New insights into the genetic component of non-infectious uveitis through an Immunochip strategy

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ABSTRACT

Objective. In recent years, large-scale genetic studies have identified several *loci* associated with specific disorders involving uveitis, such as Behçet's disease, Vogt-Koyanagi-Harada syndrome, sarcoidosis and birdshot chorioretinopathy (BSCR). The aim of the present study was to identify new genetic risk factors associated with non-infectious uveitis by performing a dense genotyping of immune-related *loci* using the Immunochip platform.

Design. Case/control study

Participants.We analysed a total of 613 cases and 3,693 unaffected controls from three independent case/control sets of European ancestry. Only patients diagnosed with non-infectious and non-anterior uveitis and without systemic features were selected for the study.

Methods. Patients were genotyped using the Immunochip array. To perform a more comprehensive analysis of the human leucocyte antigen (HLA) region, single-nucleotide polymorphisms (SNPs), classical HLA alleles (at two- and four-digit resolution) and polymorphic amino acid variants were obtained via imputation. In each cohort, genetic variants which passed quality control filters were tested for association by logistic regression. Subsequently, a meta-analysis combining the three case/control sets was conducted by the inverse variance method.

Main Outcome Measures. Association of genetic variants located in immune-related loci with the susceptibility to non-anterior non-infectious uveitis.

Results.The highest peak belonged to the HLA region. A more detailed analysis of this signal evidenced a strong association between the classical allele HLA-A*2902 and BSCR (p=3.21E-35, OR=50.95). An omnibus test on polymorphic amino acid positions yielded HLA-A 62 and 63 as relevant positions for this disease. In patients with intermediate and posterior uveitis, the strongest associations belonged to the rs7197 polymorphism, within the *HLA-DRA* gene (p=2.07E-11, OR=1.99), and the HLA-DR15 haplotype (DRB1*1501: p=1.16E-10, OR=2.08; DQA1*0102: p=4.37E-09, OR=1.77;

DQB1*0602 alleles: p=7.26E-10, OR=2.02). Outside the HLA region, the *MAP4K4*/*IL1R2* *locus* reached statistical significance (rs7608679: p=8.38E-07, OR=1.42). In addition, suggestive associations were found at five other genetic regions.

Conclusions. Through an Immunochip approach, we have further interrogated the association between the HLA region and non-infectious intermediate, posterior and panuveitis. In addition, we have identified a new susceptibility factor and proposed additional risk *loci* with putative roles in this condition.

KEYWORDS: non-infectious uveitis, posterior uveitis, intermediate uveitis, panuveitis, gene polymorphism, HLA, meta-analysis, Immunochip.

INTRODUCTION

Non-infectious uveitis is an immune-mediated condition characterized by intraocular inflammation, which mainly affects the uveal tract but also adjacent structures,including the retina and its vessels, the vitreous and the optic nerve[1]. Non-infectious uveitis comprises a heterogeneous group of disorders diagnosed according to their clinical phenotype that may be confined to the eye or associated with systemic disorders. Based on the location of the inflammation, uveitis can be classified as anterior, posterior, intermediate and panuveitis[2]. While anterior uveitis has the best visual prognosis, non-anterior uveitis patients have a greater risk of permanent vision loss, and often require systemic immunosuppressive therapy[3]. Nowadays, non-infectious uveitis is considered a major cause of visual impairment in the working age population, which is responsible for up to 10% of cases of blindness in developed countries[4].

In recent years, there have been substantial advances in our understanding of the pathogenic mechanisms leading to non-infectious uveitis[5]. Although the precise pathogenesis remains unclear, accumulating evidence points to the interplay between a complex genetic background together with a deregulated immune response in its development. In this regard, large-scale genetic studies have been performed in several systemic diseases associated with uveitis, including Behçet's disease[6-12], Vogt-Koyanagi-Harada syndrome (VKH)[13], and sarcoidosis[14-19], as well as in inflammatory disorders confined to the eye, such as birdshot chorioretinopathy (BSCR)[20]. This approach has identified several genetic risk *loci*, mainly immune/inflammatory response genes and genes of the human leukocyte antigen (HLA) region, many of which (such as *IL23R*, *STAT4* or *ERAP1/ERAP2*) are shared by the different uveitic syndromes as well as by other immune-mediated diseases.

All published large-scale genotyping scans to date have investigated specific systemic syndromes without considering the shared genetic determinants that might predispose to uveitis *per se*, independent of the clinical diagnosis. Therefore, to further discover the genetic component of non-infectious uveitis, we decided to perform a dense genotyping

of immune-related loci using the Immunochip platform in patients with non-infectious non-anterior uveitis without systemic features.

METHODS

Study population

Three independent case/control sets of European ancestry, 360 cases and 1517 unaffected controls from Spain, 142 cases and 1173 unaffected controls from the Netherlands and 111 cases and 1003 unaffected controls from the United Kingdom (UK) were included in the study. Spanish and British cohorts have been used and characterised in previous association studies [21, 22]. Only patients diagnosed with non-infectious and non-anterior uveitis (the most severe form of uveitis) were selected. In addition, to avoid the identification of signals associated with other clinical manifestations, the uveitis entities related to systemic diseases, except VKH, were excluded from the analysis. VKH was included considering that uveitis appears in 100% of the patients, with or without systemic involvement. Non-anterior uveitis patients were classified according to the anatomical location of the inflammation as posterior uveitis, intermediate uveitis and panuveitis. Informed written consent from all participants and approval from the local ethical committees were obtained in accordance with the tenets of the Declaration of Helsinki. **Supplementary Table 1** shows the main characteristics of the case cohorts included in the study.

Genotyping

Genomic DNA was extracted from saliva samples or whole blood by standard methods. The genotyping was performed at a single center on the Illumina iScan system with the Immunochip platform, which allows a dense analysis of single-nucleotide polymorphisms (SNPs), rare variants, and insertion/deletion (indel) polymorphisms. All the Spanish samples (cases and controls) as well as unaffected subjects from the Netherlands and the UK were genotyped using the HumanImmunov1.0 BeadChip (196,524 genetic markers), whereas uveitis samples from the Netherlands and the UK were genotyped using the Infinium ImmunoArray-24 v2.0 BeadChip (253,702 genetic markers). Genetic variants included in both platforms overlap by 80%.

Quality control and imputation

Data quality control was performed for each sample set separately prior to imputation. SNPs and subjects with successful call rates lower than 98% and 95%, respectively, were removed using PLINK v.1.7[23]. SNPs with minor allele frequencies lower than 0.01 and those that were not in Hardy-Weinberg equilibrium (HWE; $p < 0.001$) were also excluded. In addition, one subject per duplicate pair and per pair of first-degree relatives was also removed via the Genome function in PLINK v.1.7 with a Pi-HAT threshold of 0.4.

IMPUTE v.2. software was used to perform imputations[24], with The 1000 Genomes Phase 3 as reference panel[25]. Imputed data were subsequently subjected to stringent quality filters inPLINK v.1.7; i.e., individuals who generated genotypes <98% were removed, and SNPs with call rates <95% and those that deviated from HWE ($p < 0.001$) were also discarded. Principal-component (PC) analyses were performed to identify and exclude outliers based on their ethnicity in PLINK v.1.7 and the gcta64 and R-base under GNU Public license v.2. With this software, we calculated the ten first PCs using the markers informative of ancestry that were included in the Immunochip. Those subjects showing more than four standard deviations from the cluster centroids were excluded as outliers.

Imputation of the HLA region

HLA imputation was performed for 8,961 common SNPs, representing classical HLA alleles, amino acids, and SNPs, across the extended major histocompatibility complex region. We used the SNP2HLA method with the Beagle software package[26] and a reference panel collected by the Type 1 Diabetes Genetics Consortium comprised of 5,225 individuals of European origin[27].

Statistical analysis

The statistical analyses were performed with PLINK v.1.7 and R. First, each case/control cohort was independently analyzed by logistic regression on the best-guess genotypes (>0.9 probability) assuming an additive model with the first ten PCs as covariates. Subsequently, the three case/control sets were combined by inverse variance weighted fixed effects meta-analysis. Heterogeneity of the ORs across studies was estimated by Cochran's Q and I^2 tests. The presence of independent effects was examined using a stepwise logistic regression by conditioning on a lead SNP, the first ten PCs and the country of origin as covariates.

For the HLA region, an omnibus association test[28] was also performed to determine the influence of the polymorphic amino acid positions on disease susceptibility. For each amino acid position, a null generalized linear model, including the first ten PCs and the country of origin as covariates, was built and compared with an alternative model, including the same variables and all the possible alleles in the analyzed amino acid positions, by a likelihood ratio test (LRT). Additionally, we also conducted conditional analyses controlling by the most associated positions by including them as covariates in the models.

The Manhattan plots were obtained with an in-house modification of the R script written by Stephen Turner. Results of the imputed regions were plotted using the on line tool LocusZoom v.1.133 (http://locuszoom.sph.umich.edu/locuszoom/).

Contrary to genome-wide association studies (GWASs), the Immunochip platform is not based on SNPs tagging the whole genome, but on a fine mapping of candidate disease-associated *loci*. Thus, it has been proposed that, since tests are correlated, using the strict genome-wide significance threshold ($5x10^{-8}$) could be an over correction[29]. Taking this into account, we estimated the appropriate Bonferroni-based statistical threshold for our study using the Genetic type 1 Error Calculator (GEC) software[30], which calculates the effective number of independent tests. A significant threshold of $1.14x10^{-6}$ was obtained and, therefore, p-values below this threshold were considered as statistically

significant. A suggestive tier of association ($2.29x10^{-5}$) was also calculated using this software.

The statistical power of the study was estimated by using CaTS Power Calculator for Genetic Studies[31], setting the significance level at $1.14x10^{-6}$ (**Supplementary Table 2**).

Functional annotation

After meta-analysis, the associated SNPs, as well as those in high linkage disequilibirum (LD) ($r^2 > 0.8$) with them (using the European populations of the 1000 Genomes Project Phase III data), were interrogated for potential regulatory function. The HaploReg v4.1 database[32] was used to evaluate whether they were located within regulatory DNA elements, including regions of DNAase hypersensitivity, binding sites of transcription factors or chromatin marks. Their effect on gene expression was also explored using HaploReg v4.1 by means of *in silico* expression quantitative trait locus (eQTL) analysis.

RESULTS

Following SNP and sample quality control, we analysed a total of 187,951 genetic variants in 579 cases of non-infectious and non-anterior uveitis and 3,676 controls of European ancestry.

As shown in **Figure 1**, two signals reached the established significance threshold ($1.14x10^{-6}$) in the inverse-variance meta-analysis including the three cohorts. These peaks lied within the HLA region and the chromosomal region 2q11.2.

HLA associations

After imputation, a high association peak was observed within the HLA class I region (**Supplementary Figure 1**). Specifically, the top associated signal belonged to the classical allele HLA-A*2902 (p=1.04E-16, OR=2.41). The strong association between this HLA allele and BSCR is well established [33], and many patients with this condition (n=78/613) were included in our analysis. Therefore, in order to determine whether this signal was due to a strong association with the BSCR subgroup of patients, we decided to further stratify our analyses of the HLA region by considering the BSCR patients on one hand, and the remaining uveitis patients on the other hand.

HLA analyses in the subgroup of patients with BSRC. When BSRC patients (n=78) were independently analyzed, a stronger association between the classical allele HLA-A*2902 and this disease was evident (p=3.21E-35, OR=50.95) (**Figure 2 and Supplementary Table 3**). Although several SNPs, linked to this classical allele, also showed strong associations, HLA-A*2902 had the greatest effect size (**Supplementary Table 3**). Signals within the HLA-B, -C and class II regions were also observed; however, no independent secondary effects were found after controlling for the effect of HLA-A*2902 (**Figure 2 and Supplementary Table 3**).

Subsequently, we examined whether a specific amino acid position could be responsible for the association observed for this classical allele by means of an omnibus test (**Supplementary Figure 2 and Supplementary Table 4**). The most relevant amino acid

positions for disease risk were the positions 63 and 62 of the HLA-A molecule (P_{LRT}=1.44E-58 and P_{LRT}=3.99E-57, respectively). After performing the omnibus test conditioning on either of these two polymorphic positions, none of the other signals remained significant (**Supplementary Figure 2 and Supplementary Table 4**).

Table 1 shows the most associated amino acid residues in both positions, Leu in position 62 and Gln in position 63 (p=5.33E-35, OR=49.48), which are in complete LD. Both residues showed statistically nearly indistinguishable effects compared to the classical allele HLA-A*2902 (OR=50.95). When we performed a logistic regression analysis adjusting for HLA-A*2902, the association of Leu-62/Gln-63 with BSCR was lost (p=0.111). Similarly, after controlling for either of these two residues, the association between HLA-A*2902 and BSCR also lost its statistical significance (p=0.294) (**Figure 2 andSupplementary Table 3)**.

HLA analyses in non-infectious non-anterior uveitis patients excluding BSCR. Subsequently, we considered the remaining uveitis patients who did not have BSRC (**Supplementary Figure 3and Supplementary Table 5**). After excluding patients with BSCR, no signals in the class I region were observed; thus indicating that the association between HLA-A*2902 and uveitis observed in our initial analysis was due to the strong association of this allele with BSCR. However, a peak within the HLA class II region was evident, with a set of SNPs, located within the *HLA-DRA* gene and in close LD, showing the strongest signal (highest hit rs3129888: p=6.33E-09, OR=1.65) (**Supplementary Figure 3 and Supplementary Table 5**). Additionally, these SNPs were strongly linked to the classical alleles forming the HLA-DR15 haplotype, DRB1*1501, DQA1*0102 and DQB1*0602, which also appeared to be associated with disease (p=3.23E-08, OR=1.72;p=6.86E-08, OR=1.57; and p=3.03E-08, OR=1.73, respectively).

Since an association between the DR15 haplotype and idiopathic intermediate uveitis has been reported previously[34, 35], we reanalysed the HLA region stratifying patients according to the anatomical location of their inflammation (**Supplementary Figure 4**).

In the group of patients with intermediate uveitis, a peak within the HLA class II region was observed, although no significant associations were found. The largest signals corresponded to the set of SNPs located within *HLA-DRA* (highest hit rs7197: p=8.91E-05, OR=1.78). As previously described, the classical alleles DRB1*1501, DQA1*0102 and DQB1*0602, also appeared between the most significant variants (p=8.92-04, OR=1.72; p=9.26E-04, OR=1.58; and p=2.06E-03, OR=1.67, respectively).

Interestingly, when we analyzed the group of patients with posterior uveitis, a similar pattern was evident. Again, the highest signals arose from *DRA* polymorphisms (highest hit rs3135388: p=2.06E-09, OR=2.38) and the DRB1*1501 (p=2.93E-09, OR=2.36) and DQB1*0602 (p=8.08E-09, OR=2.30) alleles. Indeed, in this subgroup of patients, these class II signals reached the genome-wide significance level.

Finally, when we analyzed the last subgroup of uveitis patients, those with panuveitis, we did not observe any peak within the HLA region.

HLA analyses in patients with intermediate and posterior uveitis. Since a similar pattern of association between HLA-DR and -DQ alleles and either intermediate and posterior uveitis was observed, we next combined both subgroup of patients to better define the association between this HLA region and these clinical phenotypes. As expected, the strongest signals corresponded to *HLA-DRA* variants (highest hit rs7197: p=2.07E-11, OR=1.99) and the HLA-DRB1*1501 classical allele (p=1.16E-10, OR=2.08) (**Figure 3 and Supplementary Table 6**). We next carried out a step-wise conditional logistic regression analysis to identify HLA alleles that independently influenced the susceptibility. No additional independent associations were observed after conditioning on either of the top signals, rs7197 or DRB1*1501 (**Figure 3 and Supplementary Table 6**).

After performing the omnibus test, the positions 133 and 142 of the HLA-DRβ1 molecule (which are completely linked) appeared to be the most associated with posterior and intermediate uveitis (P_{LRT}=1.64E-09) (**Supplementary Figure 5 andSupplementary Table 7**). All the remaining associated positions were explained by linkage to HLA-DRβ1

133/142 after performing the conditioned omnibus test (**Supplementary Figure 5 andSupplementary Table 7**).

Table 2 shows the most associated amino acid residues with intermediate and posterior uveitis. Positions 133 and 142 were biallelic in the analysed cohort, with two possible amino acid residues present at each of them, leucine and arginine in position 133 and valine and methionine in position 142. Of these, Leu-133 and Met-142 appeared to confer risk to the disease (p=4.79E-10, OR=1.94). Interestingly, these amino acids correlated with two other residues of the HLA-DRβ molecule, proline in position 11 and arginine in position 13 (**Table 2**). Therefore, the conditional analysis adjusting for each of the amino acid residues (Leu-133, Met-142, Pro-11 or Arg-13) could not statistically distinguish which of them was driving the effect.

Finally, functional annotation analysis indicated that most of the SNPs linked to the *HLA-DRA* gene (including the SNP with the strongest association, rs7197) have a regulatory role in immune cell lines, modulating the expression of a large number of genes, including DRB1 and DQB1 (**Supplementary Table 8**).

Non-HLA associations

Outside the HLA region, a novel signal was observed in chromosome 2q11.2 (**Figure 1**). The rs7608679 genetic variant, located in an intergenic region between the *MAP4K4* (mitogen-activated protein kinase kinase kinase kinase 4) and *IL1R2* (interleukin 1 receptor type 2) genes, reached the established significance threshold in the meta-analysis (p=8.38E-07, OR=1.42) (**Table 3**). In addition, several other genetic variants in tight LD with it also showed strong associations. However, stepwise logistic regression analyses, conditioned on the most highly associated polymorphism, showed there were no other variants with independent effects (**Figure 4**).

When we evaluated the potential regulatory role of rs7608679 and its proxies, several SNPs (rs13011687, rs12473090, rs12990046 and rs1541435) overlapped with histone

marks enriched at promoters and enhancers in different immune cell lines (monocytes, neutrophils and primary B cells) (**Table 4**). Furthermore, all of them appeared to influence the expression levels of both the *IL1R2* and the *MAP4K4* genes in whole blood.

Finally, we also evaluated signals reaching our suggestive tier of association ($2.29x10^{-5}$). Genetic variants in five genetic regions passed this statistical threshold (**Figure 1 and Table 3**). The third strongest signal belonged to a SNP (rs76649453) in an intronic region of the *KIAA1109* locus (p=4.07E-06, OR=2.31). This gene is located within a large haplotype block encompassing the autoimmunity-associated genes *IL2* and *IL21*. Suggestive associations were also found for SNPs in close proximity to *RCL1*/*JAK2* (RNA terminal phosphate cyclase like 1/janus kinase 2) (rs7862852: p=8.82E-06, OR=1.35), *CASP10*/*TRAK2* (caspase 10/trafficking kinesin protein 2) (rs17672977: p=1.02E-05, OR=2.07), *ERC2* (ELKS/RAB6-interacting/CAST family member 2) (rs13098621: p=1.23E-05, OR=1.50) and *HHEX*/*EXOC6* (hematopoietically expressed homeobox/exocyst complex component 6) (rs11187157: p=1.96E-05, OR=1.33).

For all these *loci*, except for *ERC2*, the top associated SNPs, or those showing high LD with them (r^2> 0.8), seemed to have potential functional roles (**Table 4**). Enrichment for histone marks, Dnase hypersensitive sites and/or eQTLs were evident using publicly available functional annotation data. Interestingly, the lead SNP within the *HHEX/EXOC6* region influenced gene expression levels of *EXOC6* and *KIF11* (kinesin family member 11). Regarding *CASP10/TRAK2*, several proxy SNPs correlated with the expression of two genes located in the same haplotype block, *CFLAR* (CASP8 and FADD like apoptosis regulator) and *PPIL3* (peptidylprolyl isomerase like 3). Finally, the associated SNP within the *RCL1/JAK2 locus* was highly correlated with the expression of *JAK2* in whole blood.

DISCUSSION

By conducting the first large-scale genotyping study in non-infectious uveitis, we have confirmed the HLA locus as the most strongly associated region with this inflammatory condition. We have explored subphenotype associations, both shedding light into the previously reported HLA signals and identifying possible mechanisms by which these molecules are involved in the development of uveitis. Furthermore, outside the HLA region, we have detected a new risk *locus*, *MAP4K4*/*IL1R2*, and five suggestive associations with *KIAA1109*, *RCL1*/*JAK2*, *CASP10*/*TRAK2*, *ERC2* and *HHEX*/*EXOC6*.

The correlation between BSCR and the classical allele HLA-A*2902 represents one of the strongest associations between the HLA region and disease reported to date[33]. In this regard, our results suggest that this effect could be driven by the amino acid Leu62/Gln63. Indeed, in our study cohort, both residues, Leu62 and Gln63, are specific to the classical alleles HLA-A*2902 and *2901. The lack of association between HLA-A*2901 and BSCR in our dataset (p=0.201) was probably due to the lower frequency of this classical allele (0.002) compared with HLA-A*2902 (0.057). Indeed, a nominal association between HLA-A*2901 and BSCR was reported in a previous GWAS[20], in which the relative effect sizes for these classical alleles were entirely consistent with our data. It should be noted that the amino acid positions 62 and 63 of the HLA-A protein are likely to be involved in the antigen presentation process. On one hand, the position 62 within the antigen binding site is positioned to make direct contact with the T cell receptor[36], whereas the position 63 faces into the antigen binding site and is likely to be involved in peptide binding[36, 37]. Therefore, these amino acid positions could be responsible for the effect of the HLA-A molecule on the BSCR susceptibility.

Our study confirms the previously reported association between the HLA-DR15 haplotype and idiopathic intermediate uveitis[34, 35]. Interestingly, this haplotype represents the primary HLA genetic susceptibility factor for multiple sclerosis (MS) [38], an immune-mediated disease often associated with intermediate uveitis [39]. However, our data revealed that the classical alleles forming this haplotype (DRB1*1501, DQA1*0102 and

DQB1*0602) have an even stronger influence on susceptibility to posterior uveitis compared with the intermediate form of the disease. The combined analysis of both intermediate and posterior uveitis patients pointed toward positions 133 and 142 of the HLA-DRβ1 molecule as the most relevant for disease susceptibility. None of these positions have been reported to be involved in antigen presentation; however, amino acid residues conferring risk to the disease (Leu-133 and Met-142) were almost completely linked to Pro-11 and Arg-13. Both proxy positions lie within the peptide binding groove of the HLA-DR molecule and therefore, a functional role can be more reliably ascribed to them. Indeed, these same amino acid positions have been reported to be the most relevant for systemic lupus erythematosus susceptibility[40]. Additionally, a previous fine-mapping of the HLA region in MS identified the position 71 of DRβ1 as the most significant conferring risk[41]. This is also located in the peptide groove of the HLA-DR molecule and is tightly correlated with positions 11 and 13 (r^2=0.90). Indeed, in our dataset, the amino acid alanine in position 71 also showed high statistical significance, as shown in **Supplementary Table 6**.

On the other hand, the highest signals associated to intermediate and posterior uveitis belonged to a set of highly linked genetic variants located in the *HLA-DRA* gene. Many of these SNPs appeared to act as eQTLs affecting the expression levels of a number of genes, some of which encodes HLA molecules, such as *DQB1* and *DRB1*. Therefore, a potential functional effect of these polymorphisms on the pathogenesis of posterior and intermediate uveitis cannot be discounted. In this instance, polymorphisms of the HLA region in this subgroup of patients could be altering the expression of certain genes rather than directly influencing the antigen presentation process. Nevertheless, the high LD between the associated SNPs, classical alleles and amino acid residues makes it difficult to determine the true causal variant.

Outside the HLA region, we identified the *MAP4K4/IL1R2* locus as a new genetic risk factor for non-infectious non-anterior uveitis. *In silico* eQTL analysis showed several proxy SNPs affecting the expression levels of these two genes in whole blood. *MAP4K4*

encodes a member of the serine/threonine protein kinase family, which is involved in the tumour necrosis factor-alpha (TNF-α) signalling pathway, whereas *IL1R2* encodes a cytokine receptor that inhibits the activity of its ligands (IL-1A, IL-1B and IL1R1). Both proinflammatory cytokines, TNF-α and IL-1, have been detected in the eye during active inflammation[42]. Interestingly, two recent clinical trials have demonstrated an effective response to adalimumab treatment, a TNF-α inhibitor, in patients with non-infectious intermediate, posterior, or panuveitis [43, 44]. Similarly, treatment with gevokizumab, a monoclonal antibody inhibiting the activation of IL-1 receptors, has also shown therapeutic efficacy in patients with Behçet's disease uveitis [45]. Moreover, this region has been previously associated with ankylosing spondylitis (AS)[46], an inflammatory disorder that frequently presents acute anterior uveitis (30-40% of AS patients).

Evidence for eQTLs was found for three of the five *loci* showing suggestive associations with uveitis and may help annotate these signals. In this regard, the association observed in the *HHEX/EXOC6* region seemed to be due to an eQTL influencing the expression of *EXOC6* and *KIF11*. *EXOC6* is one of three genes included in a chromosomal microdeletion leading to an autosomal dominant form of non-syndromic optic nerve aplasia[47]; whereas mutations in *KIF11* cause an autosomal dominant disorder characterised by chorioretinopathy[48]. SNPs within the *CASP10/TRAK2* locus were found to affect the expression of two genes located in the same haplotype block; *CFLAR*, a regulator of apoptosis, and *PPIL3*, involved in protein folding. Finally, eQTL analysis showed that *JAK2* was the most likely causal gene within the *RCL1/JAK2 locus*. *JAK2* encodes a signal transducer that plays an important role in the differentiation of Th1 and Th17 lymphocytes and both cell types are heavily implicated in the pathogenesis of autoimmune uveitis [49].

In summary, our study has revealed new associations with non-infectious uveitis, both within and outside the HLA region. The identification of genetic risk factors influencing this disorder will further elucidate the complex mechanisms underlying uveitic conditions. Further studies in larger cohorts will be desirable in order to confirm the detected signals.

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Table 1. Amino acid residues of the HLA-A molecule showing the strongest associations with birdshot chorioretinopathy.

			Residue frequency		Meta-analysis		Spain		Netherlands	
Amino acid residue	**Binding Pocket**	**Classical HLA Alleles**	**Birdshot**	**Controls**	**P-value**	**OR [95% CI]**	**P-value**	**OR [95% CI]**	**P-value**	**OR [95% CI]**
Leu62	Yes	*29:01 and *2902	0.519	0.059	5.33E-35	49.48 [26.63-91.93]	3.22E-21	25.56 [13.05-50.03]	1.08E-12	219.20 [49.72-966.80]
Gln63	Yes									

OR, odds ratio; CI, confidence interval.

Table 2. Amino acid residues of the HLA-DRβ1 molecule showing the strongest associations with intermediate and posterior uveitis.

				Residue frequency		Meta-analysis		Spain		Netherlands		UK	
Amino acid residue	**Binding pocket**	**r^2 with Pro11**	**Classical HLA alleles**	**IU+PU**	**Controls**	**P-value**	**OR [95% CI]**	**P-value**	**OR [95% CI]**	**P-value**	**OR [95% CI]**	**P-value**	**OR [95% CI]**
Pro11	Yes	NA	15:01, 15:02 and 15:03	0.210	0.131	4.43E-10	1.94 [1.58-2.40]	5.12E-09	2.26 [1.72-2.98]	0.091	1.53 [0.93-2.49]	0.015	1.75 [1.12-2.75]
Arg13	Yes	1		0.210	0.131	4.43E-10	1.94 [1.58-2.40]	5.12E-09	2.26 [1.72-2.98]	0.091	1.53 [0.93-2.49]	0.015	1.75 [1.12-2.75]
Leu133	No	0.995		0.210	0.132	4.79E-10	1.94 [1.58-2.39]	5.55E-09	2.26 [1.72-2.97]	0.090	1.53 [0.94-2.49]	0.016	1.74 [1.11-2.74]
Met142	No	0.995		0.210	0.132	4.79E-10	1.94 [1.58-2.39]	5.55E-09	2.26 [1.72-2.97]	0.090	1.53 [0.94-2.49]	0.016	1.74 [1.11-2.74]

NA, not applicable; OR, odds ratio; CI, confidence interval; IU, intermediate uveitis; PU, posterior uveitis.

Table 3. Non-HLA loci associated with non-infectious non-anterior uveitis at the established significance level ($p<1.14\times10^{-6}$) (bold) and at the suggestive significance level ($p<2.29\times10^{-5}$).

				Allele frequency							
				Spain		Netherlands		UK		Meta-analysis	
Chromosome	***Loci***	**Most significant SNP**	**Minor allele**	**Uveitis**	**Controls**	**Uveitis**	**Controls**	**Uveitis**	**Controls**	**P-value**	**OR [95% CI]**
2	***MAP4K4 / IL1R2***	**rs7608679**	**C**	**0.301**	**0.234**	**0.357**	**0.240**	**0.277**	**0.246**	**8.38E-07**	**1.42**
4	*KIAA1109*	rs76649453	A	0.047	0.018	0.040	0.020	0.027	0.015	4.07E-06	2.31
9	*RCL1/JAK2*	rs7862852	C	0.388	0.327	0.401	0.375	0.464	0.346	8.82E-06	1.35
2	*CASP10/TRAK2*	rs17672977	G	0.033	0.020	0.075	0.033	0.055	0.024	1.02E-05	2.07
3	*ERC2*	rs13098621	A	0.160	0.108	0.147	0.102	0.150	0.115	1.23E-05	1.50
10	*HHEX/EXOC6*	rs11187157	C	0.442	0.388	0.528	0.445	0.509	0.448	1.96E-05	1.33

OR, odds ratio; CI, confidence interval.

Table 4. Functional annotation for the non-HLA genetic variants showing associations (p<1.14x10-6) or suggestive associations (p<2.29x10-5) with non-infectious non-anterior uveitis. The most highly associated polymorphisms and those in tight linkage disequilibrium with them (r^2>0.8) were used for the analysis.

Mapped genes	Lead SNP	Location	eQTL	Promoter histone marks	Enhancer histone marks	Dnase hypersensitivity
MAP4K4 / IL1R2	rs7608679	Intergenic	*IL1R2, MAP4K4*	Yes	Yes	No
KIAA1109	rs76649453	Intronic	-	Yes	Yes	No
RCL1 / JAK2	rs7862852	Intergenic	*JAK2*	Yes	Yes	No
CASP10/TRAK2	rs17672977	Intronic	*PPIL3, CFLAR*	Yes	Yes	Yes
ERC2	rs13098621	Intronic	-	No	No	No
HHEX/EXOC6	rs11187157	Intergenic	*EXOC6, KIF11*	Yes	Yes	Yes

eQTL, expression quantitative trait loci; SNP, single nucleotide polymorphism.

FIGURE LEGENDS

Figure 1. Manhattan plot of the meta-analysis of the three analysed cohorts. The red and blue lines represent the established ($p=1.14\times10^{-6}$) and the suggestive ($p=2.29\times10^{-5}$) significance levels, respectively.

Figure 2. Manhattan plot representing the results of the conditional logistic regression analysis of the HLA region in patients with birdshot chorioretinopathy. (A) Unconditioned test of the HLA region. (B) Results after conditioning on the HLA-A*2902 classical allele. (C) Results after conditioning on the amino acid residue Leu62/Gln63. The red/green color gradient represents the effect direction of each analyzed variant (red for risk and green for protection). The size of the diamonds indicates the degree of linkage disequilibrium with the classical allele HLA-A*2902. The red line represents the established significance threshold ($p<1.14x10^{-6}$).

Figure 3. Manhattan plot representing the results of the conditional logistic regression analysis of the HLA region in patients with posterior and intermediate uveitis. (A) Unconditioned test of the HLA region. (B) Results after conditioning on the rs7197 polymorphism. (C) Results after conditioning on the HLA-DRB1*1501 classical allele. The red/green color gradient represents the effect direction of each analyzed variant (red for risk and green for protection). The size of the diamonds indicates the degree of linkage disequilibrium with the classical allele HLA-DRB1*1501. The red line represents the established significance threshold($p<1.14x10^{-6}$).

Figure 4. Regional plot of the non-HLA *locus* associated with non-infectious non-anterior uveitis in the meta-analysis. (A) Unconditioned analysis. (B) Results after conditioning on the lead SNP (rs7608679).