nature genetics

Genome-wide association analysis of Vogt-Koyanagi-Harada syndrome identifies two new susceptibility loci at 1p31.2 and 10q21.3

Shengping Hou[1–3], Liping Du[1–3], Bo Lei[1–3], Chi Pui Pang[4], Meifen Zhang[5], Wenjuan Zhuang[6], Minglian Zhang[7], Lulin Huang[8], Bo Gong[8], Meilin Wang[9,10], Qi Zhang[1–3], Ke Hu[1–3], Qingyun Zhou[1–3], Jian Qi[1–3], Chaokui Wang[1–3], Yuan Tian[1–3,11], Zi Ye[1–3], Liang Liang[1–3], Hongsong Yu[1–3], Hong Li[1–3], Yan Zhou[1–3], Qingfeng Cao[1–3], Yunjia Liu[1–3], Lin Bai[1–3], Dan Liao[1–3], Aize Kijlstra[11], Jianfeng Xu[10,12], Zhenglin Yang[8,13,14] & Peizeng Yang[1–3,14]

To identify new genetic risk factors for Vogt-Koyanagi-Harada (VKH) syndrome, we conducted a genome-wide association study of 2,208,258 SNPs in 774 cases and 2,009 controls with follow-up in a collection of 415 cases and 2,006 controls and a further collection of 349 cases and 1,588 controls from a Han Chinese population. We identified three loci associated with VKH syndrome susceptibility (*IL23R-C1orf141*, rs117633859, $P_{combined} = 3.42 \times 10^{-21}$, odds ratio (OR) = 1.82; *ADO-ZNF365-EGR2*, rs442309, $P_{combined} = 2.97 \times 10^{-11}$, OR = 1.37; and *HLA-DRB1/DQA1*, rs3021304, $P_{combined} = 1.26 \times 10^{-118}$, OR = 2.97). The five non-HLA genes were all expressed in human iris tissue. *IL23R* was also expressed in the ciliary body, and *EGR2* was expressed in the ciliary body and choroid. The risk G allele of rs117633859 in the promoter region of *IL23R* exhibited low transcriptional activation in a cell-based reporter assay and was associated with diminished *IL23R* mRNA expression in human peripheral blood mononuclear cells.

VKH syndrome is a multisystemic autoimmune disorder characterized by bilateral granulomatous panuveitis frequently associated with systemic involvement[1]. This syndrome primarily affects individuals from certain countries such as China and Japan and is rare in the United States and the UK[1,2]. *HLA-DR4* and *HLA-DR53* are associated with VKH syndrome in various ethnic populations[3–8]. Previous genetic studies of VKH syndrome have identified several associated genes such as *CTLA4*, *MIF* and *SPP1* (also called *OPN*)[9–11]. To investigate additional genetic variants for VKH syndrome, we conducted the first multistage genome-wide association study (GWAS) with a total of 1,538 cases with VKH syndrome and 5,603 controls from a Han Chinese population. The characteristics of the cases and controls enrolled in the present study are summarized in **Supplementary Tables 1** and **2**. In the GWAS stage, 900,015 and 906,659 SNPs were genotyped in 795 cases and 2,046 controls, respectively (Online Methods). To further increase genome coverage, we performed an imputation analysis to infer the genotypes of additional common SNPs (Online Methods). After standard quality-control filtering for subjects and SNPs (Online Methods), we obtained data for 2,208,258 genotyped or imputed SNPs in 774 cases and 2,009 controls (GWAS stage, Chongqing and Sichuan cohort) for the subsequent analysis (**Supplementary Table 2** and **Supplementary Fig. 1**). Principal component analysis (PCA) showed that the cases and controls in this study were of Han Chinese ancestry and were well matched (**Supplementary Figs. 2** and **3**). We performed GWAS analysis using logistic regression and adjusted for the top eigenvectors using PLINK[12]. A quantile-quantile plot analysis showed that the genomic inflation factor (λ) values with and without MHC-region SNPs were 1.044 and 1.039, respectively (**Fig. 1a** and **Supplementary Fig. 4a**), indicating that population stratification had negligible effects on the genetic analysis in our study samples. In addition to the previously reported MHC class II region (**Supplementary Fig. 5**), multiple SNPs in *IL23R-C1orf141* at 1p31.2 showed strong association, with *P* values that reached a genome-wide significance of $P < 3.13 \times 10^{-7}$ ($P < 3.13 \times 10^{-7}$ was considered as genome-wide significant, with a total of 159,799 independent blocks among the controls in the study assuming an r^2 value of 0.5) (**Table 1**, **Figs. 1b** and **2a**, **Supplementary Fig. 4b** and **Supplementary Tables 3**–**5**).

[1]The First Affiliated Hospital of Chongqing Medical University, Chongqing, China. [2]Chongqing Key Laboratory of Ophthalmology, Chongqing, China. [3]Chongqing Eye Institute, Chongqing, China. [4]Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China. [5]Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China. [6]Department of Ophthalmology, People's Hospital of Ningxia Hui Autonomous Region, Yinchuan, China. [7]Department of Ophthalmology, Xingtai Eye Hospital, Xingtai, China. [8]The Sichuan Provincial Key Laboratory for Human Disease Gene Study, Hospital of the University of Electronic Science and Technology of China and Sichuan Provincial People's Hospital, Chengdu, China. [9]Department of Occupational Medicine and Environmental Health, School of Public Health, Nanjing Medical University, Nanjing, China. [10]Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA. [11]University Clinic for Ophthalmology Maastricht, Maastricht, the Netherlands. [12]School of Public Health, Fudan University, Shanghai, China. [13]School of Medicine, University of Electronic Science and Technology of China, Chengdu, China. [14]These authors jointly directed this work. Correspondence should be addressed to P.Y. (peizengycmu@126.com) or Z. Yang (zliny@yahoo.com).

Received 3 April; accepted 17 July; published online 10 August 2014; doi:10.1038/ng.3061

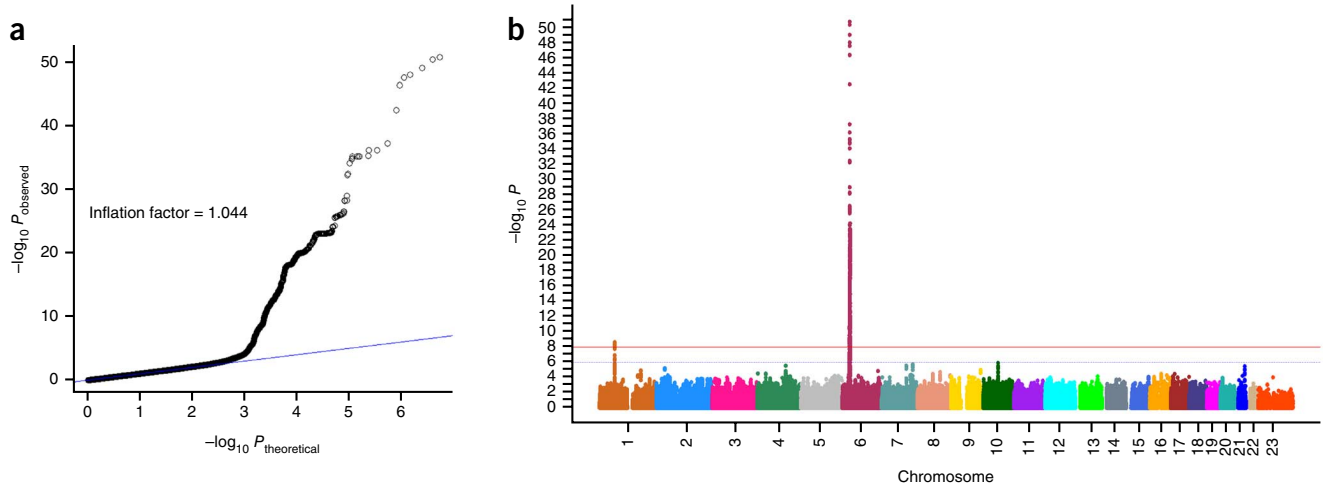

Figure 1 Genome-wide association results for 774 cases with VKH syndrome and 2,009 controls from the Han Chinese population. (**a**) Quantile-quantile plot of the observed (*y* axis) and expected (*x* axis) *P* values from the genome-wide association results for all SNPs. (**b**) Manhattan plot of *P* values on the $-\log_{10}$ scale for 2,208,258 SNPs in the GWAS stage (774 cases and 2,009 controls). The red line represents $P = 1 \times 10^{-8}$, and the blue dashed line represents $P = 1 \times 10^{-6}$.

To confirm the association obtained during the GWAS stage (**Figs. 1b** and **2**), we selected 47 independently associated SNPs for a replication study (Online Methods) in 415 cases and 2,006 controls (replication 1, Guangdong and Hong Kong cohort) (**Supplementary Note**). Three loci on chromosomes 1, 6 and 10 were successfully replicated: rs3021304 at 6p21.3 showed the strongest association with VKH syndrome ($P = 1.84 \times 10^{-38}$), followed by rs78377598 at 1p31.2 ($P = 1.60 \times 10^{-5}$) and rs442309 at 10q21.3 ($P = 6.15 \times 10^{-3}$) (**Table 1**). To assess the enrichment of VKH syndrome–associated SNPs and genes (2,295 SNPs, which had $P < 1.0 \times 10^{-4}$ in the GWAS stage, were enrolled in this analysis), we performed pathway-based analysis using GenGen software (see URLs)[13,14]. The result showed that 20 SNPs and genes were enriched in the transmembrane receptor activity pathway (the GO0004888 pathway) (size of 20, nominal $P < 0.000001$, false discovery rate <0.0001), suggesting that cellular surface signal transduction may be involved in the development of VKH syndrome. However, the association with VKH syndrome of these 20 SNPs and genes enriched in the GO0004888 pathway was not replicated in the first replication study.

To further confirm the association of the three loci with VKH syndrome, we genotyped three SNPs in a second replication collection

Table 1 Association results for three loci in the GWAS and replication studies

SNP	Chr.	Position	Genes	MA	Stage	MAF (case/control)	*P*	OR (95%CI)	*Q*	I^2
rs78377598	1p31.2	67612502	*IL23R*, *C1orf141*	T	GWAS	15.3/8.7	2.31×10^{-9}	1.83 (1.50–2.23)		
					Replication 1	14.9/9.8	1.60×10^{-5}	1.62 (1.30–2.01)		
					Replication 2	15.6/10.7	3.57×10^{-4}	1.52 (1.21–1.91)		
					Combined	15.4/9.7	6.00×10^{-16}	1.67 (1.47–1.89)	0.46	0
rs117633859	1p31.2	67627828	*IL23R*, *C1orf141*	G	GWAS	15.0/8.5	5.38×10^{-9}	1.81 (1.49–2.22)		
					Replication 1	17.3/10.1	6.85×10^{-9}	1.89 (1.53–2.53)		
					Replication 2	16.4/10.0	2.91×10^{-6}	1.73 (1.38–2.18)		
					Combined	16.0/9.5	3.42×10^{-21}	1.82 (1.60–2.05)	0.86	0
rs114800139	6p21.3	32428715	*HLA-DRA*, *HLA-DRB5*	A	GWAS	63.5/34.9	5.17×10^{-51}	3.07 (2.66–3.56)		
					Replication 1	63.1/34.6	1.27×10^{-43}	3.17 (2.69–3.73)		
					Replication 2	57.9/33.7	5.79×10^{-29}	2.77 (2.32–3.31)		
					Combined	62.3/34.5	1.16×10^{-119}	3.02 (2.75–3.31)	0.52	0
rs3021304	6p21.3	32575658	*HLA-DRB1*, *HLA-DQA1*	G	GWAS	62.6/34.4	5.08×10^{-47}	2.88 (2.49–3.32)		
					Replication 1	62.3/36.5	1.84×10^{-38}	2.93 (2.49–3.44)		
					Replication 2	60.7/32.3	3.30×10^{-24}	3.18 (2.66–3.81)		
					Combined	62.2/34.6	1.26×10^{-118}	2.97 (2.71–3.26)	0.67	0
rs442309	10q21.3	64490495	*ADO*, *ZNF365*, *EGR2*	T	GWAS	30.9/24.6	1.15×10^{-6}	1.44 (1.24–1.66)		
					Replication 1	31.1/26.4	6.15×10^{-3}	1.26 (1.07–1.48)		
					Replication 2	33.0/25.8	2.82×10^{-3}	1.43 (1.19–1.72)		
					Combined	31.5/25.6	2.97×10^{-11}	1.37 (1.25–1.51)	0.43	0
rs224058	10q21.3	64498865	*ADO*, *ZNF365*, *EGR2*	A	GWAS	30.4/24.4	4.08×10^{-6}	1.41 (1.22–1.63)		
					Replication 1	31.1/26.6	7.96×10^{-3}	1.25 (1.06–1.48)		
					Replication 2	33.5/26.0	4.93×10^{-5}	1.47 (1.22–1.77)		
					Combined	31.4/25.6	5.79×10^{-11}	1.37 (1.25–1.51)	0.40	0

Chr., chromosome; position, position on NCBI human reference genome build 37; MA, minor allele; MAF, minor allele frequency; OR: odds ratio for the minor allele; 95% CI, 95% confidence intervals; *Q*, statistic for the meta results; I^2, value for the combined results.

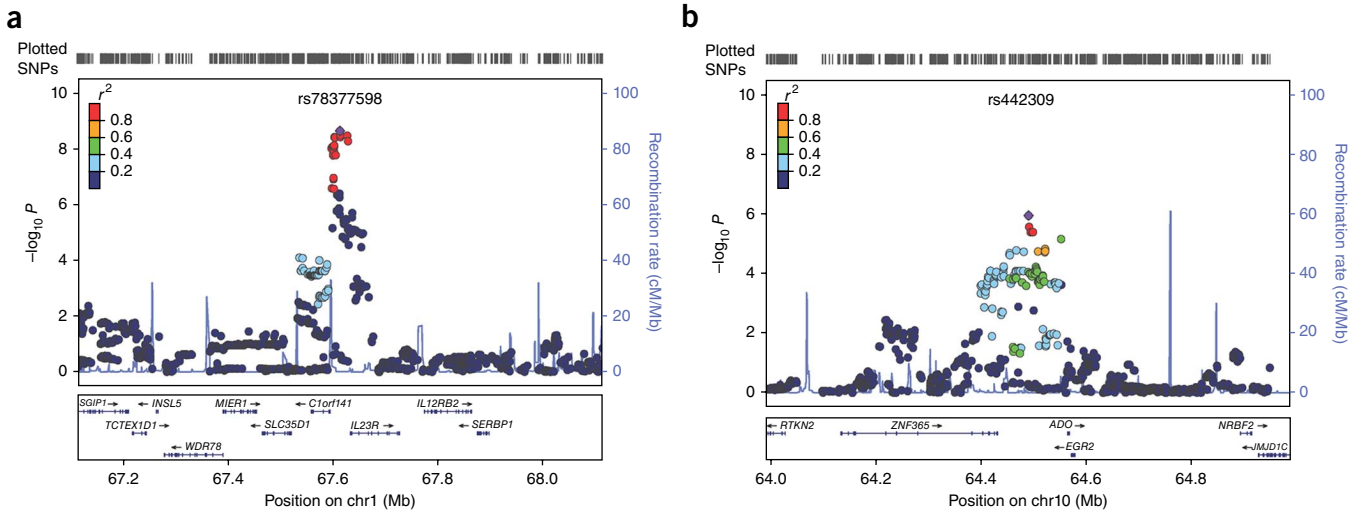

Figure 2 Regional plots of association results for the two newly identified susceptibility loci for VKH syndrome at 1p31.2 and 10q21.3. (**a**,**b**) Regional plots at 1p31.2 (**a**) and 10q21.3 (**b**). Results ($-\log_{10} P$) are shown for SNPs in the regions flanking 500 kb on either side of the index SNPs (purple diamond). The association results for both genotyped and imputed SNPs are shown with recombination rates estimated from the 1000 Genomes Project CHB and JPT data. The index SNPs are shown as diamonds, and the r^2 values of the remaining SNPs are indicated by color. The genes within the region are annotated and indicated by arrows.

comprising 349 cases and 1,588 controls (replication 2, Beijing cohort) and genotyped seven additional SNPs in the same linkage disequilibrium (LD) block with the three associated SNPs at 1p31.2 (four SNPs), 6p21.3 (one SNP) and 10q21.3 (two SNPs) in the GWAS and two replication cohorts (GWAS and replications 1 and 2). We were able to confirm an association with VKH syndrome for all five SNPs in *IL23R-C1orf141* at the 1p31.2 locus ($3.57 \times 10^{-4} \leq P \leq 2.91 \times 10^{-6}$; **Table 1** and **Supplementary Table 5**), leading to highly significant observations when we performed meta-analysis on data from the GWAS and first and second replication collection (total sample of 1,538 cases and 5,603 controls, OR = 1.82, $P_{combined} = 3.42 \times 10^{-21}$ for the lead SNP rs117633859) (**Table 1** and **Supplementary Table 5**). Three SNPs in *ADO-ZNF365-EGR2* at the 10q21.3 locus were also significantly associated with VKH syndrome in the second replication ($P_{combined} = 2.97 \times 10^{-11}$ and OR = 1.37 for the lead SNP rs442309; **Table 1**). Two SNPs in the *HLA* region at 6p21.3 also showed a strong association with VKH syndrome ($P_{combined} = 1.26 \times 10^{-118}$, OR = 2.97 for SNP rs3021304; and $P_{combined} = 1.16 \times 10^{-119}$, OR = 3.02 for SNP rs114800139; **Table 1**). We further performed an interaction analysis for the three associated SNPs, including rs117633859 at 1p31.2, rs3021304 at 6p21.3 and rs442309 at 10q21.3, and did not detect any gene-gene interactions between these three loci (**Supplementary Table 6**). As we performed the present GWAS using Affymetrix and Illumina platforms, we calculated the logistical *P* value for the three significant SNPs (rs117633859, rs3021304 and rs442309) after adjusting for the different platforms. These significantly associated SNPs were not confounded by the use of two different platforms (**Supplementary Table 7**). Additionally, we used a genome-wide complex trait analysis to assess the variance demonstrated by these three associated SNPs (rs117633859, rs3021304 and rs442309) assuming a VKH syndrome prevalence of 0.000006 according to Japanese data[15], in view of the lack of Chinese incidence data for this syndrome. The result showed that the estimated proportion of genetic variance regarding rs117633859, rs3021304 and rs442309 over the total variance was 0.000812 ($P < 0.001$).

To focus further on the HLA association, we performed an imputation analysis for the association of rs3021304 at *HLA-DRB1/DQA1* with VKH syndrome. The results indicated that only some HLA alleles were associated with risk of VKH syndrome (the most significant HLA allele was *HLA-DQB1*301*, $P = 8.88 \times 10^{-13}$) (**Supplementary Table 8**). However, the most significant allele, *HLA-DQB1*301*, showed weak LD with rs3021304 ($r^2 = 0.107$), suggesting that rs3021304 is an independent risk locus for VKH syndrome.

As mentioned above, our study identified two new non-HLA susceptibility loci for VKH syndrome: *IL23R-C1orf141* at 1p31.2 and *ADO-ZNF365-EGR2* at 10q21.3. To investigate whether the identified risk allele in *IL23R-C1orf141* contributed to the functional effects, we explored its biological functions. rs117633859, located ~4 kb upstream from *IL23R*, showed the strongest association with VKH syndrome. rs117633859 is in the same LD block as rs1884444 in an exon of *IL23R* ($D' = 1.0$, $r^2 = 0.284$) but is in a different LD block than rs7528804 in *C1orf141* (**Supplementary Fig. 6**). We examined the expression of *IL23R* in human uveal tissues (iris, choroid and ciliary body) using RT-PCR (**Supplementary Table 9**). Although *IL23R* was expressed in both the iris and ciliary body of all four controls (**Supplementary Fig. 7a**,**b**), this expression was likely from resident macrophages and dendritic cells, which are abundant in these tissues[16]. We found expression of *C1orf141* only in the iris (**Supplementary Fig. 8a**,**e**). These findings suggest that the association signal observed at 1p31.2 was more likely to be linked to *IL23R* than to *C1orf141*. Bioinformatics analysis showed that rs117633859 affects transcription factor binding (JASPAR; **Supplementary Table 10**)[17], which may lead to altered *IL23R* expression. We therefore focused on the effect of genotypes at rs117633859 on *IL23R* expression in human peripheral blood mononuclear cells. We found decreased *IL23R* expression in normal controls with the risk GG genotype of rs117633859 ($P = 0.011$; **Supplementary Fig. 7c**). Public data (GSE6536)[18] also showed diminished mRNA expression of *IL23R* in individuals with the risk G allele of rs117633859, despite this result being inconsistent with other public data[19] (**Supplementary Fig. 9**). We also performed an *in vitro* luciferase reporter gene assay to test whether this polymorphism affected *IL23R* transcription directly. We observed lower transcription activity in HEK-293A cells containing the risk G allele of rs117633859 ($P = 1.10 \times 10^{-13}$; **Supplementary Fig. 7d**). Taken together, these data suggest that genetic variants of *IL23R* may be a risk factor for

VKH syndrome caused by low expression of this gene. We also evaluated *IL23R* expression in iris specimens between patients with VKH syndrome without active inflammation (obtained during iridectomy surgery) and controls. The result showed no significant differences in *IL23R* mRNA expression between the patients and controls ($P = 0.201$; **Supplementary Fig. 8f**). This finding may be explained by the absence of active inflammation in the enrolled patients.

A previous study showed that four SNPs in *IL23R* are not associated with VKH syndrome[20]. The inconsistent result may be explained by the lack of strong LD and the location in a different LD block between the four SNPs reported in that study and VKH syndrome–associated SNPs in *IL23R* found in the present study using the 1000 Genomes Project CHB data ($r^2 \leq 0.04$; **Supplementary Fig. 10a**), suggesting that SNPs within the same gene may have different contributions to disease. Recently, GWAS and candidate studies have identified multiple risk genes, such as *IL23R*, for Behçet's disease, another common uveitis entity in the Chinese population[21–27]. The reported SNPs in *IL23R* that are associated with Behçet's disease[21,22] are located in a different LD block than the SNPs contributing to the risk of VKH syndrome (**Supplementary Fig. 10**). We therefore tested the association of VKH syndrome–associated SNPs at 1p31.2 with Behçet's disease in a Chinese Han population. The results did not show an association of the examined SNPs with Behçet's disease ($P_{Bonferroni} > 0.05$; **Supplementary Table 11**). *IL23R* variants have also been found to be associated with various diseases such as psoriasis, Crohn's disease and ankylosing spondylitis[28–37] (**Supplementary Table 12**). However, the SNPs in *IL23R* that are associated with psoriasis, Crohn's disease and ankylosing spondylitis are located in a different LD block than the VKH syndrome–associated SNPs identified in this study (**Supplementary Fig. 10b,c**). These findings suggest that genetic variants in *IL23R* are a shared common risk factor for multiple autoimmune diseases and that the contribution of this risk factor to VKH syndrome may be caused by the transcriptional regulation of this gene rather than a change in protein structure or activity, which may be involved in the association of *IL23R* with Crohn's disease, psoriasis and ankylosing spondylitis.

Our study also identified a new susceptibility locus for VKH syndrome at *ADO-ZNF365-EGR2* (rs442309). To investigate the relationship of rs442309 with *ADO*, *ZNF365* and *EGR2*, we performed functional analysis on HAPMAP3_EXPRESSION data (E-MTAB-264)[19]. The results did not show any effects of rs442309 on the expression of the three genes ($P > 0.05$; **Supplementary Fig. 11**). These results suggest that this SNP or the SNPs strongly linked with it may be involved in the development of VKH syndrome through the regulation of mRNA stability or the splicing of these genes. *ADO*, *ZNF365* and *EGR2* were all expressed in the iris (**Supplementary Table 9** and **Supplementary Fig. 8b–d**), and we also found *EGR2* expression in the ciliary body and choroid (**Supplementary Fig. 8d**). These results suggest that these genes, along with their interacting genes that are also expressed in ocular tissue, may contribute to the pathogenesis of VKH syndrome. Variants at *ZNF365* have been shown to be associated with Crohn's disease[29,34,38,39], atopic dermatitis[40,41] and breast cancer[42]. *EGR2* has important roles in the regulation of the adaptive immune responses and the homeostasis of B and T cells[43,44]. *ADO* has also been identified as a risk locus for Crohn's disease[34]. Because similar genetic associations have been observed for VKH syndrome, Crohn's disease, psoriasis and ankylosing spondylitis, we summarized the known risk loci for these diseases (**Supplementary Table 13**). Although no non-HLA SNPs (excluding SNPs at 1p31.2 and 10q21.3) reached genome-wide significance in the present GWAS, 11 of the 166 loci and genes showed a suggestive association with VKH syndrome ($P = 0.046$ to 0.013; **Supplementary Table 13**). However, whether genetic overlap for multiple autoimmune diseases is indicative of a dysregulated immune or metabolic response or is instead specific to a disease or tissue remains unclear. We also reevaluated a variety of genetic variants that have been linked to VKH syndrome and published in previous reports[10,11] using the present GWAS data. Although none of these SNPs demonstrated genome-wide significance in this GWAS, 8 out of 12 genes described in the earlier reports showed a suggestive association with this syndrome ($P = 0.044$ to 2.28×10^{-4}; **Supplementary Table 14**). Further studies using larger samples or other ethnic populations will be needed to elucidate the true contributors to VKH syndrome.

In summary, we identified two new risk loci for VKH syndrome at 1p31.2 and 10q21.3 and confirmed the association between *HLA* genes and VKH syndrome.

URLs. EIGENSOFT, http://genetics.med.harvard.edu/reich/Reich_Lab/Software.html; IMPUTE, https://mathgen.stats.ox.ac.uk/impute/impute.html; HapMap, http://hapmap.ncbi.nlm.nih.gov/; LocusZoom, http://csg.sph.umich.edu/locuszoom/; PLINK 1.07, http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml; R statistical software, http://www.r-project.org/; Gene Expression Omnibus (GEO), http://www.ncbi.nlm.nih.gov/geo/; 1000 Genomes Project, http://www.1000genomes.org/; GenGen, http://www.openbioinformatics.org/gengen/; JASPAR, http://jaspardev.genereg.net/; HIBAG, http://www.biostat.washington.edu/~bsweir/HIBAG/; Gene Ontology, http://www.geneontology.org/.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation Major International (Regional) Joint Research Project (81320108009) (P.Y.), the National Basic Research Program of China (973 Program) (2011CB510200) (P.Y.), the Key Project of the Natural Science Foundation (81130019) (P.Y.), the National Natural Science Foundation Project (31370893 (P.Y.), 81270990 (S.H.) and 81025006 (Z. Yang)), the Research Fund for the Doctoral Program of Higher Education of China (20115503110002) (P.Y.), the Clinic Key Project of the Ministry of Health (201002019) (P.Y.), the Basic Research program of Chongqing (cstc2013jcyjC10001) (P.Y.), the Chongqing Key Laboratory of Ophthalmology (CSTC, 2008CA5003) (P.Y.), the National Key Clinical Specialties Construction Program of China (P.Y.), the Key Project of Health Bureau of Chongqing (2012-1-003) (P.Y.), the Fund for PAR-EU Scholars Program (P.Y.) and The Youth Outstanding-notch Talent Support Program of Chongqing (S.H.). We thank H. Zhou (Zhongshan Ophthalmic Center, Sun Yat-sen University), X. Liu (Department of Ophthalmology, The Second Hospital of Jilin University), Y. Wang (The Eye Hospital of Wenzhou Medical University), H. Wang (Department of Ophthalmology, Beijing Tongren Hospital, Capital Medical University), L. Xing (Ophthalmology of Department, the First Affiliated Hospital, Harbin Medical University), R. Zhang (Department of Ophthalmology, Eye and ENT Hospital of Fudan University), Y. Shi (Department of Ophthalmology, Shanxi Eye Hospital) and C. Zhao (Department of Ophthalmology, Nanjing General Hospital of the Nanjing Military Region) for helping with sample collection, and we also thank the individuals, their families and friends who participated in this project. Some samples of cases and controls were collected in the Zhongshan Ophthalmic Center, Sun Yat-sen University. We thank Genergy Bio-technology Corporation (Shanghai) and CapitalBio Corporation (Beijing) for helping with the microarray experiment, and we also thank Beijing Genomics Institute for luciferase reporter analysis.

AUTHOR CONTRIBUTIONS

P.Y., Z. Yang and S.H. conceived and designed the study. S.H., M.W. and J.X. analyzed the GWAS data. P.Y., Z. Yang, L.D., C.P.P., Meifen Zhang, W.Z., Minglian Zhang, Q. Zhang, K.H., Q. Zhou and H.L. recruited subjects and participated in

the diagnostic evaluations. S.H., L.D., L.H., K.H., J.Q., C.W., Y.T., Z. Ye, Y.Z., Y.L., L.B. and D.L. contributed to the genotyping. L.L., H.Y. and Q.C. contributed to the reagents, materials and analysis tools. S.H., B.L., B.G. and D.L. contributed to the RT-PCR or real-time PCR. S.H. and P.Y. drafted the manuscript. B.L., C.P.P., J.X., A.K. and Z. Yang helped revise the manuscript. P.Y., Z. Yang and S.H. obtained funding for this study. All authors reviewed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Yang, P. *et al.* Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. *Ophthalmology* **114**, 606–614 (2007).
2. Moorthy, R.S., Inomata, H. & Rao, N.A. Vogt-Koyanagi-Harada syndrome. *Surv. Ophthalmol.* **39**, 265–292 (1995).
3. Kim, M.H. *et al.* Association of HLA with Vogt-Koyanagi-Harada syndrome in Koreans. *Am. J. Ophthalmol.* **129**, 173–177 (2000).
4. Weisz, J.M. *et al.* Association between Vogt-Koyanagi-Harada syndrome and HLA-DR1 and -DR4 in Hispanic patients living in southern California. *Ophthalmology* **102**, 1012–1015 (1995).
5. Zhao, M., Jiang, Y. & Abrahams, I.W. Association of HLA antigens with Vogt-Koyanagi-Harada syndrome in a Han Chinese population. *Arch. Ophthalmol.* **109**, 368–370 (1991).
6. Zhang, X.Y., Wang, X.M. & Hu, T.S. Profiling human leukocyte antigens in Vogt-Koyanagi-Harada syndrome. *Am. J. Ophthalmol.* **113**, 567–572 (1992).
7. Islam, S.M. *et al.* HLA class II genes in Vogt-Koyanagi-Harada disease. *Invest. Ophthalmol. Vis. Sci.* **35**, 3890–3896 (1994).
8. Hou, S. *et al.* Small ubiquitin-like modifier 4 (*SUMO4*) polymorphisms and Vogt-Koyanagi-Harada (VKH) syndrome in the Chinese Han population. *Mol. Vis.* **14**, 2597–2603 (2008).
9. Chu, M. *et al.* Elevated serum osteopontin levels and genetic polymorphisms of osteopontin are associated with Vogt-Koyanagi-Harada disease. *Invest. Ophthalmol. Vis. Sci.* **52**, 7084–7089 (2011).
10. Du, L. *et al.* Association of the *CTLA-4* gene with Vogt-Koyanagi-Harada syndrome. *Clin. Immunol.* **127**, 43–48 (2008).
11. Zhang, C. *et al. MIF* gene polymorphisms confer susceptibility to Vogt-Koyanagi-Harada syndrome in a Han Chinese population. *Invest. Ophthalmol. Vis. Sci.* **54**, 7734–7738 (2013).
12. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
13. Wang, K., Li, M. & Bucan, M. Pathway-based approaches for analysis of genomewide association studies. *Am. J. Hum. Genet.* **81**, 1278–1283 (2007).
14. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. USA* **102**, 15545–15550 (2005).
15. Murakami, S., Inaba, Y., Mochizuki, M., Nakajima, A. & Urayama, A. A nationwide survey on the occurrence of Vogt-Koyanagi-Harada disease in Japan. *Jpn. J. Ophthalmol.* **38**, 208–213 (1994).
16. McMenamin, P.G., Crewe, J., Morrison, S. & Holt, P.G. Immunomorphologic studies of macrophages and MHC class II–positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. *Invest. Ophthalmol. Vis. Sci.* **35**, 3234–3250 (1994).
17. Wasserman, W.W. & Sandelin, A. Applied bioinformatics for the identification of regulatory elements. *Nat. Rev. Genet.* **5**, 276–287 (2004).
18. Stranger, B.E. *et al.* Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science* **315**, 848–853 (2007).
19. Stranger, B.E. *et al.* Patterns of *cis* regulatory variation in diverse human populations. *PLoS Genet.* **8**, e1002639 (2012).
20. Jiang, Z., Yang, P., Hou, S., Li, F. & Zhou, H. Polymorphisms of *IL23R* and Vogt-Koyanagi-Harada syndrome in a Chinese Han population. *Hum. Immunol.* **71**, 414–417 (2010).
21. Mizuki, N. *et al.* Genome-wide association studies identify *IL23R-IL12RB2* and *IL10* as Behcet's disease susceptibility loci. *Nat. Genet.* **42**, 703–706 (2010).
22. Remmers, E.F. *et al.* Genome-wide association study identifies variants in the MHC class I, *IL10*, and *IL23R-IL12RB2* regions associated with Behcet's disease. *Nat. Genet.* **42**, 698–702 (2010).
23. Jiang, Z. *et al. IL23R* gene confers susceptibility to Behcet's disease in a Chinese Han population. *Ann. Rheum. Dis.* **69**, 1325–1328 (2010).
24. Xavier, J.M. *et al.* Association study of *IL10* and *IL23R-IL12RB2* in Iranian patients with Behcet's disease. *Arthritis Rheum.* **64**, 2761–2772 (2012).
25. Kirino, Y. *et al.* Targeted resequencing implicates the familial Mediterranean fever gene *MEFV* and the toll-like receptor 4 gene *TLR4* in Behcet disease. *Proc. Natl. Acad. Sci. USA* **110**, 8134–8139 (2013).
26. Hughes, T. *et al.* Identification of multiple independent susceptibility loci in the HLA region in Behcet's disease. *Nat. Genet.* **45**, 319–324 (2013).
27. Kirino, Y. *et al.* Genome-wide association analysis identifies new susceptibility loci for Behcet's disease and epistasis between *HLA-B*51* and *ERAP1*. *Nat. Genet.* **45**, 202–207 (2013).
28. Duerr, R.H. *et al.* A genome-wide association study identifies *IL23R* as an inflammatory bowel disease gene. *Science* **314**, 1461–1463 (2006).
29. Barrett, J.C. *et al.* Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* **40**, 955–962 (2008).
30. Cargill, M. *et al.* A large-scale genetic association study confirms *IL12B* and leads to the identification of *IL23R* as psoriasis-risk genes. *Am. J. Hum. Genet.* **80**, 273–290 (2007).
31. McGovern, D.P. *et al.* Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat. Genet.* **42**, 332–337 (2010).
32. Silverberg, M.S. *et al.* Ulcerative colitis-risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. *Nat. Genet.* **41**, 216–220 (2009).
33. Burton, P.R. *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* **39**, 1329–1337 (2007).
34. Rioux, J.D. *et al.* Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat. Genet.* **39**, 596–604 (2007).
35. Evans, D.M. *et al.* Interaction between *ERAP1* and *HLA-B27* in ankylosing spondylitis implicates peptide handling in the mechanism for *HLA-B27* in disease susceptibility. *Nat. Genet.* **43**, 761–767 (2011).
36. Nair, R.P. *et al.* Genome-wide scan reveals association of psoriasis with IL-23 and NF-κB pathways. *Nat. Genet.* **41**, 199–204 (2009).
37. Strange, A. *et al.* A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between *HLA-C* and *ERAP1*. *Nat. Genet.* **42**, 985–990 (2010).
38. Haritunians, T. *et al.* Variants in *ZNF365* isoform D are associated with Crohn's disease. *Gut* **60**, 1060–1067 (2011).
39. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
40. Hirota, T. *et al.* Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. *Nat. Genet.* **44**, 1222–1226 (2012).
41. Sun, L.D. *et al.* Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. *Nat. Genet.* **43**, 690–694 (2011).
42. Lindström, S. *et al.* Common variants in *ZNF365* are associated with both mammographic density and breast cancer risk. *Nat. Genet.* **43**, 185–187 (2011).
43. Okamura, T., Fujio, K., Sumitomo, S. & Yamamoto, K. Roles of LAG3 and EGR2 in regulatory T cells. *Ann. Rheum. Dis.* **71** (suppl. 2), i96–i100 (2012).
44. Li, S. *et al.* The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells. *Immunity* **37**, 685–696 (2012).

ONLINE METHODS

Recruitment of patients and normal controls. We performed a three-stage case-control study, including an initial GWAS stage and two replication stages, with a Han Chinese population (**Supplementary Tables 1** and **2** and **Supplementary Fig. 1**). The GWAS stage included 795 cases with VKH syndrome and 2,046 normal controls. The replication stages included 764 cases and 3,594 normal controls. The enrolled samples were recruited from multiple ophthalmic centers in China. All cases were diagnosed with VKH syndrome by senior ophthalmologists based strictly on the revised diagnostic criteria 2001 for VKH syndrome[45]. If the diagnosis was uncertain, the cases were excluded from the study. The study of Behçet's disease consisted of 509 cases with Behçet's disease recruited from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) and the Zhongshan Ophthalmic Center, Sun Yat-sen University (Guangzhou, China). The diagnosis of Behçet's disease was based on the criteria of the International Study Group[46]. A total of 4,012 normal controls used in the Behçet's disease study were recruited from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China), Sun Yat-sen University (Guangzhou, China) and The Sichuan Provincial Key Laboratory for Human Disease Gene Study, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital (Chengdu, China) and Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong (Hong Kong, China). All participants provided written informed consent. The present study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (permit number: 2009-201008) and adhered to the tenets of the Declaration of Helsinki.

DNA extraction. Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na)-anticoagulated venous blood samples were collected from all participants. Genomic DNA was extracted from peripheral blood using a QIAGEN QIAamp DNA Mini Blood Kit (Hilden, Germany) according to the manufacturer's recommendations.

GWAS genotyping, quality control and imputation analysis. Genome-wide genotyping was performed using the HumanOmniZhongHua-8 BeadChip (Illumina) or Affymetrix GeneChip Genome Wide SNP 6.0 arrays. Before subsequent GWAS analysis, we conducted quality-control filtering of the GWAS data. The SNPs were excluded from this analysis if they had MAF < 5%, deviated from Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-3}$) or had a genotyping call rate <95%. As the reference panel, we imputed ungenotyped SNPs using the IMPUTE program (v2.0; see URLs) according to the CHB and JPT data from the 1000 Genomes Project integrated phase 1 release (see URLs)[47]. Imputed SNPs were excluded if they had (i) call rate <95%, (ii) MAF < 5% or (iii) Hardy-Weinberg equilibrium P value in controls $<1.0 \times 10^{-3}$. A total of 2,208,258 SNPs passed all the quality-control criteria and were used in the subsequent analysis. For sample filtering, individuals with generated genotypes with a call rate of less than 95% were excluded (15 samples). We also conducted identity-by-state probabilities for all subjects to search for duplicates and closely related individuals among the samples. After sample filtering, 21 cases and 37 controls were excluded from the study. A total of 774 cases and 2,009 controls were enrolled in the subsequent analysis. The imputation analysis of the *HLA* region was also performed on the basis of known SNP genotypes using the R package HIBAG (see URLs)[48]. The reference panels for imputation were based on the resources provided by HIBAG (the four-digit resolution of multiple GlaxoSmithKline clinical trials of Asian ancestry (east and south Asia) (referred to as 'HLARES') and HapMap Phase 2).

SNP selection for replication study. The criteria used to select SNPs for validation from the discovery stage were $P < 1.0 \times 10^{-4}$ in the GWAS stage, MAF > 5% and $r^2 < 0.5$ (removing the SNPs in high LD using clumping analysis). Forty-seven SNPs remained after filtering. Genotyping for the replication 1 study was performed using the Sequenom MassARRAY system (Sequenom Inc.) according to the manufacturer's instructions. The associated SNPs in the replication 1 stage were genotyped in another Chinese cohort using an ABI SNPshot method according to the manufacturer's manual. Seven additional SNPs in three loci including 1p31.2 (*IL23R-C1orf141*), 6p21.3 (*HLA-DRA/DRB1*) and 10q21.3 (*ADO-ZNF365-EGR2*) were also included and genotyped using the ABI SNPshot method to further validate and augment the credibility of the associated loci. DNA samples were genotyped using TaqMan SNP genotyping assays for rs117633859 (assay ID: AHVJKBP; Applied Biosystems) (**Supplementary Fig. 12**). We also evaluated the accuracy of nine SNPs at 1p31.2, 6p21.3 and 10q21.3 imputed with VKH syndrome by genotyping these SNPs using a randomly selected sample of cases and controls tested during the GWAS stage (179 cases and 929 controls) with the iPLEX MassARRAY platform or AB TaqMan probe. The concordant call (%) between the imputed and genotyped data was more than 98.2% (**Supplementary Table 15**).

Genome-wide pathway association analysis. GenGen was used to perform pathway-based analysis for the GWAS data (see URLs). The associated 2,295 SNPs (the SNPs with $P < 1.0 \times 10^{-4}$ in the GWAS stage), which mapped to a gene or to less than 500 kb from the closest gene, were considered in this analysis. A Kolmogorov-Smirnov–like statistic was then used to assess the enrichment of the genes within pathways as described[13]. The candidate pathway was compiled from the Gene Ontology database (see URLs).

Statistical analyses. The association of each SNP with the risk of VKH syndrome in the GWAS, replication and meta-analysis stages was carried out with an additive model in logistic regression using PLINK v1.07 (see URLs)[12]. ORs and 95% CIs were adjusted for the top five eigenvectors in the logistic regression analysis. Ancestry and population stratification were assessed using PCA implemented in the EIGENSOFT package (see URLs). The first PCA was used to evaluate the population structure of the samples genotyped during the GWAS stage and the data from four populations (CHB, JPT, Utah residents of northern and western European ancestry (CEU) and Yoruba from Ibadan, Nigeria (YRI)) from the HapMap2 project (see URLs) (**Supplementary Fig. 2**). The second PCA was carried out for cases and controls genotyped in the GWAS stage (**Supplementary Fig. 3**). Heterogeneity was examined using Cochran's Q and I^2 statistics. A fixed-effects (Mantel-Haenszel) model was applied for the meta-analysis if P_{het} for Q was >0.05; a random-effects model was adopted if P_{het} for Q was ≤0.05. Regional plots were generated using LocusZoom31 (see URLs). R was used to create quantile-quantile plots to evaluate the overall significance of the GWAS results (see URLs) (**Fig. 1** and **Supplementary Fig. 4a**,**b**). The difference in luciferase activity was assessed with an independent-samples t-test. $P < 0.05$ was considered significant, and all statistical tests were two sided.

45. Read, R.W. *et al.* Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. *Am. J. Ophthalmol.* **131**, 647–652 (2001).
46. International Study Group for Behçet's Disease. Criteria for diagnosis of Behcet's disease. *Lancet* **335**, 1078–1080 (1990).
47. Howie, B., Marchini, J. & Stephens, M. Genotype imputation with thousands of genomes. *G3 (Bethesda)* **1**, 457–470 (2011).
48. Zheng, X. *et al.* HIBAG—HLA genotype imputation with attribute bagging. *Pharmacogenomics J.* **14**, 192–200 (2014).

doi:10.1038/ng.3061