Sympathetic ophthalmia (SO) is a bilateral granulomatous panuveitis that can occur after penetrating ocular trauma to one eye or following ocular surgery. It may take several days or even years following the ocular insult before the disease manifests itself. The exciting eye and sympathizing eye are the special terms for the injured eye and the fellow eye, respectively. The incidence of SO has been estimated at 0.03/100,000. These figures may not be reliable in view of the fact that the disease is quite rare and that laboratory tests for its diagnosis are lacking. Although the pathogenesis and etiology of SO are not fully understood, SO has been shown to be associated with VKH disease in earlier studies and also associated with HLA-DR4, 6 with genes encoded by the human leukocyte antigen (HLA) pathways.

Study Population

The study included 114 SO patients and 1230 healthy controls who were recruited at the First Affiliated Hospital of Chongqing Medical University between May 2008 and September 2016. Normal unrelated individuals with comparable age, race, and geographic origin were randomly selected as the study population.
controls. Both controls and patients were Han Chinese. The diagnosis of SO was made according to history and clinical examination.27

**Ethical Considerations**

Informed consent was acquired and signed by all research participants. The experimental research program was agreed by the Clinical Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. The study was executed in accordance with the rules of Helsinki’s Declaration. All experiments were carried out in accordance with the regulations and approved guidelines.

**SNP Choice and Genotypes**

SNPs were chosen as based on previously published studies showing a statistically significant (P < 0.05) association with VKH disease or the occurrence of its clinical manifestations. HaploView 4.2 was used to screen the candidate SNPs through a r² critical value of 0.8, as well as a minor allele frequency (MAF) larger than 0.05. Twenty-four SNPs from 19 genes were identified and included: one SNP (rs231775) of CTLA-4,10 one SNP (rs2227981) of PDCD1,11 one SNP (rs7574865) of STAT4,12 one SNP (rs76481776) of miR-182,22 one SNP (rs310230) of IL-12B,23 three SNPs (rs78377598, rs117633859) of IL23R-C1orf141,24 one SNP (rs2488457) of PTPN22,21 one SNP (rs13210247) of TNIP1,20 one SNP (rs17728383) of MIF,18 two SNPs (rs4705863) of AGT10,26 two SNPs (rs442309, rs224058) of ADO, ZNF365, EGR2,24 two SNPs (rs12569232, rs6540679) of TRAF5,19 one SNP (rs49148855) of TNFAIP3,15 three SNPs (rs3102350, rs310236, rs310241) of JAK1,16 one SNP (rs2501436) of FGR10P,17 one SNP (rs755622) of MIF,18 two SNPs (rs12569232, rs6540679) of TRAF5,19 one SNP (rs13210247) of TRAF5IP2,20 one SNP (rs7728338) of TNIP1,20 one SNP (rs2488457) of PTPN22,21 one SNP (rs76481776) of miR-182,22 one SNP (rs3212227) of IL-12B,23 two SNPs (rs442309, rs224058) of ADO, ZNF365, EGR2,24 two SNPs (rs85777598, rs117633859) of IL23R-C1orf141,24 one SNP (rs6498169) of CLEC16A,25 and one SNP (rs4703863) of AGT10 (Table 1).26

HLA typing was not performed in our SO patients.

**DNA Extraction and Genotyping**

Genomic DNA from blood samples of SO patients and controls were extracted through the QIamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA) and stored in 3.2% sodium citrate-treated tubes at −80°C. Later on, the extracted DNA sample was kept at −20°C. Polymerase chain reaction (PCR) was executed on a 9700 Thermal Cycler of the ABI Gene Amp PCR System based on the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). Genotypes were ascertained through the MassARRAY platform (Sequenom, San Diego, CA, USA) and iPLEX Gold Assay. Genotyping primers were designed through the MassARRAY Assay design software. The experimental data were analyzed with TYPER software version 4.0.

**Statistical Analysis**

The SHEsis website was applied to test for Hardy-Weinberg equilibrium in healthy controls, and the genotype frequencies were approximated by straightforward counting. Differences in allele and genotype frequencies between healthy controls and patients were analyzed using the χ² test and Fisher’s exact correction with the SPSS 19.0 statistical package (version 19.0, SPSS, Chicago, IL, USA). The P value was corrected for multiple comparisons (Pc) using the Bonferroni correction method. A P < 0.05 was regarded as statistically significant.

**RESULTS**

**Characteristics of Patients With SO**

The clinical characteristics, age, and gender distribution of selected SO patients and healthy controls are shown in Table 2. The mean age of SO cases was 43.2 ± 15.2 years (range, 11–70 years), and included 97 males (85.1%) and 17 females (14.9%). The time interval from ocular trauma or surgery to onset of SO ranged from 5 days to 46 years, with 28.1% of the cases

| Table 1. Candidate SNPs Shown in Earlier Studies to be Associated With VKH Disease |
|---|---|---|---|
| Number | SNP | Gene | P Value |
| 1 | rs231775 | CTLA-4 | 3.70 × 10⁻² |
| 2 | rs2227981 | PDCD1 | 1.30 × 10⁻² |
| 3 | rs7574865 | STAT4 | 1.30 × 10⁻² |
| 4 | rs765780 | IL-17F | 3.00 × 10⁻² |
| 5 | rs4754 | OPN | 9.20 × 10⁻³ |
| 6 | rs9494885 | TNFAIP3 | 3.70 × 10⁻³ |
| 7 | rs310230 | JAK1 | 3.70 × 10⁻⁷ |
| 8 | rs310236 | JAK1 | 3.70 × 10⁻⁸ |
| 9 | rs310241 | JAK1 | 3.70 × 10⁻⁹ |
| 10 | rs2301436 | FGR10P | 1.30 × 10⁻² |
| 11 | rs755622 | MIF | 1.30 × 10⁻² |
| 12 | rs12569232 | TRAF5 | 3.70 × 10⁻¹⁰ |
| 13 | rs6540679 | TRAF5 | 3.70 × 10⁻¹¹ |
| 14 | rs13210247 | TRAF3IP2 | 3.70 × 10⁻¹² |
| 15 | rs17728383 | TNIP1 | 9.20 × 10⁻⁴ |
| 16 | rs2488457 | PTPN22 | 3.70 × 10⁻⁴ |
| 17 | rs76481776 | miR-182 | 3.00 × 10⁻⁵ |
| 18 | rs5212227 | IL-12B | 3.00 × 10⁻⁴ |
| 19 | rs442309 | ADO, ZNF365, EGR2 | 9.20 × 10⁻⁴ |
| 20 | rs224058 | ADO, ZNF365, EGR3 | 3.70 × 10⁻¹⁵ |
| 21 | rs78577598 | IL23R-C1orf141 | 1.30 × 10⁻² |
| 22 | rs117633859 | IL23R-C1orf142 | 3.00 × 10⁻⁴ |
| 23 | rs6498169 | CLEC16A | 3.70 × 10⁻¹⁴ |
| 24 | rs4705863 | AGT10 | 3.70 × 10⁻¹⁵ |
Association of a PDCD1 Polymorphism With SO

In this study, we investigated genetic susceptibility to SO using 19 genes that had earlier been identified to be associated with a uveitis entity known as VKH and only showed a significant effect for PDCD1/rs2227981. Individuals carrying the G allele or GG genotype showed protection against developing SO following ocular trauma or surgery. In an earlier study, we showed that PDCD1 was not directly associated with VKH disease itself but that certain PDCD1 gene polymorphisms protected patients from developing the extraocular manifestations of VKH. Why PDCD1 is involved in the extraocular manifestations of VKH, whereas it directly affects SO, is not yet clear. SO is a sight threatening panuveitis and is thought to be mediated by an immune response against a broad range of ocular antigens, although the exact antigens remain to be identified. In contrast to VKH, the autoimmune response in SO may not only be confined to melanocyte associated antigens, but may also involve a T cell-mediated immune response to retinal photoreceptor antigens, such as S antigen and IRBP. This hypothesis is mainly based on the findings in experimental models of uveitis whereby immunization of animals with these antigens can cause ocular disease that resembles SO in humans. The fact that VKH is associated with many more immune response genes than SO may be due to the fact that SO is an autoimmune disease directed against a broader range of autoantigens than VKH.

Many studies on the pathogenesis of SO have focused on the role of cytokines. Gelatinase B, monocyte chemotactic protein-1 (CCL2/MCP-1), and stromal cell-derived factor-1 (CXCL12/SDF-1) were shown to be involved in the formation of granulomas in SO patients by promoting intraocular leukocyte recruitment. Other cytokines such as IL-17, IL-18, IL-23, CCL1, and CXCL11 were also found to contribute to the granulomatous inflammation in SO, whereas IFN-γ and CCL17 may play a role in the nongranulomatous reaction. Others provided evidence showing that TNF-α and inducible nitric oxide synthase (iNOS) may play a role in the nongranulomatous reaction. In contrast to VKH, the autoimmune response in SO may not only be confined to melanocyte associated antigens, but may also involve a T cell-mediated immune response to retinal photoreceptor antigens, such as S antigen and IRBP. This hypothesis is mainly based on the findings in experimental models of uveitis whereby immunization of animals with these antigens can cause ocular disease that resembles SO in humans. The fact that VKH is associated with many more immune response genes than SO may be due to the fact that SO is an autoimmune disease directed against a broader range of autoantigens than VKH.

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Despite the observation that genotypes of HLA-DRB1*04, HLA-DQA1*05, and HLA-A11 have been shown to be associated with SO, further research addressing a possible role for genetic variants of non-HLA immune response genes in SO pathogenesis has been lacking. The study presented here was set up to address this issue. Instead of performing a genome-wide association study (GWAS), we decided to choose a
number of variants in genes that had earlier been shown to have an association with VKH uveitis. An argument for the choice of this set of genes came from the fact that, as mentioned above, both diseases show some resemblance in both the ocular as well as the extraocular features. The association of SO with gene variants of PDCD1 has, to the best of our knowledge, not yet been reported earlier. programmed death 1 (PD-1) molecule is a well-characterized suppressor of B and T cells. As a member of the differentiation CD28/B7 family's cluster, PD-1 carries an immunoreceptor tyrosine-based motif (ITIM) that, once activated, can induce an inhibitory signal attenuating proliferation and T-lymphocyte activation, restrain cytokine secretion, and induce T-cell apoptosis, thereby maintaining peripheral tolerance.\textsuperscript{32} PD-L1 has been shown to be expressed on human ocular cells and can suppress ocular inflammation by inhibiting the release of proinflammatory and Th2 cytokines by activated T cells.\textsuperscript{33} The human gene that encodes PD-1 is located on 2q37.3 and is named PDCD1. PDCD1 gene polymorphisms have been shown to be associated with several autoimmune diseases like VKH disease,\textsuperscript{34,35} systemic lupus erythematosus (SLE),\textsuperscript{34,35,36} rheumatoid arthritis (RA),\textsuperscript{36,37} type 1 diabetes (TID),\textsuperscript{38} multiple sclerosis (MS),\textsuperscript{39} and ankylosing spondylitis (AS).\textsuperscript{40} The protective locus described in our study, rs2227981, is located at exon 5 and may have an impact on gene expression and function. However, the biologic function of this locus has not yet been studied. This SNP rs2227981 has also been identified to play a role in several diseases, such as SLE,\textsuperscript{35} as well as epithelial ovarian,\textsuperscript{41} esophagus,\textsuperscript{42} and lung cancers.\textsuperscript{43}

Our study has a number of limitations. Firstly, owing to sample size limitations, we cannot exclude the possibility of making a statistics type II error. To improve the reliability of our study, we used a very large control group. We furthermore chose a relatively reliable method, MassARRAY platform and iPLEX Gold Assay, for genotyping. The other limitation lies in the fact that the relationship between SO and the candidate genes can still not be fully revealed, because our study only tested a restricted number of candidate gene SNPs and we might have missed many SNPs. A multicenter trial involving a larger number of SO patients and involving GWAS should be performed to address this issue.

In conclusion, comparison of the genetic susceptibility to VKH and SO reveals that both diseases, although sharing some predisposing features, such as the HLA association, are separate clinical entities involving different immunogenetic backgrounds.

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References


