**JAK1, but Not JAK2 and STAT3, Confers Susceptibility to Vogt–Koyanagi–Harada (VKH) Syndrome in a Han Chinese Population**

Ke Hu,1 Shengping Hou,1 Fuzhen Li,1 Qin Xiang,1 Aize Kijlstra,2 and Peizeng Yang1

1The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, People’s Republic of China
2University Eye Clinic Maastricht, Maastricht, Limburg, The Netherlands

**Citation:** Hu K, Hou S, Li F, Xiang Q, Kijlstra A, Yang P, JAK1, but not JAK2 and STAT3, confers susceptibility to Vogt–Koyanagi–Harada (VKH) syndrome in a Han Chinese population. Invest Ophthalmol Vis Sci. 2013;54:3360–3365. DOI:10:1167/iovs.13-11615

**METHODS.** A case-control study was performed in 737 Chinese VKH syndrome patients and 809 healthy controls from a Han Chinese population. The genotypes of three single-nucleotide polymorphisms (SNPs) (rs10758669, rs7857730, rs10119004) in JAK1 were performed using an SNP genotyping system. Three SNPs (rs10758669, rs7857730, rs10119004) in JAK2 and four SNPs (rs6503695, rs744166, rs2293152, and rs12948909) in STAT3 were analyzed using polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP). Hardy–Weinberg equilibrium (HWE) was tested using the \( \chi^2 \) test. Genotype frequencies were estimated through direct counting. Allele and genotype frequencies were compared between patients and controls using the \( \chi^2 \) test.

**RESULTS.** There was no deviation from the HWE in all controls tested. Three SNPs, including rs510230, rs510236, and rs510241, in JAK1 were significantly associated with VKH syndrome (\( P = 0.008, 0.005, 0.001 \), respectively). None of the tested SNPs of JAK2 and STAT3 was associated with VKH syndrome. Stratification analysis according to headache, dysacusis, alopecia, poliosis, and vitiligo for VKH syndrome did not reveal an association.

**CONCLUSIONS.** These results suggest that JAK1 genetic polymorphisms, but not JAK2 and STAT3, are associated with the susceptibility to VKH syndrome.

Keywords: JAK1, JAK2, STAT3, VKH, polymorphisms

Vogt–Koyanagi–Harada (VKH) syndrome is one of the most common uveitis entities in China. The major clinical manifestations of VKH syndrome include headache, dysacusis, alopecia, poliosis, and vitiligo. Although the etiology of VKH syndrome remains unclear, a number of studies have revealed that an autoimmune response against melanocytes plays a key role in the initiation and maintenance of this disease. However, the factors, which trigger the autoimmune response, are still unknown. Like most complex trait diseases, a widely accepted hypothesis is that viral infection or cutaneous injury triggers an inappropriate, overactive Th1 and Th17 cell differentiation. These results suggest that JAK1, JAK2, and STAT3 are three important molecules in the immune response as well as in autoimmune or immune-mediated diseases. Recent studies have reported that polymorphisms of rs10758669 in JAK2 and rs744166 in STAT3 were associated with the susceptibility to inflammatory bowel disease (IBD). The protective haplotype for multiple sclerosis (MS) in STAT3 rs744166 is a risk allele for Crohn’s disease, implying that STAT3 represents a shared risk locus for at least two autoimmune diseases. SNPs rs10119004 and rs7857730 in JAK2 and rs2293152 and rs6503695 in STAT3 were shown to be a risk factor for ankylosing spondylitis (AS) in a Han Chinese population. Additionally, we recently found that three SNPs of JAK1 polymorphisms were found to be associated with another uveitis entity, Behcet’s disease (unpublished data). Previous studies showed that the same gene variants could be involved in a common pathway of pathogen-
JAK1 Confers Susceptibility to VKH Syndrome

Table 1. Primers and Restriction Enzymes Used for RFLP Analysis of JAK2 and STAT3

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Primers</th>
<th>Tm, °C</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
<td>rs7857730</td>
<td>5'-CCCTTTATTGATTAGTTGATGCCAT-3'</td>
<td>37</td>
<td>HIN1II</td>
</tr>
<tr>
<td>JAK2</td>
<td>rs10758669</td>
<td>5'-GAAGCAAGGACATGCTGAAGTCG-3'</td>
<td>37</td>
<td>HPYCH4V</td>
</tr>
<tr>
<td>JAK2</td>
<td>rs10119004</td>
<td>5'-AGATCAGGAAATTGAATGAGGTGCTCC-3'</td>
<td>65</td>
<td>BSENI</td>
</tr>
<tr>
<td>STAT3</td>
<td>rs2293152</td>
<td>5'-ACAGGGGTCGCTGCTG-3'</td>
<td>55</td>
<td>KPN2I</td>
</tr>
<tr>
<td>STAT3</td>
<td>rs6503695</td>
<td>5'-CAGCAATATTAAAGGAAAGATAATT-3'</td>
<td>65</td>
<td>TASI</td>
</tr>
<tr>
<td>STAT3</td>
<td>rs744166</td>
<td>5'-GGGATAGCATTCCGGAAATGTCATGC-3'</td>
<td>37</td>
<td>HINDIII</td>
</tr>
<tr>
<td>STAT3</td>
<td>rs12948909</td>
<td>5'-GCGATCTACACAGGGTCACACTGCCA-3'</td>
<td>65</td>
<td>TASI</td>
</tr>
</tbody>
</table>

sis in a number of autoimmune diseases.25 Based on these findings, the question was raised as to whether variants of JAK1, JAK2, and STAT3 were possibly associated with the susceptibility to VKH syndrome. In the study presented here, we analyzed the association of 10 SNPs of the JAK1, JAK2, and STAT3 gene with VKH syndrome in a Han Chinese population and found a significant association with JAK1 polymorphisms.

Materials and Methods

Clinical Samples

The study group comprised 809 healthy subjects and 737 VKH patients referred to the Zhongshan Ophthalmic Center, Sun Yat-sen University and the First Affiliated Hospital of Chongqing Medical University (People's Republic of China) between January 2005 and November 2011. All control subjects were matched ethnically and geographically with the patients. The proportion of male versus female was 1:1 to 1:1 both in the cases and controls. The diagnosis of VKH syndrome was made according to the criteria of the First International Workshop criteria for VKH syndrome.26 The clinical characteristics of VKH syndrome patients were assessed at the time of diagnosis.

Ethics Statement

The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China (Permit Number: 2009-201011), and written informed consent was obtained from all the study subjects. All procedures followed the tenets of the Declaration of Helsinki.

Genomic DNA Extraction and Genotyping

Blood samples were collected in EDTA tubes and kept at −70°C until used. Genomic DNA was extracted by a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The target DNA in the JAK2 and STAT3 genes was amplified by PCR using primers presented in Table 1. Each PCR reaction was performed in 10 μL containing 5 μL of commercial premix (Premix Taq; Promega, Madison, WI), 20 pmol primers, and 0.2 μg of genomic DNA. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes followed by 37 cycles of denaturation at 94°C for 30 seconds, annealing at different temperatures (rs310230, rs310236, rs310241, 58°C for rs10758669, 56°C for rs744166, 58°C for rs2293152, and 60°C for rs12948909) for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. These SNPs were genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) analysis. PCR products of rs310230, rs310236, rs310241, rs10758669, rs7857730, rs10119004, rs6503695, rs744166, rs2293152, and rs12948909 polymorphisms were respectively digested with 4 U of HPYCH4V, HIN1II, BSENI, TASI, HINDIII, KPN2I, and TASI restriction enzymes (Table 1) in a 10 μL reaction volume overnight. Digestion products were visualized on a 4% agarose gel and stained (GoldView; SBS Genetech, Beijing, China). Randomly selected subjects (10% of all samples) were directly sequenced (Invitrogen Biotechnology, Shanghai, China) to validate the PCR–RFLP results. The genotyping for the three candidate SNPs in the JAK1 was performed (Sequenom iPLEX system; Sequenom, Inc., San Diego, CA). The genotyping primers of JAK1 gene are shown in Table 2.

Statistical Methods

Hardy–Weinberg equilibrium was tested using the χ² test. The variable age, described as mean ± SD, median, and range, between cases and controls was compared using the Student’s t-test. Genotype frequencies were estimated by direct counting. This statistical method was also performed to analyze the association between the SNPs and various clinical features of VKH syndrome. All statistical tests were two-sided. The P values were corrected (Pc) with the Bonferroni correction by multiplying with the number of analyses performed. The Pc < 0.05 was considered significant.

Results

No statistical differences were observed in the age distribution for the Chinese Han case–control cohorts (P > 0.05). There was also no significant difference in the distribution of sex between cases and controls. The age and sex distribution of the VKH syndrome patients and healthy controls are shown in Table 3. The clinical features of the VKH syndrome patients included in our study are presented in Table 4.

A total of 737 VKH patients and 809 healthy controls from a Han Chinese population were genotyped for three SNPs (rs310230, rs310236, rs310241) of JAK1, three SNPs of JAK2 (rs10758669, rs7857730, rs10119004), and four SNPs (rs6503695, rs744166, rs2293152, and rs12948909) of STAT3. The genotype and allele frequencies of these 10 SNPs in the three genes examined in VKH syndrome patients and healthy controls are summarized in Table 5. The results showed that the distribution of genotypes and alleles did not deviate from
When the Bonferroni correction was applied ($n = 30$), the frequency of the GG genotype of JAK1 rs310236, GG genotype of rs310236, and TT genotype of rs310241 differed significantly between VKH patients and controls ($P_c = 0.008, 0.005, \text{and } 0.001, \text{respectively}$). The frequency of the G allele of rs310230 and rs310236, T allele of JAK1 rs310241 was significantly lower in VKH patients as compared with that observed in healthy controls ($P_c = 0.001, 8.84 \times 10^{-4}, \text{and } 2.05 \times 10^{-4}, \text{respectively}$). There were no differences concerning the genotype or allele frequencies of the other seven SNPs in JAK2 and STAT3 between VKH syndrome patients and healthy controls. The 10 SNPs investigated in our study are all located in the intron of the three genes studied. We also performed a linkage disequilibrium analysis using our data for 10 SNPs of three genes. The analysis showed that the genetic polymorphisms in the three genes were not in linkage disequilibrium.

In addition, we investigated whether the 10 SNPs were associated with the various clinical features of VKH syndrome, such as dysacusis, alopecia, poliosis, and vitiligo. The analysis did not show any significant association between these parameters and the tested 10 SNPs of three genes ($P > 0.05$).

**DISCUSSION**

This study shows that three SNPs of the JAK1 gene are associated with VKH syndrome in a Han Chinese population. The frequencies of the GG genotype and G allele of rs310230 and rs310236 in the test group were much lower than those in healthy controls. The same was found for the TT genotype and T allele of rs310241.

To our knowledge this is the first report addressing an association between genetic variants of JAK1 and VKH syndrome. A recent study from our group also showed the association of JAK1 (rs310230, rs310236, rs310241) with Behçet’s disease, another uveitis entity (manuscript in preparation). These data suggest that JAK1 may be a common risk gene for clinical uveitis. We found no association of seven SNPs in JAK2 and STAT3 with VKH syndrome and healthy controls. The 10 SNPs investigated in our study are all located in the intron of the three genes studied. We also performed a linkage disequilibrium analysis using our data for 10 SNPs of three genes. The analysis showed that the genetic polymorphisms in the three genes were not in linkage disequilibrium.

In addition, we investigated whether the 10 SNPs were associated with the various clinical features of VKH syndrome, such as dysacusis, alopecia, poliosis, and vitiligo. The analysis did not show any significant association between these parameters and the tested 10 SNPs of three genes ($P > 0.05$).
Behçet’s disease is currently considered an autoinflammatory disease, whereas VKH syndrome is seen as an autoimmune disease. Some of the genes investigated may play an important role in the initial regulation of the immune response responsible for disease development, whereas others may be more important in the final intraocular pathway of inflammation. The eye has only a limited repertoire to react against an insult and it is possible that similar polymorphisms of genes involved in these pathways are shared by different uveitis entities. Further studies in other uveitis entities are needed to provide evidence for this hypothesis. In view of the large sample sizes needed for gene association studies we have focused on VKH syndrome and Behçet’s disease, since these two entities occur in a relatively high frequency in our country.

The JAK1–JAK2–STAT3 pathway is a signaling target of a multitude of cytokines that are thought to play significant biological roles in autoimmune disease. This pathway is essential in Th1 cell differentiation and proliferation. Addi-
tionally, it is also important for the development of the Th17 cells. These Th1 and Th17 cells play critical roles in autoimmune or autoinflammatory diseases such as VKH syndrome and Behcet’s disease, through the production of IFN-γ and IL-17. Therefore JAK1, JAK2, and STAT3 may exert their influence in autoimmune diseases including VKH syndrome through modulating the function of Th1 and Th17 cells. Recently JAK1, JAK2, and STAT3 have been investigated for their association with a number of autoimmune diseases including inflammatory bowel disease, multiple sclerosis, and ankylosing spondylitis in a variety of ethnic cohorts. 

Numerous factors have been reported to influence the results of the study on the association of gene polymorphisms with disease. We made the following efforts to ensure correctness of the results. The VKH syndrome patients were selected strictly according to the criteria of the First International Workshop criteria for VKH syndrome. The healthy individual controls were recruited from the same geographical regions as those of the patients. No statistical differences were observed in the distribution of age and sex for the collected Han Chinese case-control cohorts. To validate the results of genotyping by the PCR-RFLP, 10% of the samples were randomly chosen and confirmed by direct sequencing.

Like other candidate gene research, there are some limitations in our study. Even though the sample size of our patients is relatively large, it is possible that even more patients need to be studied to show a small pathogenic role of the three genes investigated. The patients were recruited from a Han Chinese population only from Southern China and our findings concerning the association of JAK1, JAK2, and STAT3 polymorphisms with VKH syndrome should be studied in other ethnic populations but should also be confirmed in other regions of our country. A significant association was found for the whole group of VKH uveitis patients but was not specifically associated with the subgroups we made, depending on the presence of symptoms such as headache, dysacusis, alopecia, poliosis, or vitiligo. This may be due to the fact that the sample size became too low after the subdivision into smaller groups. As yet no published data are available concerning a possible functional role for the JAK1 SNPs. Preliminary unpublished data from our group suggest that rs310241 probably does not affect gene expression, but additional studies are needed to clarify this issue. It cannot be excluded that the association found is not causative, but that it reflects an association, which is in linkage disequilibrium with the causative locus. We are currently looking into these issues.

In conclusion, this study indicates that the gene encoding JAK1, but not JAK2 and STAT3, showed an association with susceptibility to VKH syndrome in a Han Chinese population.

Acknowledgments

The authors thank technician Hongyang Zhou for her assistance in collection of blood samples from patients and controls in the Zhongshan Ophthalmic Center, and all patients and controls enrolled in the present study. Supported, in part by Project of Medical Science and Technology of Chongqing, Key Project of Health Bureau of Chongqing Grant 2012-1-003; Chongqing Key Laboratory of Ophthalmology Grant CSTC, 2008CA5003; Project of Health Bureau of Chongqing Grant 20092357; National Natural Science Foundation Project Grants 81100657 and 30975242; Key Project of Natural Science Foundation Grant 81130019; and Fund for PAR-EU Scholars Program, Clinic Key Project of Ministry of Health, Basic Research Program of Chongqing. The authors alone are responsible for the content and writing of the paper.

Disclosure: K. Hu, None; S. Hou, None; F. Li, None; Q. Qiang, None; A. Kijlstra, None; P. Yang, None

References


Program of Chongqing. The authors alone are responsible for the content and writing of the paper.

Disclosure: K. Hu, None; S. Hou, None; F. Li, None; Q. Qiang, None; A. Kijlstra, None; P. Yang, None


