0.06$  and SNP rs6759298  $P=0.05$ ). The lead AAU associated *ERAP1* SNP had a significantly stronger effect size in the AS with AAU versus HC compared to AS without AAU versus HC, 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 the AS with AAU compared to AS without AAU (OR=0.97, 95% CI 0.93 – 1.01,  $P=0.14$ ).

### ANTXR2 Odds Ratio Comparison

The AS associated *ANTXR2* SNP rs4389526 showed a significant difference in effect size between AS with AAU and AS without AAU in model 2 ( $P=0.04$ ). The rs4389526 SNP had an effect in the AS without AAU cohort ( $P=5.6\times 10^{-3}$ , OR=0.98, 95% CI 0.97 – 0.99) but no effect in the AS with AAU cohort ( $P=0.77$ , OR=1.00, 95% CI 0.99 – 1.02).

### Analysis of ANTXR2 expression in the eye using real-time quantitative RT-PCR

Given the negative association between *ANTXR2* SNP rs4389526 and AS 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 the tested eye compartments, as well the lung (Figure 2). *ANTXR1* expression was equivalent in the iris/ciliary body, cornea and choroid/sclera, and slightly lower in the retina (Figure 2A). By contrast, *ANTXR2* was expressed most abundantly in the choroid/sclera, iris/ciliary body and retina, compared with the cornea, where expression of *ANTXR2* was minimal (Figure 2B).

### Previous AAU associations

A number of previous non-MHC associations have been reported for AAU. Therefore we sought to examine whether there was evidence to support their reported association. While

not all previously reported AAU genetic associations were on the Immuchip 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 AAU versus HC analysis (Table 3). After controlling for the lead MHC SNP (rs116488202), no residual association was seen with *TNF* SNPs previously reported to be AAU-associated, although nominal association was seen with *TNF*-238 (rs1800629,  $P=0.003$ ). This association disappeared after conditioning on the next two 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 AAU-associated in *IL10* and *CYP27B1* (*IL10*, rs6703630,  $P=0.04$  and *CYP27B1*, rs703842,  $P=0.003$ ). As mentioned above, much stronger association was seen in the current study with the *IL10* SNP rs17351243; this SNP is in moderately strong LD with the previously reported *IL10* association rs6703630 ( $D'=0.87$  and  $r^2=0.24$  current dataset;  $D'=0.71$  and  $r^2=0.09$  1000 Genomes)[14]. This suggests that they may tag the same association, as controlling for association at rs17351243 means rs6703630 becomes non-significant ( $P=0.7$ ).

### AS associations

Given the extensive co-morbidity between AS and AAU we examined all SNPs previously reported to be associated with AS in the AS with AAU versus AS without AAU, and the all AAU versus HC studies. The strength of association is significantly weaker than reported for AS, likely explained by small numbers of participants and hence greatly reduced power. The results are reported in Supplementary Table 8 and 9.

## DISCUSSION

This study reports 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 two related diseases.

We found evidence to suggest that MHC class I or class II alleles besides *HLA-B27* contribute to AAU susceptibility. *HLA-B08*, which we found to be tagged by rs115937001 in *HLA-B27* negative patients, has been associated previously with AAU and in addition 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 comparing AAU cases with and without AS, confirming the long held view that *HLA-B27* is an AAU risk gene regardless of whether AS is present or not.

We observed a number of genetic associations shared with IBD[40, 41]. The *IL10* gene is associated with IBD, and IL-10 is important in an animal model of experimentally-induced uveitis, as treatment with IL-10 abrogates disease in this model [42]. It has also been shown recently that IL-10 is important in IL-35 induced B regulatory cell suppression of EAU[43]. In humans with AAU, peripheral blood mononuclear cells (PBMCs) show up-regulation of both IL-10 and IL-19[44]. Patients with AS who also have IBD have much lower rates of

*HLA-B27*, offering relative protection from AAU. In patients with IBD up to 3.8% develop uveitis, a rate substantially lower than in AS[45].

The suggestive association of the IL-18 receptor highlights the importance of the innate immune recognition 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-18R alpha chain has been noted in CD4 T cells[48].

In considering the shared associations between AS and AAU, the intergenic region chromosome 21q22 and *ANTXR2* have an effect in only AS patients without AAU, and not in AS patients with AAU. Further, the chromosome 2p15 intergenic region shows significantly greater association in AS without AAU. These findings suggest that there may be genetic subgroups in AS with heterogeneity in the genetic profiles of AS patients, and that genetic factors influence which AS patients develop AAU and which do not. A suggestive association between SNPs 17kb upstream of *ANTXR2* and myopia has been described[49]. But with no effect in the AS with AAU cohort it seems *ANTXR2* is potentially an AS risk locus alone. However, the difference in *ANTXR2* expression across the different components of the eye is of interest in view of the positive genetic associations. The mechanisms for disease association have not been identified at either chromosomes 2p15 or 21q22; long non-coding 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 additional cases are likely to result in new associations. In addition, the suggestive associations require replication to be considered robustly associated. The use of self-report AAU is a potential limitation, although self-report has been shown to differ little from ophthalmologist diagnosis in SpA patients previously[51]. The high incidence of AAU in AS, and the progressive increase in AAU penetrance with disease duration, means that many AS cases currently classified as AAU-negative may ultimately develop AAU (Figure 3). There may also have been issues with under diagnosis of AAU in subclinical cases, AS treatment suppressing AAU manifesting or where the diagnosis was missed even if clinically apparent. Accurately quantifying the power of the study is not possible because the size of these effects is completely unknown. Finally there are environmental factors in the etiology of AAU, and 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 found with the AAU patient sets compared to HC in the small sample sizes used were highly likely to be AAU associated loci. From this analysis we have been able to identify a number of novel immune related loci associated with AAU. The two shared associations with IBD are of particular interest because these are not associations shared with AS. The pathways identified will help identify novel treatment

strategies for this common and important disease and highlights further pathways shared between AS, IBD and AAU.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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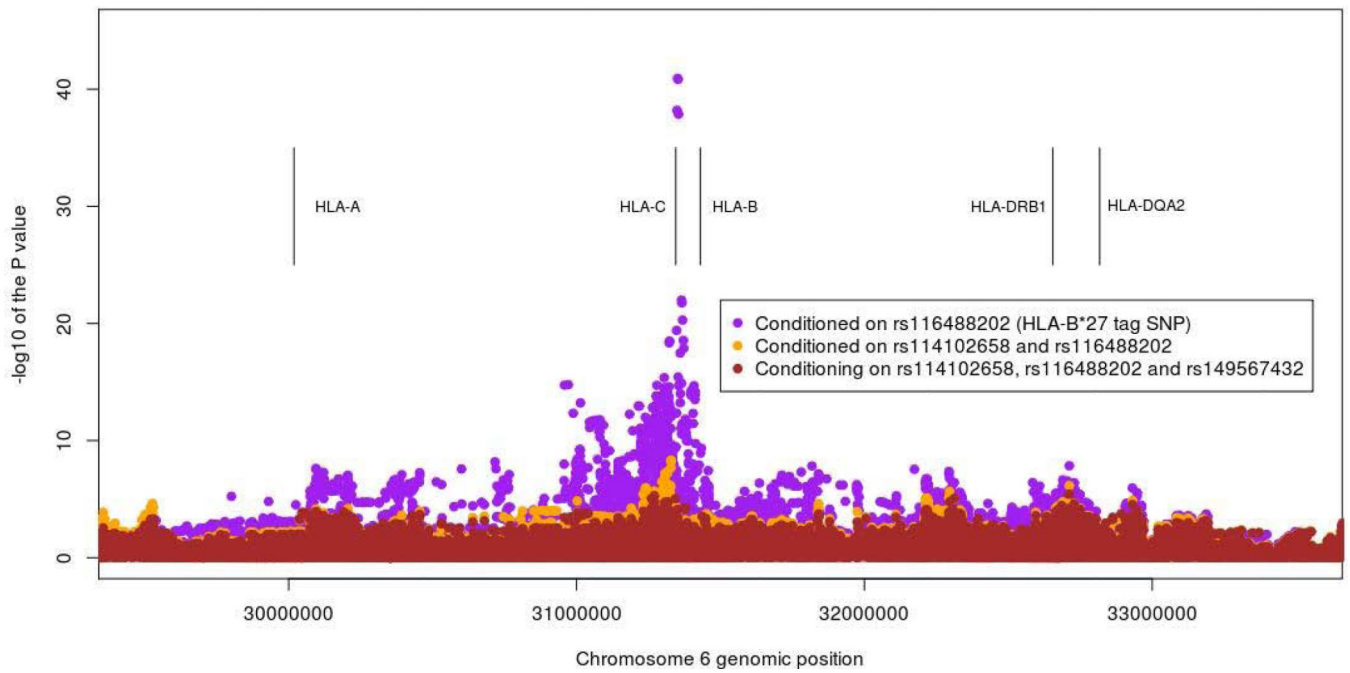
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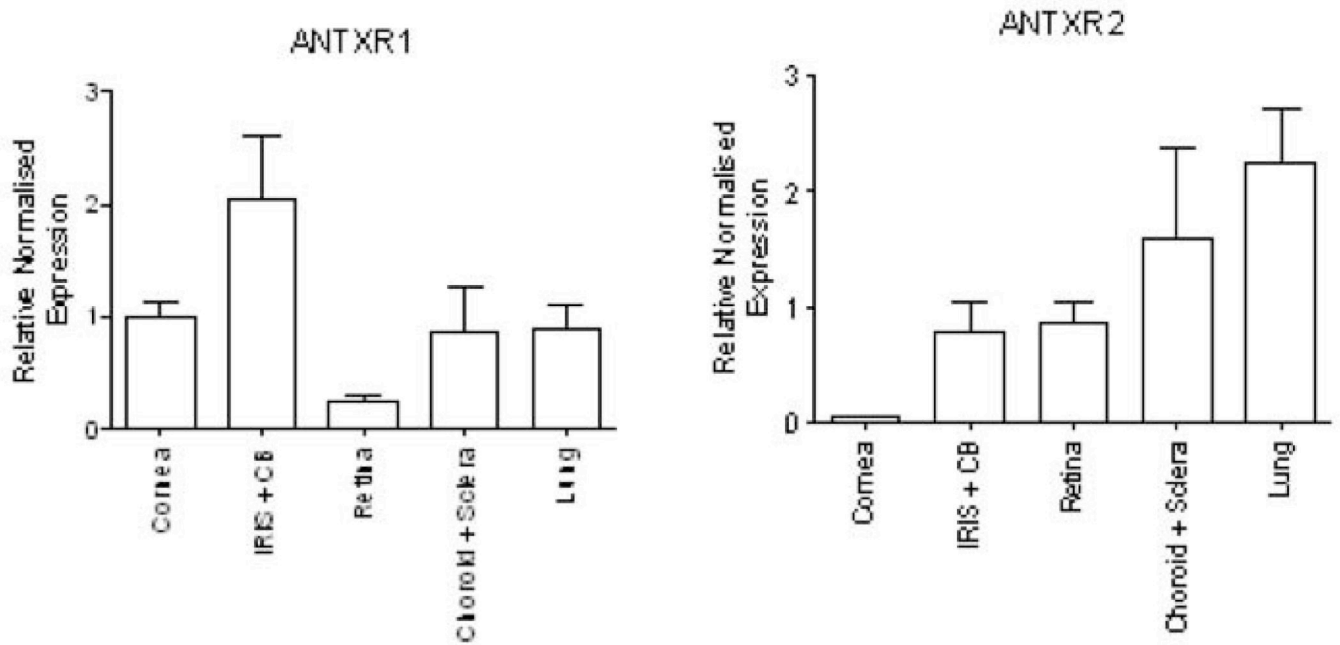
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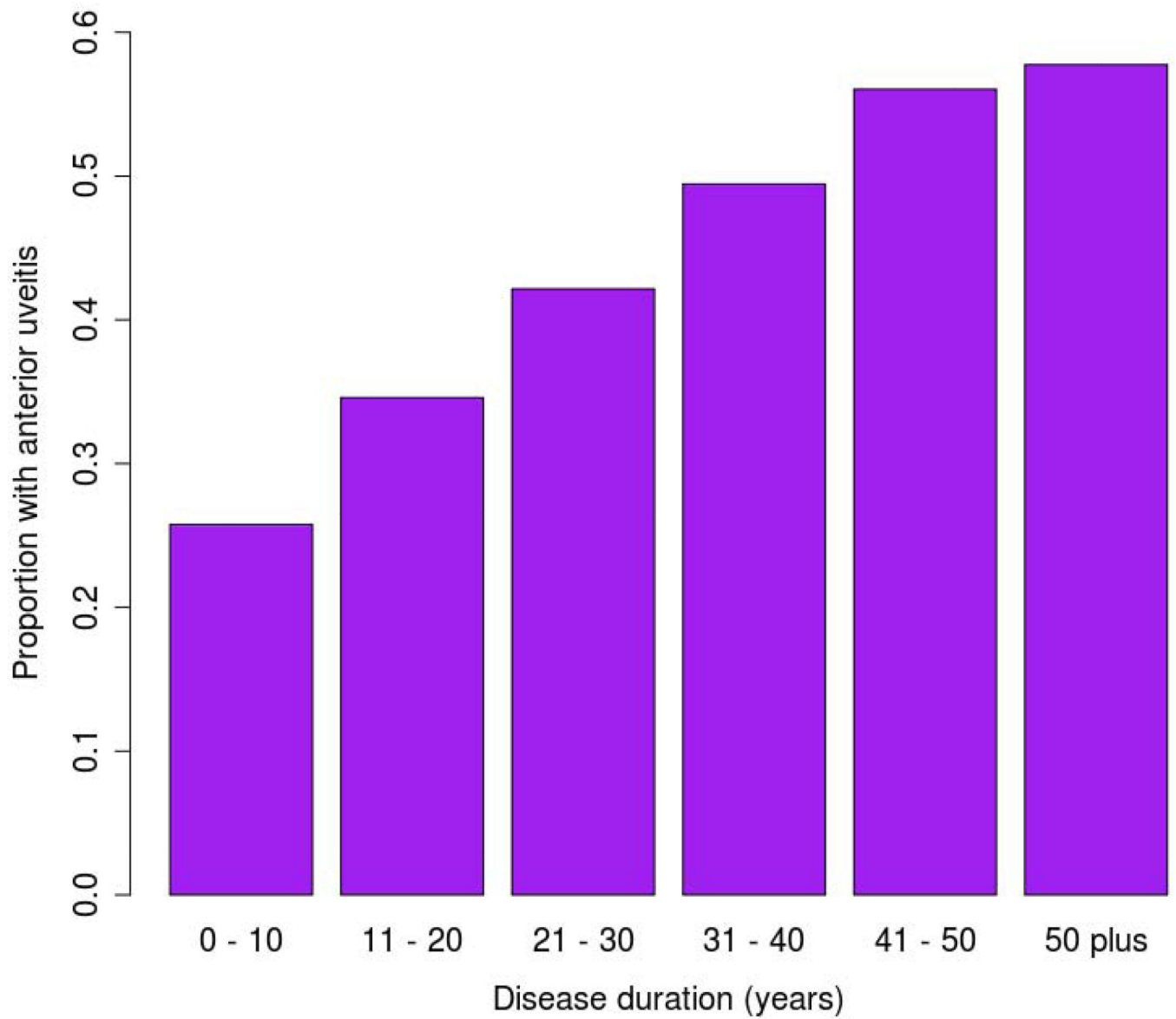
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**Figure 1.**  
Major histocompatibility complex association plot for all acute anterior uveitis versus healthy controls



**Figure 2.** Expression of ANTXR1 and ANTXR2 in eye compartments. Total RNA was isolated from different compartments of the eye, as well as the lung and real-time RT-PCR performed to determine the relative abundance of ANTRX1 and ANTRX2 mRNA by reference to L32 mRNA. The results represent mean and SEM from three independent experiments, each including triplicate samples



**Figure 3.** Proportion of ankylosing spondylitis patients with acute anterior uveitis by ankylosing spondylitis disease duration. Source: Ankylosing spondylitis GWAS patient cohort

**Table 1**

Association results for the AS with AAU versus AS without AAU analysis

rsid	Chr	Position *	P value	Risk Allele	Protective Allele	RAF (Cases/Controls)	OR Lower	OR Upper	Nearby Genes	Also AS Associated
rs2032890	5q15	96,121,152	2.11E-16	A	C	0.77/0.69	1.51	1.37	ERAP1/ERAP2/LNPEP	Yes
rs4672507	2p15	62,570,573	2.05E-12	T	A	0.43/0.36	1.38	1.27	intergenic	Yes
rs79755370	1p31	67,699,915	1.27E-08	C	A	0.96/0.94	1.80	1.45	IL23R	Yes
rs12132349	1q32	200,875,242	1.57E-07	T	A	0.76/0.71	1.31	1.19	KIF21B-C orf106	Yes
rs6690230	1q21	154,432,877	1.09E-06	C	G	0.41/0.36	1.23	1.13	IL6R	Yes
rs17351243	1q32	206,959,527	1.46E-06	A	G	0.51/0.45	1.24	1.14	IL10-IL19	No
rs10197284	2q12	102,982,703	1.67E-06	G	A	0.26/0.22	1.25	1.14	IL18R1-IL1R1	No
rs665873	6q12	65,298,551	4.99E-06	A	G	0.98/0.97	2.03	1.48	EYS	No

Chr Chromosome,

\* Human genome build 19, RAF Risk allele frequency, OR Odds ratio, AS ankylosing spondylitis, AAU acute anterior uveitis

**Table 2**  
Heterogeneity test results between AS with AAU versus healthy controls and AS without AAU versus healthy controls

SNP	Gene/Region	Model 1: SNP + Principle Components			Model 2: SNP + HLA-B27 + Principle Components			Interaction term		
		AS with AAU P value	AS without AAU P value	AS with AAU OR	AS without AAU P value	AS with AAU P value	AS without AAU OR	Model 1 P value	Model 2 P value	
rs2283790	UBE2LE	0.87	1.001	0.007	0.98	0.999	0.12	0.992	0.049	0.29
rs2836883	21q22	0.0059	1.02	0.03	1.03	1.005	0.00017	1.019	0.19	0.04
rs2032890	ERAP1	1.80E-17	1.06	3.30E-04	1.03	1.02	0.02	1.01	0.001	0.08
rs30187	ERAP1	4.80E-19	1.06	1.04E-07	1.04	1.02	0.003	1.02	0.02	0.24
rs10045403	ERAP1	1.10E-14	1.05	5.30E-04	1.02	3.60E-04	0.03	0.01	0.005	0.5
rs2910686	ERAP2	0.18	1.01	0.03	0.99	1.00	0.14	0.99	0.01	0.43
rs6759298	2p15	8.50E-16	1.06	1.70E-22	1.07	2.00E-06	9.70E-12	1.04	0.13	0.05
rs4333130	ANTXR2	0.048	0.98	3.00E-04	0.97	1.002	5.60E-03	0.98	0.28	0.04
Suggestive										
rs7282490	ICOSLG	0.98	1.00	0.007	1.02	1.004	0.01	1.013	0.054	0.17
rs665873	EYS	6.10E-05	0.92	0.14	0.97	0.002	0.96	0.98	0.09	0.3
rs4672507	2p15	4.00E-16	1.06	2.90E-22	1.07	2.60E-07	1.10E-12	1.04	0.17	0.06

SNP – single nucleotide polymorphism, AS ankylosing spondylitis, AAU acute anterior uveitis, OR odds ratio

**Table 3**

Previous AAU associations

Chr	Position*	rsid	Gene	Uncond P value	B27 Cond P value	Top two Cond P value	Top Three Cond P value	Reference
1	206948639	rs6703630	<i>IL10</i>	0.0381204				14
1	206945381	rs2222202	<i>IL10</i>	0.550083				14
1	206945311	rs3024490	<i>IL10</i>	0.12393				14
6	31542482	rs1799724	<i>TNF-857</i>	7.04E-40	0.178664	0.01679	0.00718782	17,18
6	31543101	rs361525	<i>TNF-308</i>	2.74E-11	0.0680665	0.244505	0.361933	17,18
6	31543031	rs1800629	<i>TNF-238</i>	7.79E-32	0.00296247	0.0158237	0.0760507	17,18
6	31542308	rs1799964	<i>TNF-I03</i>	2.67E-18	0.0645271	0.337868	0.303424	17
6	31542476	rs1800630	<i>TNF-863</i>	3.58E-09	0.244742	0.580368	0.440478	17
6	31914935	rs1048709	<i>CFB</i>	4.11E-09	0.202212	0.749028	0.404371	16
12	58162739	rs703842	<i>CYP27B1</i>	0.00279726				13

\* Human genome build 19, Uncond: Unconditioned result. B27 Cond: Conditioned on HLA-B27 dose as imputed by SNP2HLA. Top two Cond: Result after conditioning on rs116488202 and rs114102658. Top Three Cond: Result after conditioning on rs116488202, rs114102658 and rs149567432