A genome-wide association study identifies a functional ERAP2 haplotype associated with birdshot chorioretinopathy

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Birdshot chorioretinopathy (BSCR) is a rare form of autoimmune uveitis that can lead to severe visual impairment. Intriguingly, >95% of cases carry the HLA-A29 allele, which defines the strongest documented HLA association for a human disease. We have conducted a genome-wide association study in 96 Dutch and 27 Spanish cases, and 398 unrelated Dutch and 380 Spanish controls. Fine-mapping the primary MHC association through high-resolution imputation at classical HLA loci, identified HLA-A*29:02 as the principal MHC association (odds ratio (OR) = 157.5, 95% CI 91.6–272.6, \( P = 6.6 \times 10^{-274} \)). We also identified two novel susceptibility loci at 5q15 near ERAP2 (rs7705093; OR = 2.3, 95% CI 1.7–3.1, for the T allele, \( P = 8.6 \times 10^{-8} \)) and at 14q32.31 in the TECPR2 gene (rs150571175; OR = 6.1, 95% CI 3.2–11.7, for the A allele, \( P = 3.2 \times 10^{-8} \)). The association near ERAP2 was confirmed in an independent British case–control samples (combined meta-analysis \( P = 1.7 \times 10^{-6} \)). Functional analyses revealed that the risk allele of the polymorphism near ERAP2 is strongly associated with high mRNA and protein expression of ERAP2 in B cells. This study further defined an extremely strong MHC risk component in BSCR, and detected evidence for a novel disease mechanism that affects peptide processing in the endoplasmic reticulum.

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INTRODUCTION

Birdshot chorioretinopathy (BSCR; MIM605808) is a rare ocular disorder that has a very strong HLA association, with >95% of cases carrying the HLA-A29 allele (1–3). BSCR manifests as a severe progressive intraocular inflammation of the posterior eye segment, typically leading to extensive retinal atrophy and blindness (4,5). The disease is most often observed in middle-aged and elderly individuals of European descent and has a slight female predominance (3,6). Current treatment strategies include various immunosuppressive medications but fail to prevent or cease the retinal atrophy. HLA-A29 is relatively common in European populations, but only a tiny subset of HLA-A29-positive individuals develops BSCR (6). It is therefore assumed that other genes than HLA-A and additional exogenous factors are involved in the development of the disease.

RESULTS

To identify BSCR susceptibility genes and to fine-map the association within the MHC region, we conducted a genome-wide association study in 96 Dutch and 27 Spanish BSCR cases and 398 unaffected Dutch and 380 Spanish controls from European ancestry. After quality control and genome-wide SNP imputation, we analyzed a total of 9,932,851 SNPs in 117 cases and 693 controls (Materials and Methods; Supplementary Material, Fig. S1). We did not see evidence for population stratification. After quality control and genome-wide SNP imputation, we analyzed a total of 9,932,851 SNPs in 117 cases and 693 controls (Materials and Methods; Supplementary Material, Table S1). We did not see evidence for population stratification (AOGC = 0.96; Supplementary Material, Fig. S2).

The strongest association signal was located within the HLA class I region for rs142115394 (P = 6.3 × 10^-15). We next imputed and tested classical alleles and amino acid polymorphisms in HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1 and HLA-DRB1 following a recently described procedure (7). The strongest association of all variants tested mapped to the classical HLA-A*29:02 allele (P = 6.6 × 10^-74) (Fig. 1 and Table 1; Supplementary Material, Table S1). We found HLA-A*29:01 to be nominally associated (P = 0.02) but not significant once the A*29:02 effect was accounted for (P = 0.093), indicating that the classical A29 effect can be primarily attributed to the A*29:02 allele. No polymorphic amino acid position could explain the data better than A*29:02 (Supplementary Material, Table S2). Controlling for the HLA-A*29:02 effect, no other classical HLA-A allele was significantly associated (best adjusted P = 3.0 × 10^-4 for HLA-A*30:01).

Outside the MHC region, a novel association was found at 5q15 for rs7705093 (P = 8.6 × 10^-8) within the LNPEP gene encoding leucyl/cystinyl aminopeptidase (Table 1). This association was further strengthened in 30 British cases and 2793 control samples (combined meta-analysis P = 1.7 × 10^-9). Due to linkage disequilibrium (LD), several other SNPs across the locus also showed strong association (Supplementary Material, Fig. S3). This locus includes the three members of the M1 family of aminopeptidases; ERAP1, ERAP2 and the LNPEP gene (8). ERAP1 and ERAP2 encode endoplasmic reticulum aminopeptidase 1 and 2, respectively, and are both involved in antigen processing and presentation by HLA class I molecules to T cells (8–10). The LNPEP gene encodes leucyl/cystinyl aminopeptidase that specifically functions in peptide trimming for cross-presentation in dendritic cells (11).

To explore the biological relevance of the associated variation in the 5q15 locus, we first investigated whether the associated SNPs also influence expression levels of these three genes using the expression quantitative trait loci (eQTL) database GeneVar (12). The strongest QTL for ERAP2 expression in LCL cells (P = 2.0 × 10^-114 from Genevar) is observed for rs10044354 (P = 1.2 × 10^-7 for association with BSCR), which is almost in perfect LD with the lead SNP rs7705093 (r^2 = 0.98 in the controls; Supplementary Material, Fig. S4). These SNPs are statistically indistinguishable with respect to BSCR risk (P > 0.2). The rs10044354 (and SNPs in LD with rs10044354) appeared to have no significant impact on LNPEP or ERAP1 expression (P > 0.2 from GeneVar).

Subsequently, we tested if this variant is also correlated to protein levels of ERAP2 in B-cell lines from individuals from the Centre d’Etude du Polymorphisme Humain (CEPH) panel (13) and five BSCR patients. Indeed, homozygous carriers for
the rs10044354 C allele showed little or no ERAP2 protein expression in a quantitative western blot analysis, whereas hetero- or homozygous carriers of the rs10044354T risk allele showed higher protein levels of ERAP2 ($P = 0.009$) in cases and controls (Fig. 2). In contrast, we did not observe an effect of this SNP on LNPEP expression, consistent with the mRNA findings from GeneVar (Fig. 2, bottom).

Another novel association was detected at 14q32.31 in the TECPR2 gene (rs150571175, $P = 3.2 \times 10^{-8}$) (Table 1; Supplementary Material, Fig. S3). However, this association could not be confirmed in the British cohort ($P = 1.0$; combined meta-analysis $P = 1.6 \times 10^{-7}$). The protein encoded by TECPR2 (tectonin beta-propeller repeat-containing 2) interacts with Atg8 orthologs and functions in autophagosome accumulation for autophagy (14).

Because BSCR is considered an autoimmune disease and shares signatures of T helper 17-cell responses with other autoimmune disorders (15–19), we hypothesized that known risk loci for other autoimmune diseases may also be associated with BSCR. Therefore, we tested 289 bona fide non-HLA SNP associations in autoimmune disorders (Supplementary Material, Table S3). For all BSCR Dutch and Spanish cases and controls, we computed a weighted genetic risk score based on the observed number of risk alleles and the known effect sizes for each autoimmune disease separately. We performed a similar test for SNPs associated to either height or LDL levels as two control traits. After correction for multiple testing, we obtained no significant association between increased risk scores and BSCR risk for any of the autoimmune diseases tested, except for wGRS Psoriasis (higher for BSCR) and LDL (lower in BSCR) (Supplementary Material, Table S4). However, these associations were only nominally significant in the Dutch cohort but not in the Spanish cohort. Although this would suggest that BSCR shares no additional susceptibility loci with other autoimmune diseases, it is possible that the sample size and power was simply too low to detect such an overlap.

**DISCUSSION**

To date, the association of HLA-A29 with BSCR is the strongest association described between an HLA class I allele and human disease (2,6). Although this association has been known for over three decades (1), lack of understanding of the contribution of HLA-A29 to the pathogenesis of BSCR, impeded it to become an absolute criterion for diagnosis (22). HLA-A29 can be subdivided in at least 17 subtypes (23), but most common in Caucasians are the subtypes HLA-A*29:01 and HLA-A*29:02 that have both been most associated with BSCR (24). However, the imputation analyses of the HLA region revealed that the nominal association of HLA-A*29:01 disappeared once the A*29:02 effect was accounted for, indicating that the classical A29 effect in BSCR can be primarily attributed to the A*29:02 allele and comes with unusually large effect size in terms of explained variance (Nagelkerke’s index $r^2$ [controlled for top 4 PCs and cohorts] = 0.595). However, since HLA-A29 is relatively common in European populations (7%) and very few HLA-A29-positive individuals develop BSCR (6), other additional genetic or exogenous factors are required for the development of the disease. Here, we provide evidence for an
association with ERAP2 and speculate about a possible association of TECPR2.

The ERAP2 gene is located on chromosome 5 and is positioned between the closely related ERAP1 and LNPEP genes (8,9). The ERAP2 gene encodes for an aminopeptidase located in the endoplasmic reticulum (ER) that is involved in the final processing and presentation of antigenic precursors, together with the closely related and better characterized ERAP1 (10). In fact, ERAP2 and ERAP1 were shown to form a heterodimer to combine their restricted sequence specificities for peptide trimming (10). Notably, some antigenic peptides, including the immunodominant epitope derived from HIV gag protein, have been shown to be solely dependent upon ERAP2 trimming (25).

Recent genome-wide association studies have implicated a role for ERAP1 and ERAP2 in several HLA-associated autoimmune diseases such as psoriasis (26), ankylosing spondylitis (20,21) and Crohn’s disease (27), or juvenile idiopathic arthritis (28), suggesting an important functional role for these genes in the immunopathology of autoimmune diseases. Of note, all these ERAP1- or ERAP2-associated diseases share a major HLA class I association that contrasts the major HLA class II association found in many other autoimmune diseases not associated to ERAP1 or ERAP2. In this respect, our study substantiates an emerging concept as we also found a strong link between ERAP2 and BSCR, an autoimmune disease affecting the eye that manifests almost exclusively in HLA-A29-positive Caucasians.

Similar to HLA genes, ERAP2 has been shown to undergo balancing selection, which maintains two main haplotypes A and B, with highly differential protein expression due to alternative mRNA splicing (29). We discovered that the SNPs associated with BSCR at 5q15 also serve as tags for these haplotypes, with high expression levels of ERAP2 in BSCR patients and no or low expression in homozygous negative healthy individuals. Based on our results, and given the fact that ERAP2 has nonoverlapping trimming capacities (10) with ERAP1, it is likely that ERAP2 has a specific contribution to the immunopathology of BSCR, by differential generation or destruction of immunodominant epitopes capable of binding to HLA-A29. Modulation of the antigen processing and presentation pathway by targeting ERAP2 in BSCR is particularly of interest since, in contrast to ERAP1, a rather high frequency of ERAP2-deficient individuals is maintained in the population without any clear adverse effects on their immunity and healthy (29). However, the efficacy of selective inhibition of ERAP2 in BSCR, while remaining ERAP1 function, remains to be clarified and involves the use of novel inhibitors that targets this family of pleiotropic aminopeptidases (30).

We found a significant association for TECPR2 in the Dutch and Spanish cohorts, but these findings could not be replicated in British patients. TECPR2 encodes for TECPR2 (tectonin beta-propeller repeat-containing 2) that interacts with Atg8 orthologs and is a positive regulator of autophagy and functions in autophagosome accumulation (14). A mutation in the TECPR2 gene was recently implicated in hereditary spastic paraparesis (31). The eye maintains an unconventional form of autophagy that is crucial for the function and health of the photoreceptors in the retina (32,33). Interestingly, interfering with autophagy by rapamycin, a potent inducer of autophagy, can paradoxically reduce or exacerbate uveitis (34,35). Moreover, autophagy-related genes have also been associated with other autoimmune diseases, including SLE and Crohn’s disease (36,37). Further investigation of the association of the TECPR2 gene in other BSCR populations is necessary to clarify its possible contribution to the pathogenesis of BSCR.

In conclusion, we identified HLA-A*29:02 as the principal MHC association and report variants near the ERAP2 gene at 5q15 that predispose to BSCR. Expression studies indicate that the disease-associated variants at 5q15 correlate to mRNA and protein expression levels of ERAP2, suggesting that differential peptide processing and antigen presentation to T cells is an essential disease mechanism for BSCR.

MATERIALS AND METHODS

Subject collection
A total of 96 unrelated Dutch BSCR cases, 27 Spanish BSCR cases and 398 unrelated Dutch healthy controls and 380 Spanish controls, all from European ancestry, are enrolled in this study. BSCR diagnosis was based on criteria established by international consensus (22). Dutch cases were recruited at the Department of Ophthalmology at the University Medical Center Utrecht, the Eye Hospital Rotterdam and Radboud University Nijmegen Medical Center, the Netherlands. These three institutes represent the largest registry of BSCR patients in the Netherlands; we estimate that >90% of all BSCR cases in the Netherlands are included in this cohort. Spanish cases were recruited at the department of ophthalmology at the Hospital Clinico San Carlos, Madrid, Hospital Clinico San Cecilio, Granada, Hospital de Cruces, Bilbao, Hospital Clinic Barcelona, Hospital Carlos Haya, Malaga and Hospital Universitario, Leon.
Dutch controls were collected at UMC Utrecht and Spanish controls were recruited from the National bloodbank. For replication, we included an additional 30 UK BSCR cases recruited at the department of ophthalmology at the Moorfields Eye Hospital NHS Foundation Trust, London. As controls, we used genotype data from the UKBS and 58BC controls, which are part of the WTCCC study (38). The UKBS cohort contained 1397 control individuals selected from a UK sample of blood donors, and the 58BC contained 1396 control individuals from the 1958 British Birth Cohort (39). This study was performed in compliance with the guidelines of the Declaration of Helsinki and has the approval of the local Institutional Review Boards. Study details were explained to all cases and controls, and the collection of blood was carried out after obtaining written informed consent from each participant.

Genotyping, quality control and association analysis
All Dutch and Spanish samples were genotyped using the Illumina Human OmniExpress BeadChip, which contains 730 525 SNPs. Data for the British 1958 Birth Cohort and UKBS were obtained from the European Genome-Phenome Archive at https://www.ebi.ac.uk/ega/. These individuals were genotyped using the Affymetrix GeneChip 500K platform and the Illumina Immunochip platform. We used PLINK (40) to clean the genotype data using standard quality control parameters: we removed SNPs with low frequency (<0.02), out of Hardy–Weinberg equilibrium ($P < 10^{-6}$), or with excess missingness (>5%). We also checked for relatedness and inconsistent gender. Principal components analysis was performed to detect population stratification. Overall, we excluded two Dutch and four Spanish cases, and four Dutch and 85 Spanish controls, and kept a total of 393 831 SNPs for subsequent analyses. Imputation of untyped SNPs was performed using Minimac (41). As reference panel, we used the 998 phased haplotypes from the Genome of the Netherlands Project release 4 (42). As previously described (7), we imputed classical alleles and amino acid polymorphisms in HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1 and HLA-DRB1 at a four-digit resolution, as well as an additional 3,117 SNPs across the MHC, using a large reference panel of 2,767 individuals of European descent. We imputed cases and controls together. For the MHC, we removed SNPs with low frequency ($<1\%$ or with imputation quality (info score) $<0.01$ were excluded from analyses. Genome-wide distribution of the test statistic indicated no evidence for population stratification (QQ plots Supplementary Material, Fig. S2). As parametric one-way ANOVA; Kruskal–Wallis test with Dunn’s multiple-comparison post hoc test in GraphPad Prism 5 software. Statistical significance was accepted at a significance level of $P < 0.05$.

protein expression assays
We assessed ERAP2 and LNPEP protein levels in EBV transformed B cells (EBV-LCL), from 19 individuals from the CEPH panel and EBV-LCLs generated from peripheral blood mononuclear cells of five BSCR patients, by western blotting (13). From the CEPH panel, we selected six heterozygotes for the C allele, six heterozygotes and seven homozygotes for the T allele (NA07022, NA07029, NA07056, NA07345, NA07357, NA10838, NA10846, NA10854, NA10863, NA11839, NA12003, NA12056, NA12144, NA12145, NA12239, NA12249, NA12812, NA12815 and NA12872). Protein extracts (20 μg/lane) from EBV-LCL cells were separated on a 4–12% NuPage Bis–Tris gel (Invitrogen) and transferred to a PVDF membrane. Proteins were detected using a 1:2500 dilution of primary antibody (goat anti-ERAP2 polyclonal antibody (AF3830, R&D Systems), mouse anti-ERAP2 polyclonal (ab699037, Abcam), sheep anti-LNPEP polyclonal antibody (AF6386, R&D Systems) and anti-α/β-tubulin monoclonal prepared in Rabbit (#2148, Cell Signaling)). Anti- and anti-rabbit secondary antibodies conjugated to Alexa Fluor 680 (Molecular Probes; 1:2500) and anti-goat secondary antibody conjugated to IRDye800 (Li-Cor; 1:2500) were used to probe primary antibodies. Protein bands were detected and quantified by western blotting with the Odyssey system (Li-Cor). The ratio of the intensity of the ERAP2-α/β-tubulin band of CC-homozygotes to CT-heterozygotes and TT-homozygotes was calculated using ImageJ.

Transcriptional analysis
eQTL analysis was performed based on data available from the Sanger Institute GENEVAR project for lymphoblastoid-cell lines from 726 HapMap 3.0 CEU individuals and three tissue types (LCL cell type was used in the analysis) collected from 856 healthy female twins of the MuTHER resource (12,43). Differences in the levels of ERAP2 in B-cell line between groups according to rs10044354 genotype were analyzed using non-parametric one-way ANOVA; Kruskal–Wallis test with Dunn’s multiple-comparison post hoc test in GraphPad Prism 5 software. Statistical significance was accepted at a significance level of $P < 0.05$.

Genetic risk score
To test the cumulative effect of validated SNPs associated with autoimmune diseases (ankylosing spondylitis, rheumatoid arthritis, Crohn’s disease, ulcerative colitis, psoriasis, vitiligo, type 1 diabetes and multiple sclerosis) (20,26,44–49), we computed a weighted genetic risk score in all cases and controls of our study. We summed the estimated dosages of the known risk alleles (for a given autoimmune disease) weighted by the natural log of the published odds ratios (Supplementary Material, Table S3). We tested the genetic risk scores for each disease as a continuous variable for association to BSCR status using logistic regression, including the top four principal components as covariates. We corrected for multiple testing of 10 traits in total (including height (50–52) and LDL (53,54) as a negative control).

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

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REFERENCES


