Replication of Genome-Wide Association Analysis Identifies New Susceptibility Loci at Long Noncoding RNA Regions for Vogt-Koyanagi-Harada Disease

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Purpose. This study was aimed at investigating the association of long noncoding RNA (lncRNA)–related single nucleotide polymorphisms (SNPs) with Vogt-Koyanagi-Harada (VKH) disease.

Methods. LncRNA-related SNPs were selected by multi-omics analysis. Genotyping, expression of lncRNA and mRNA, cell proliferation, and cytokine production were tested by MassARRAY System, real-time PCR, CCK8, and ELISA.

Results. A significant association with VKH was found for lnc-TOR3A-1:1/rs3829794, which is located in a non-*HLA* region (CC genotype: Bonferroni corrected *P* values [P_C] = 2.98×10^{-8}, odds ratio [OR] = 0.62; TT genotype: $P_C = 1.64 \times 10^{-8}$, OR = 1.57; C allele: $P_C = 1.39 \times 10^{-12}$, OR = 0.71). Additionally, an association was found for four lncRNA SNPs located in the *HLA* region. Functional experiments in rs3829794 genotyped individuals showed decreased *ABL2* (ABL proto-oncogene 2, nonreceptor tyrosine kinase) expression, decreased proliferation of anti-CD3 plus anti-CD28–stimulated peripheral blood mononuclear cells (PBMCs), and an increased production of IL-10 in CC carriers compared to TT carriers ($P = 0.0073$, $P = 0.0011$, and $P = 0.002$, respectively).

Conclusions. Our study identified five new loci associated with VKH susceptibility and identified a functional variant (lnc-TOR3A-1:1/rs3829794) that confers risk for VKH, which is possibly mediated by modulating gene expression, proliferation of lymphocytes, and regulation of anti-inflammatory cytokine production.

Keywords: lncRNA, Vogt-Koyanagi-Harada disease, multi-omics analysis

Uveitis is one of the main global causes of blindness[1]; Vogt-Koyanagi-Harada (VKH) disease is a subtype of uveitis and is among the most prevalent uveitis entities seen in China.[2] It is a refractory autoimmune disease directed against melanosome-associated antigens and affects tissues expressing these antigens.[3,4] It is characterized by granulomatous bilateral panuveitis usually with vitiligo, alopecia, poliosis, and central nervous system signs.[5,6] The pathogenesis of VKH disease is not yet clear, but infectious triggers and other environmental factors may cause this disease in genetically susceptible individuals.[7] It is well known that the intracellular sensors Nod1 and Nod2 are critical for bacterial recognition and host defense.[8] Recently, we have found that the higher expression of NOD1 and NOD2 is associated with VKH syndrome, which strengthens the hypothesis that systemic microbial infection may trigger this disease.[9] In fact, the high inducibility of Epstein-Barr virus replication has been found in B lymphocytes in VKH syndrome.[10] Earlier studies[11–13] have also identified several *HLA* (human leukocyte antigen) genes such as *HLA-DR4* (human leukocyte antigen DR4/HLA-DRB1*04) as well as non-*HLA* genes like *CTLA4* (cytotoxic T-lymphocyte–associated antigen-4), *MIF* (macrophage migration inhibitory factor), and *JAK1* (Janus kinase 1) to be associated with VKH disease. Most of these genes play a role during inflammation, underlining the role of these factors in the pathogenesis of the T-cell–mediated autoimmune diseases such as VKH, which is directed against one or more antigens associated with melanocytes, melanin, and RPE cells.[14]

An earlier study[15] has shown that more than one-third of the susceptibility gene loci identified by genome-wide association analysis (GWAS) are mapped to noncoding intervals. Many of these noncoding transcripts can be assigned to long noncoding RNAs (lncRNAs).[16] LncRNAs are emerging as important regulators of inflammatory immune responses, whereby genetic variants may affect their biologic function.[17,18] The role of genetic polymorphisms in lncRNAs in the predisposition to uveitis has not been widely studied and was therefore the aim of the study described here.[19]

In a recent GWAS study in VKH disease we have found an association with a susceptibility locus that maps to long noncoding genome regions with unknown function.[19,20] Using microarray analysis, abnormal expression of lncRNA was found in VKH disease patients as compared to healthy controls. Abnormal expression of lncRNA has also been observed earlier in prostate cancer, breast cancer, and hepatocellular cancer.[21,22] LncRNA has also been shown to play an important

Table 1. Selection of Potential LncRNA Susceptibility Loci Identified by Multi-omics Analysis

No.	CHR	SNP	*P* Value in Our GWAS Data	Related LncRNA	Tag SNP in Reported GWAS by Other Teams	Related Disease	PMID†
1	1	rs733887	4.23×10^{-4}	lnc-CASP9-1:1	rs12046278	Systolic blood pressure	19430479
2	1	rs3829794*	1.49×10^{-4}	lnc-TOR3A-1:1	rs1325195	IgE grass sensitization allergic rhinitis	22036096
3	5	rs2339962	1.69×10^{-4}	lnc-CPEB4-5:1	rs889014	Height	20881960
4	6	rs1063355	1.08×10^{-27}	lnc-HLA-DQA1-5:1	rs9275524	Autism spectrum disorder, schizophrenia	23453885
5	6	rs2516511	7.83×10^{-14}	lnc-ATP6V1G2-DDX39B-1:1	rs2248462	Hodgkin's lymphoma	22286212
6	6	rs3101942	1.87×10^{-7}	lnc-PSMB9-6:1	rs6936004	Disc degeneration (lumbar)	22993228
7	6	rs3117098	3.57×10^{-5}	lnc-HLA-DRA-1:1	rs3117098	Asthma	21804548
8	6	rs3130151	2.36×10^{-13}	lnc-SLC39A7-2:1	rs1883414	Nephropathy	21399633
9	6	rs3817979	5.80×10^{-161}	lnc-HLA-DRA-1:2	rs3817963	Lung adenocarcinoma	22797724
10	6	rs4713654	8.12×10^{-4}	lnc-C6orf125-1:1	N/A	N/A	N/A
11	6	rs2071463*	1.88×10^{-21}	lnc-HLA-DQA2-10:2	rs9296092	Chronic obstructive pulmonary disease	21685187
12	6	rs2284178*	2.43×10^{-9}	lnc-MICB-3:1	rs2255221	HIV-1 control	21051598
13	6	rs2523852*	1.55×10^{-13}	lnc-MUC22-1:2	rs9368677	Atopic dermatitis	23042114
14	6	rs12181270*	2.56×10^{-17}	lnc-HLA-DQA2-1:1	rs1049110	IgG glycosylation	23382691
15	17	rs1051855	7.19×10^{-3}	lnc-WDR45L-1:2	N/A	N/A	N/A
16	18	rs206526	4.92×10^{-4}	lnc-PIEZO2-5:2	N/A	N/A	N/A

CHR, chromosome; N/A, not applicable.
* Represents the potential susceptibility loci found to have a significant association in the current study.
† Represents the PubMed unique identifier numbers of each article.

role in the pathogenesis of autoimmune diseases.[23–25] This has been confirmed by the finding that lncRNA-related single nucleotide polymorphisms (SNPs) can confer risk to several immune-related diseases.[26] Our group[19] has recently found a strong association of the lncRNA rs6871626 with VKH disease by using a candidate gene approach. In this study we expanded these findings by using a multi-omics approach whereby we tested VKH susceptibility for all known SNPs located in long noncoding regions from our previous GWAS data. We identified a functional variant, lnc-TOR3A-1:1/rs3829794, which confers disease risk for VKH disease. This variant was shown to affect gene expression and proliferation of lymphocytes, and regulates the production of an anti-inflammatory cytokine.

Materials and Methods

Study Population

A total of 1500 VKH patients and 3000 unrelated healthy controls were recruited from The First Affiliated Hospital of Chongqing Medical University (Chongqing, China) between May 2008 and June 2017. The diagnosis of VKH was strictly performed according to International Workshop criteria.[27] In the meantime, a total of 3000 normal individuals, having the same ethnic background and geographic area as the VKH patients, were enrolled and were considered as normal controls. The controls were matched for age and sex with the VKH patients. The study was performed in two stages, whereby patients and controls for the first stage were from Southwest China, including Chongqing, Sichuan, Yunnan, and Guizhou province. A confirmatory second-stage study was conducted with a cohort of patients from North China (Hebei, Beijing), Central China (Henan, Hubei), and East China (Anhui, Shandong, and Zhejiang). The Student's *t*-test or the nonparametric Mann-Whitney *U* test was used to compare the differences concerning the clinical VKH characteristics between the first and second disease groups. No significant difference could be detected between the first- and second-stage group ($P > 0.05$). This study was performed according to the tenets of the Declaration of Helsinki and received approval from the Ethics Research Committee at the Chongqing Medical University (permit No. 2009-201008). Before participating in this study, all the VKH patients and normal controls were informed about the study and provided written informed consent. The present study was carried out with approval from the Institutional Review Board of the First Affiliated Hospital of Chongqing Medical University.

Selection of Single Nucleotide Polymorphisms

The selection of lncRNAs-related SNPs was based on multi-omics analysis (GWAS, lncRNA, and mRNA microarray) and three databases including UCSC Genome Browser Home (http://genome-asia.ucsc.edu/; in the public domain) lncRNASNP-human (http://bioinfo.life.hust.edu.cn/lncRNASNP/; in the public domain), and lincSNP (http://210.46.80.146/lincsnp/, in the public domain).[28,29] Based on the multi-omics analysis, the potential susceptibility loci were included when they had a Bonferroni corrected *P* values (P_C) value $\leq 10^{-3}$ for both GWAS and genome-wide gene expression studies. Furthermore, the lncRNA SNPs were considered as the regions 10 kb upstream or downstream of lncRNA gene transcripts. Linkage disequilibrium (LD) was tested between the chosen SNPs. LD was identified as $r^2 > 0.3$ or $D' > 0.7$ in the Ensembl genome browser. Finally, a total of 16 candidate SNPs were selected for this study (Table 1).

DNA Extraction and Genotyping

Genomic DNA from peripheral blood of the VKH patients and healthy controls was extracted with the QIAamp DNA Mini blood kit (QIAGEN, Valencia, CA, USA), based on the manufacturer's protocols. The extracted DNA was diluted and then stored at –80°C until used. The genotypes of lncRNAs SNPs were examined by MassARRAY System (Sequenom, San Diego, CA, USA). The primers used for genotyping were made according to MassARRAY Assay design software. All SNPs were genotyped by using the Sequenom method (Sequenom MassARRAY system) based on the manufacturer's manuals. The results of genotyping assay data were calculated by TYPER software version 4.0 (Sequenom) or TaqMan Genotyper Software. The eQTL (expression quantitative trait loci) analysis was performed by using two bioinformatics tools including the GTeX (http://www.gtexportal.org/home/; in the public domain) and haploreg v4 (http://www.broadinstitute.org/

Table 2. Clinical Features of Participants Enrolled in This Study

	First–Stage (Southwest China)	%	Second–Stage (North China, Central China, and East China)	%	Total	%
Patients with VKH	500	–	1000	–	1500	–
Age, mean ± SD, y	38.1 ± 11.3	–	35.3 ± 11.7	–	36.2 ± 11.6	–
Male	273	54.6	575	57.5	848	56.5
Female	227	45.4	425	42.5	652	43.5
Uveitis	500	100	1000	100	1500	100
Nuchal rigidity	76	15.2	130	13.0	206	13.7
Headache	212	42.4	391	39.1	603	40.2
Scalp allergy	88	17.6	154	15.4	242	16.1
Tinnitus	242	48.4	453	45.3	695	46.3
Dysacusia	180	36.0	318	31.8	498	33.2
Alopecia	191	38.2	420	42.0	611	40.7
Poliosis	186	37.2	408	40.8	594	39.6
Vitiligo	98	19.6	216	21.6	314	20.9
Healthy controls	1000	–	2000	–	3000	–
Age, mean ± SD, y	39.9 ± 11.2	–	39.7 ± 11.0	–	39.8 ± 11.1	–
Male	515	51.5	1060	53.0	1575	52.5
Female	485	48.5	940	47.0	1425	47.5

mammals/haploreg/haploreg.php; in the public domain) databases.[30,31]

Cell Culture and Detection

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. One part of the isolated PBMCs (unstimulated) was used for testing the expression of ABL2, the other was cultured with RPMI 1640 complete medium at a concentration of 1×10^6 cells/mL. These latter PBMCs were stimulated with lipopolysaccharide (LPS) (100 ng/mL; Sigma-Aldrich Corp., St. Louis, MO, USA) for 24 hours at 37°C under 5% CO_2 atmosphere to test the production of MCP-1, IL-1β, TNF-α, IL-8, and IL-6 according to a previous report.[32] To investigate IL-10, IFN-γ, and IL-17 production, the PBMCs were stimulated with anti-CD3 plus anti-CD28 antibodies (5:1; Miltenyi Biotec, Palo Alto, CA, USA) for 72 hours.

Detection of the Expression of LncRNA and mRNA

Total RNA including lncRNA and mRNA was extracted from unstimulated PBMCs with TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Real-time PCR was performed on the ABI7500 Fast System (Applied Biosystems, Foster City, CA, USA). Primers for the detection of lncRNA and mRNA were designed by KangChen Bio-tech (Shanghai, People's Republic of China). Using the $2^{-\Delta\Delta CT}$ method, the relative expression of lncRNA or mRNA was normalized to the expression of β-actin (internal control).

Microarray Analysis

Microarray analysis was performed by KangChen Bio-tech Company. In brief, the labeled RNAs were detected with the human LncRNA and mRNA Array (Arraystar, Rockville, MD, USA) according to the manufacturer's instructions.

Detection of Cell Proliferation

PBMCs were stimulated with LPS (100 ng/mL; Sigma-Aldrich Corp.) for 24 hours or anti-CD3 plus anti-CD28 antibodies (5:1; Miltenyi Biotec) for 72 hours at 37°C under 5% CO_2 atmosphere. The detection of cell proliferation was performed with the Cell Counting kit-8 (Sigma-Aldrich Corp.) following the manufacturer's instructions. The mean optical density was detected by using an ELISA reader at 450 nm (SpectraMax M2e; Molecular Devices, Sunnyvale, CA, USA).

Measurement of Cytokine Production by ELISA

The supernatant of cultured and stimulated PBMCs was collected and then stored at −80°C until cytokine measurement. The production of IL-10, IL-6, MCP-1, IL-1β, IL-8, IFN-γ, IL-17, and TNF-α was measured by Duoset ELISA development kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Statistical Analysis

Hardy-Weinberg equilibrium in the healthy control group was detected by using SHEsis software (Shanghai JiaoTong University, Shanghai, China). The genotype and allele frequencies were compared between VKH patients and normal controls by SPSS software (v. 17.0; SPSS, Inc., Chicago, IL, USA) with the χ^2 test. The P_c were calculated by multiplying with the number of comparisons performed. Results of gene expression and cell proliferation were analyzed by Student's *t*-test. Cytokine expression was analyzed by the nonparametric Mann-Whitney *U* test. Results were considered to be significantly different when $P < 0.05$. Data in figure legends are shown as mean ± SD.

Results

Clinical Features of VKH Disease Patients

The distribution of clinical features and demographic characteristics of the enrolled VKH patients are shown in Table 2. The enrolled VKH patients consisted of a posterior uveitis group (168 patients, 11.2%), anterior uveal involvement group (409 patients, 27.3%), and recurrent granulomatous anterior uveitis group (923, 61.5%). The following clinical features were noted: 100% with uveitis, 13.7% with nuchal rigidity, 40.2% with headache, 16.1% with scalp allergy, 46.3% with tinnitus, 33.2% with dysacusia, 40.7% with alopecia, 39.6% with poliosis, and 20.9% with vitiligo. As previously mentioned, reduced doses of

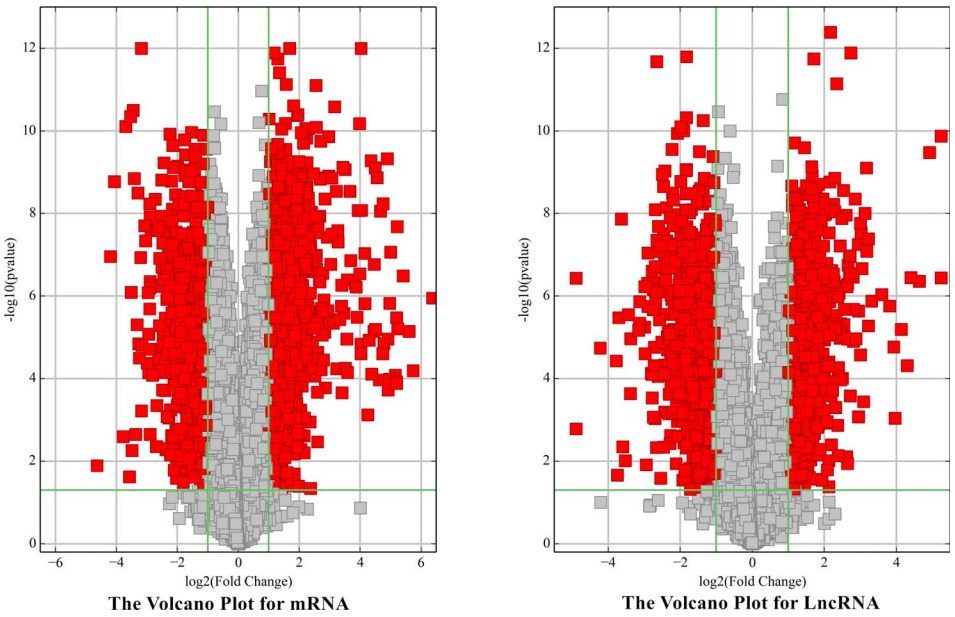

Figure 1. The volcano plot for mRNAs and lncRNAs in matched VKH disease and normal control samples.

corticosteroids combined with immunosuppressive agents were used to treat these patients.[33]

Expression Profile of LncRNA and mRNA in VKH Disease

Microarray analysis showed that many lncRNAs were abnormally expressed in VKH. Among these, 152 were upregulated, whereas 159 were downregulated (fold change $\geq$2.0, $P <$ 0.05). A total of 243 mRNAs showed a significant difference in their expression when the VKH group was compared with controls: 91 were upregulated, and 152 were downregulated (fold change $\geq$2.0, $P <$ 0.05). This analysis thus revealed a different expression pattern of mRNAs and lncRNAs in matched VKH disease patients versus normal controls (Fig. 1).

Gene Ontology (GO) and KEGG Pathway Analysis

GO enrichment analysis was performed to identify the significantly dysregulated lncRNA and mRNA species in VKH disease. Our data demonstrated that the abnormal expression of lncRNAs involved various biological functions such as protein binding, nucleic acid binding, and transmembrane signaling receptor activity with involvement in different signaling pathways, especially those involved in the regulation of the immune system (Figs. 2, 3).

Genotype and Allele Frequencies of Detected SNPs in VKH Patients and Normal Controls for the First-Stage Study

A total of 16 SNPs were successfully genotyped in 500 VKH patients and 1000 normal controls for the first-stage study. The selected SNPs did not deviate from the Hardy-Weinberg equilibrium in the normal controls. Eight of the 16 detected lncRNA SNPs were significantly associated with VKH disease. Among these, only one SNP (rs3829794) was located in a non-*HLA* region, whereas the rest were all located in the *HLA* gene region (Table 3). Four novel susceptibility SNPs (rs12181270, rs2284178, rs2523852, rs2071463) were identified in the *HLA* region; these showed a weak LD with rs3021304 and rs114800139, which were shown earlier by our GWAS data to have a significant association with VKH.[20] The weak LD suggests that these four susceptibility loci may represent an

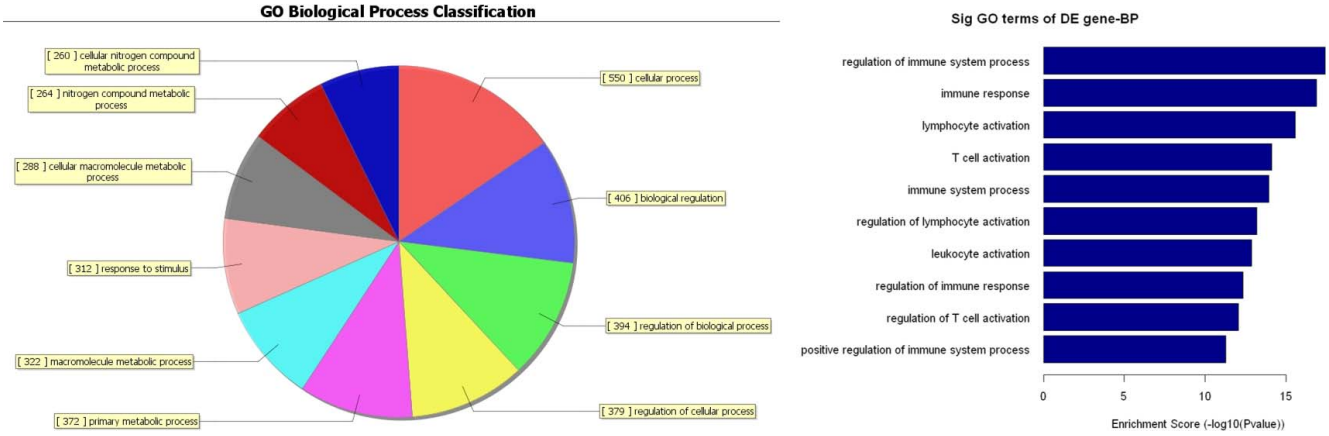

Figure 2. The abnormal expression of lncRNAs involves various biological functions including regulation of the immune system.

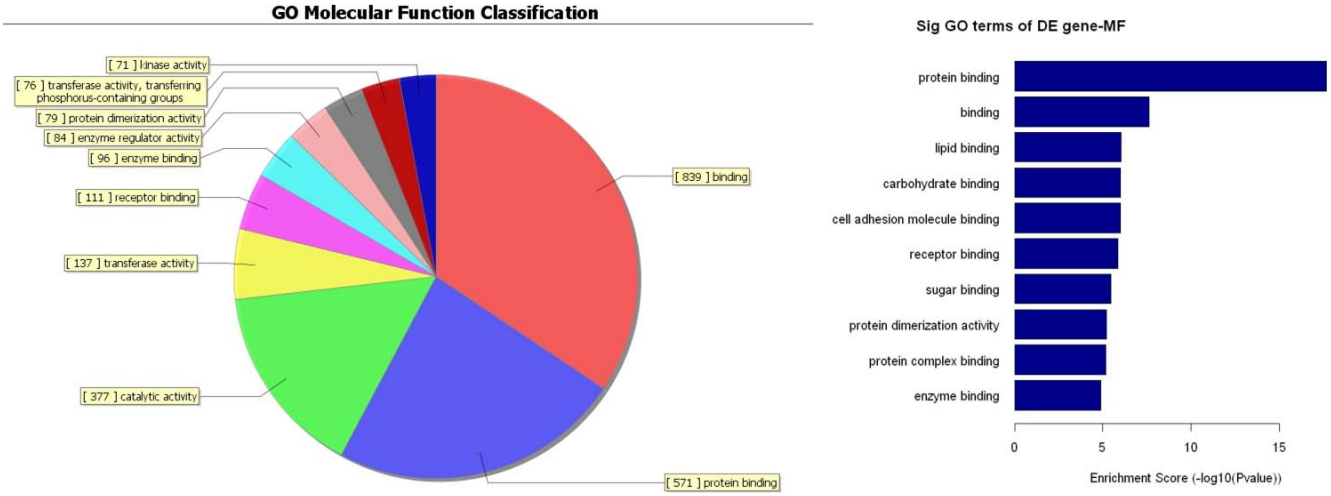

Figure 3. The abnormal expression of lncRNAs involves various biological functions including regulation of the protein binding.

independent risk locus for VKH (Table 4). These five new susceptibility loci (rs3829794, rs12181270, rs2284178, rs2523852, rs2071463) are considered to affect gene regulation as shown by the two bioinformatics tools GTeX and haploreg v4 (Table 5). However, *HLA* alleles are highly polymorphic and difficult to investigate owing to interference of different alleles and scarcity of some alleles.[34,35] In our subsequent experiments we therefore focused on the non-*HLA* region and performed experiments to investigate whether rs3829794 had a biological function.

Table 3. Allele and Genotype Frequencies of Eight SNPs Significantly Associated With VKH in the First-Stage Study

SNPs	Geno/Allele	VKH	Control	χ^2	*P* Value	P_c Value	OR (95% CI)
Rs1063355	GG	325	443	57.17	4.01×10^{-14}	1.92×10^{-12}	2.34 (1.87–2.92)
lnc-HLA-DQA1-5:1	GT	154	461	32.28	1.35×10^{-8}	6.48×10^{-7}	0.52 (0.42–0.65)
*GWAS: 1.08×10^{-27}	TT	21	96	13.52	2.37×10^{-4}	0.011	0.41 (0.25–0.67)
	G	804	1347	55.95	7.42×10^{-14}	3.56×10^{-12}	1.99 (1.66–2.39)
Rs12181270	CC	309	423	50.73	1.06×10^{-12}	5.09×10^{-11}	2.21 (1.77–2.75)
lnc-HLA-DQA2-1:1	CG	168	445	16.39	5.16×10^{-5}	2.48×10^{-3}	0.63 (0.51–0.79)
GWAS: 2.56×10^{-17}	GG	23	132	26.61	2.49×10^{-7}	1.20×10^{-5}	0.32 (0.20–0.50)
	C	786	1291	61.78	3.84×10^{-15}	1.84×10^{-13}	2.01 (1.69–2.41)
Rs2284178	CC	91	256	10.27	1.36×10^{-3}	NS	0.65 (0.50–0.85)
lnc-MICB-3:1	CT	240	486	0.048	0.826	NS	0.98 (0.79–1.21)
GWAS: 2.43×10^{-9}	TT	169	258	10.48	1.21×10^{-3}	NS	1.47 (1.16–1.85)
	C	422	998	15.86	6.84×10^{-5}	3.28×10^{-3}	0.73 (0.63–0.85)
Rs2523852	CC	75	195	4.573	0.032	NS	0.73 (0.55–0.98)
lnc-MUC22-1:2	CG	192	493	15.96	6.74×10^{-5}	3.24×10^{-3}	0.64 (0.52–0.80)
GWAS: 1.55×10^{-13}	GG	233	312	34.18	5.04×10^{-9}	2.42×10^{-7}	1.92 (1.54–2.40)
	C	342	883	27.32	1.73×10^{-7}	8.30×10^{-6}	0.66 (0.56–0.77)
Rs3117098	CC	24	92	9.044	2.64×10^{-3}	NS	0.50 (0.31–0.79)
lnc-HLA-DRA-1:1	CT	174	410	5.389	0.021	NS	0.77 (0.62–0.96)
GWAS: 3.57×10^{-5}	TT	302	498	15.05	1.05×10^{-4}	5.04×10^{-3}	1.54 (1.24–1.91)
	C	222	594	18.94	1.35×10^{-5}	6.48×10^{-4}	0.68 (0.57–0.81)
Rs3817979	TT	309	785	47.09	6.77×10^{-12}	3.25×10^{-10}	0.44 (0.35–0.56)
lnc-HLA-DRA-1:2	TC	168	198	34.41	4.46×10^{-9}	2.14×10^{-7}	2.05 (1.61–2.61)
GWAS: 5.80×10^{-161}	CC	23	17	10.80	1.01×10^{-3}	0.048	2.79 (1.48–5.27)
	T	786	1768	50.59	1.14×10^{-12}	5.47×10^{-11}	0.48 (0.39–0.59
Rs2071463	TT	77	112	5.339	0.021	NS	1.44 (1.06–1.97)
lnc-HLA-DQA1-5:1	TC	239	425	3.795	0.051	NS	1.24 (0.99–1.54)
GWAS: 1.88×10^{-21}	CC	184	463	12.27	4.62×10^{-4}	0.022	0.68 (0.54–0.84)
	T	393	649	13.80	2.03×10^{-4}	9.74×10^{-3}	1.35 (1.15–1.58)
Rs3829794	CC	96	278	13.17	2.84×10^{-4}	0.014	0.62 (0.48–0.80)
lnc-TOR3A-1:1	CT	252	506	0.005	0.94	NS	0.99 (0.80–1.23)
GWAS: 1.49×10^{-4}	TT	152	216	13.94	1.89×10^{-4}	9.07×10^{-3}	1.59 (1.24–2.02)
	C	444	1062	20.18	7.03×10^{-6}	3.37×10^{-4}	0.71 (0.61–0.82)

NS, not significant.

* The *P* value of GWAS comes from the previous GWAS study for VKH disease by our group.

Table 4. Summary of VKH-Associated LncRNA-Related SNPs Located in the HLA Region

No.	CHR	SNP	*P* Value in GWAS	OR in GWAS	r^2 With rs3021304	D′ With rs3021304	r^2 With rs114800139	D′ With rs114800139
1	6	rs1063355	1.08×10^{-27}	0.4577	0.120	0.427	0.378	0.999
2	6	rs12181270	2.56×10^{-17}	0.5437	0.067	0.268	0.146	0.618
3	6	rs2284178	2.43×10^{-9}	0.6903	0.054	0.299	0.061	0.396
4	6	rs2523852	1.55×10^{-13}	0.6282	None	None	0.076	0.342
5	6	rs3117098	3.57×10^{-5}	0.7414	0.132	0.750	0.069	0.596
6	6	rs3817979	5.80×10^{-161}	6.455	0.172	0.999	0.071	0.351
7	6	rs2071463	1.88×10^{-21}	1.816	0.083	0.327	0.052	0.270

Allele and Genotype Frequencies of Detected SNPs in VKH Patients and Controls for the Combined Study

As eight lncRNA-related SNPs showed a significant association with VKH disease in the first stage, we validated the result of these SNPs in independent cohorts that contained another 1000 VKH patients and 2000 controls in a second-stage study. The combined studies confirmed the association of rs3829794 with VKH disease, which as mentioned earlier, is located in a non-*HLA* region and has been identified as lnc-TOR3A-1:1 (CC genotype: $P_C = 2.98 \times 10^{-8}$, odds ratio [OR] = 0.62; TT genotype: $P_C = 1.64 \times 10^{-8}$, OR = 1.57; C allele: $P_C = 1.39 \times 10^{-12}$, OR = 0.71). The association with the other four lncRNA SNPs located in the *HLA* region were also confirmed (Table 6).

The Influence of rs3829794 on the Expression of ABL2

To address the biological function of lnc-TOR3A-1:1, we examined the role of various genotypes of rs3829794 on the expression of the adjacent *ABL2* gene. The expression of ABL2 was detected in unstimulated PBMCs obtained from unselected 41 normal individuals. Healthy genotyped controls were used, since the inflammatory response and treatment with immunosuppressive drugs in the VKH group might affect expression of this gene. Our study showed a significantly decreased expression of ABL2 in CC carriers compared to TT carriers (Fig. 4; $P = 0.007$).

The Effect of rs3829794 on the Proliferation of PBMCs

In view of the important role of ABL2 in cell proliferation and oncogenesis,[36] our further study examined the effect of rs3829794 on the proliferation of PBMCs. Our results showed a significantly decreased proliferation of anti-CD3 plus anti-CD28–stimulated PBMCs in CC carriers compared to TT carriers (Fig. 5; $P = 0.0011$). However, no significant association between individuals with different genotypes of rs3829794 was observed for the proliferation of LPS-stimulated PBMCs (Fig. 5).

The Influence of rs3829794 on Cytokine Production

A cytokine network represented by IFN-γ, IL-10, IL-17, IL-8, IL-6, MCP-1, IL-1β, and TNF-α has been shown to play a role in the pathogenesis of VKH.[37–39] We subsequently investigated whether the different genotypes of rs3829794 had an effect on cytokine production. An increased production of the anti-inflammatory cytokine IL-10 by stimulated PBMCs was found in CC carriers compared to TT or CT carriers (Fig. 6A; $P = 0.002$ and $P = 0.029$, respectively). However, no significant association was found for the production of TNF-α, IL-8, IL-6, IL-17, IFN-γ, and IL-1β by stimulated PBMCs among the different genotype carriers (Figs. 6B–H).

Discussion

In this study, we investigated the association of the related SNPs located in lncRNA regions with VKH disease in a Chinese Han population and identified five lncRNA-related susceptibility loci. Further study showed that individuals with the CC genotype of rs3829794 had decreased expression of the *ABL2* gene, increased production of IL-10, and decreased proliferation of anti-CD3 plus anti-CD28–stimulated PBMCs, which implied that this is a functional variant. Our study not only identified several new susceptibility loci for VKH in the lncRNA region, but also added to the existing knowledge that lncRNAs play important roles in the development of autoimmune disease.[25,40]

We recently have reported a GWAS study[20] and identified several non-*HLA* and *HLA* gene loci that show a significant association with VKH. However, the molecular mechanisms underlying the causality of VKH risk–associated SNPs are not fully understood.[37] As with many other complex diseases, these risk-associated SNPs often map to noncoding regions of the genome and their role in the control of adjacent genes is often not known.[41] The potential functional SNPs are usually close to both lncRNAs and protein-coding genes.[22] The number of lncRNAs located in these noncoding SNPs far exceeds that of protein-coding genes, providing a theoretical support to investigate the functional link between noncoding SNPs and lncRNAs.[42] Owing to the importance of multi-omics

Table 5. The Potential Functional Activity of LncRNA-Related SNPs for Gene Regulation

SNP	Chrom	Gencode ID	Gene Symbol	Transcript Type	Variant ID	*P* Value	NES
rs3829794	1	ENSG00000143322.15	ABL2	Protein coding	1_179072728_T_C_b37	2.2×10^{-9}	−0.44
rs2071463	6	ENSG00000250535.1	STK19P	Pseudogene	6_32812528_C_T_b37	6.5×10^{-7}	0.44
rs2523852	6	ENSG00000228789.2	HCG22	Protein coding	6_31023868_G_C_b37	5.7×10^{-29}	−0.62
rs2284178	6	ENSG00000204525.10	HLA-C	Protein coding	6_31432125_C_T_b37	6.5×10^{-24}	−0.51
rs12181270	6	ENSG00000232080.3	XXbac-BPG254F23.7	Long noncoding RNA	6_32685894_G_C_b37	2.2×10^{-48}	−0.61

Bioinformatic analysis was conducted by GTeX and haploreg v4. Chrom, chromosome; NES, normalized effect size.

Table 6. Allele and Genotype Frequencies of Tested SNPs in VKH Patients and Controls for the Combined Study

SNPs	Geno/ Allele	VKH	Control	χ^2	*P* Value	P_c Value	OR (95% CI)
Rs12181270	CC	928	1271	152.18	5.79×10^{-35}	2.78×10^{-33}	2.21 (1.94–2.51)
lnc-HLA-DQA2-1:1	CG	498	1340	54.42	1.62×10^{-13}	7.78×10^{-12}	0.62 (0.54–0.70
GWAS: 2.56×10^{-17}	GG	74	389	69.91	6.19×10^{-17}	2.97×10^{-15}	0.35 (0.27–0.45)
	C	2354	3882	178.13	1.24×10^{-40}	5.95×10^{-39}	1.99 (1.80–2.20)
Rs2284178	CC	273	761	29.02	7.16×10^{-8}	3.43×10^{-6}	0.66 (0.56–0.76)
lnc-MICB-3:1	CT	734	1459	0.036	0.849	NS	1.01 (0.89–1.15)
GWAS: 2.43×10^{-9}	TT	493	780	23.24	1.43×10^{-6}	6.86×10^{-5}	1.39 (1.22–1.60)
	C	1280	2981	39.50	3.28×10^{-10}	1.57×10^{-8}	0.75 (0.69–0.82)
Rs2523852	CC	227	585	12.89	3.30×10^{-4}	0.016	0.74 (0.62–0.87)
lnc-MUC22-1:2	CG	580	1479	45.56	1.48×10^{-11}	7.10×10^{-10}	0.65 (0.57–0.74)
GWAS: 1.55×10^{-13}	GG	693	936	97.42	5.60×10^{-23}	2.69×10^{-21}	1.89 (1.67–2.15)
	C	1034	2649	77.57	1.28×10^{-18}	6.14×10^{-17}	0.67 (0.61–0.73)
Rs2071463	TT	232	336	16.51	4.85×10^{-5}	2.33×10^{-3}	1.45 (1.21–1.74)
lnc-HLA-DQA1-5:1	TC	717	1273	11.68	6.33×10^{-4}	0.030	1.24 (1.10–1.41)
GWAS: 1.88×10^{-21}	CC	551	1391	37.83	7.72×10^{-10}	3.71×10^{-8}	0.67 (0.59–0.76)
	T	1181	1945	42.62	6.66×10^{-11}	3.20×10^{-9}	1.35 (1.24–1.48)
Rs3829794	CC	290	834	38.254	6.21×10^{-10}	2.98×10^{-8}	0.62 (0.54–0.72)
lnc-TOR3A-1:1	CT	758	1518	0.002	0.97	NS	0.99 (0.88–1.13)
GWAS: 1.49×10^{-4}	TT	452	648	39.426	3.41×10^{-10}	1.64×10^{-8}	1.57 (1.36–1.80)
	C	1338	3186	57.80	2.90×10^{-14}	1.39×10^{-12}	0.71 (0.65–0.78)

analysis in the study of the pathogenesis of complex diseases, we decided to integrate the results of multi-omics analysis including GWAS and genome-wide gene expression study as a method to select the candidate SNP loci. A total of 16 lncRNA susceptibility loci were selected by multi-omics analysis. The tag SNPs of these genetic variants have been shown to be associated with several inflammatory and allergic diseases as well as with certain types of cancer.[43–46] Examples of the association with allergic disease include IgE grass pollen, allergic rhinitis, and atopic dermatitis.[44,47,48] The findings mentioned above indicate that genetic variants in the lncRNA regions are probably important in the control of pathways that are shared by both allergic and autoimmune disease. Although the GWAS tagSNP rs1325195 for rs3829794/lnc-TOR3A-1:1 has an association with allergic rhinitis and grass sensitization,[48] our study is the first to report that rs3829794/lnc-TOR3A-1:1, which is located in a non-*HLA* region on chromosome 1, is associated with a complex disease such as VKH disease.[48] Moreover, we also identified several new susceptibility loci located in the *HLA* region and found that these SNPs might have a biological function by bioinformatics prediction. As mentioned above, SNP rs3829794, which is located upstream of the *ABL2* gene, showed the strongest association with VKH. According to bioinformatics information provided by the GTEx database, SNP rs3829794 has the potential to affect the expression of the *ABL2* gene.[31] We were able to prove this assumption and showed a significantly decreased expression of ABL2 in rs3829794 CC carriers when compared to TT carriers. ABL2 plays an important role in the proliferation and invasion of cancer cells[36] and although a potential role of ABL2 has been reported in immune disorders such as multiple sclerosis and diabetes, the exact role of ABL2 in these diseases is not yet clear.[49,50] The findings presented here suggest that the rs3829794 CC genotype, which protects against acquiring VKH, might be due to a decreased expression of ABL2. Recent studies have shown that inhibition of ABL kinases ameliorates experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis,[49] which is further supported by our hypothesis.

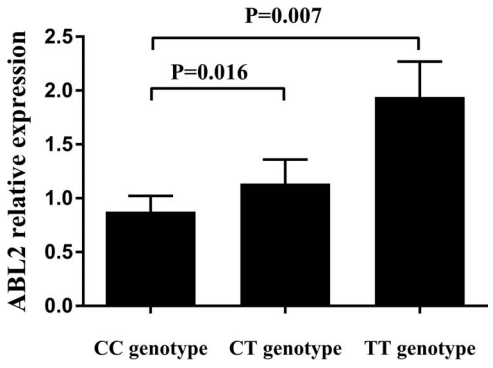

Figure 4. The influence of rs3829794 on the expression of ABL2. ABL2 expression in unstimulated PBMCs from healthy controls with different genotypes of rs3829794 (CC = 12, CT = 20, TT = 9).

This SNP also had an effect on the proliferation of PBMCs and the production of anti-inflammatory cytokines that are potentially involved in the pathogenesis of VKH. As mentioned earlier, we only detected the influence of rs3829794 in normal controls because the patient groups are extremely heterogeneous owing to the different immunosuppressive drug treatment and the variable inflammatory course. Our results showed a significantly decreased proliferation of anti-CD3 plus anti-CD28–stimulated PBMCs in CC cases compared with TT cases. An earlier study[51] has shown that ABL kinase activity is required for IL-2 production and proliferation of primary T cells. Although the key role of ABL2 in cell proliferation and tumorigenesis has been widely reported, our results failed to detect an effect of rs3829794 genotypes on the proliferation of LPS-stimulated PBMCs. One of the possible reasons for this discrepancy may be the use of different types of immune cells (lymphocytes versus monocytes). It is well known that cytokines play an important role in the pathogenesis of uveitis.[52] We therefore investigated whether the different genotypes of rs3829794 affected these cytokines, such as IFN-γ, IL-10, IL-17, IL-8, IL-6, MCP-1, IL-1β, and TNF-α. Unexpectedly, we found an increased production of IL-10 in the CC genotype, which is different from its stimulatory effect on other cytokines. One possible reason is that ABL2 affects not only Flt3 (fms-like tyrosine kinase 3 ligand) signaling but also AKT (also known as protein kinase B) signaling.[53] All these factors have been shown to be involved in the regulation of IL-

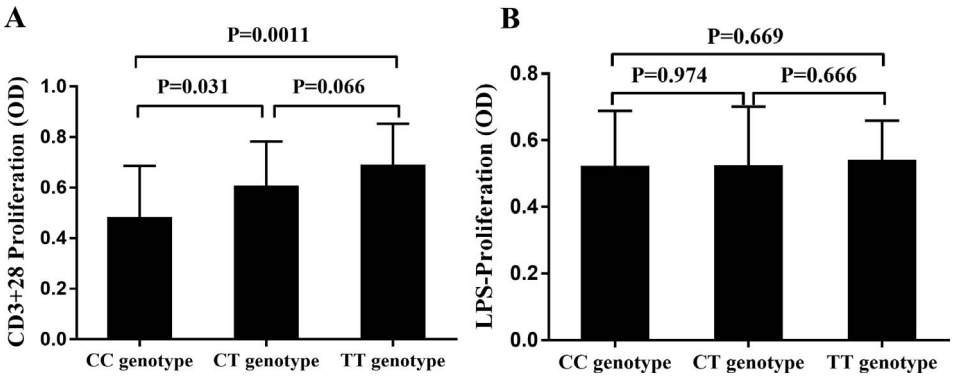

FIGURE 5. The effect of rs3829794 on the proliferation of PBMCs. (**A**) The proliferation of anti-CD3 plus anti-CD28–stimulated PBMCs from healthy controls with different genotypes of rs3829794 (CC = 12, CT = 20, TT = 9). (**B**) The proliferation of LPS-stimulated PBMCs from healthy controls with different genotypes of rs3829794 (CC = 12, CT = 20, TT = 9).

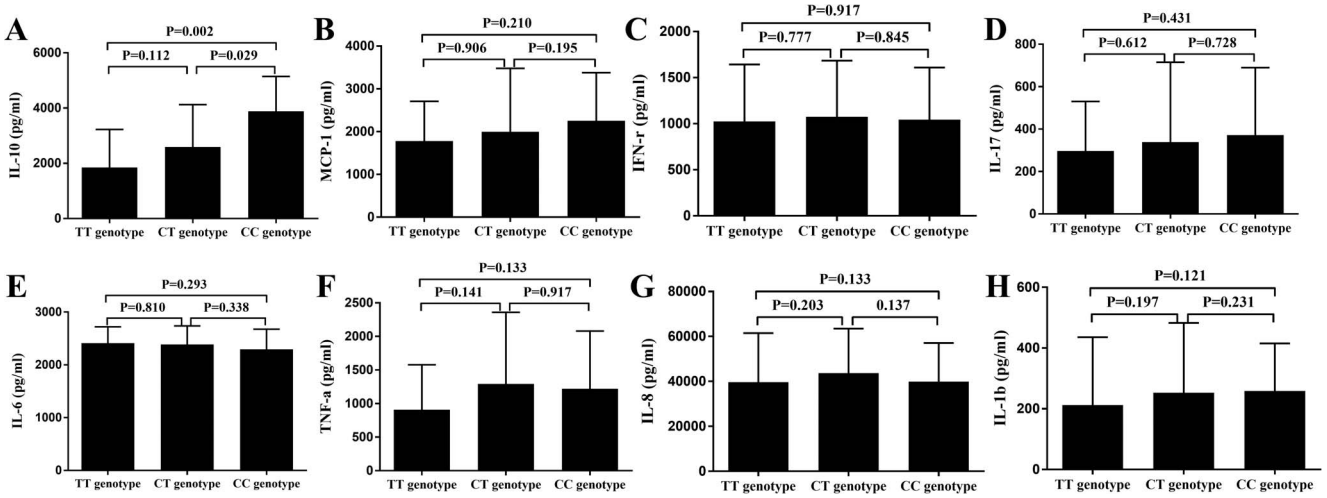

FIGURE 6. The effect of rs3829794 on the production of cytokines. The production of IL-10 (**A**), MCP-1 (**B**), IFN-γ (**C**), IL-17 (**D**), IL-6 (**E**), TNF-α (**F**), IL-8 (**G**), and IL-1β (**H**) by stimulated PBMCs from healthy controls with different genotypes of rs3829794 (CC = 12, CT = 20, TT = 9).

10 signaling.[54,55] In fact, T cells lacking ABL kinases exhibit reduced proliferation and production of IL-2 and IFN-γ but display similar levels of IL-4 in response to T cell receptor stimulation.[56] The possible reason for this discrepancy may be the use of different types of cells (purified T cells versus impure PBMCs). Therefore, the definite mechanism whereby ABL2 affects IL-10 signaling is not yet clear and requires further investigation. Taken together, these results imply that the functional variant rs3829794/lnc-TOR3A-1:1 may protect against VKH disease not only by suppressing the proliferation of lymphocytes but also by regulating anti-inflammatory cytokine production.

There were several limitations in our study. First of all, the selection of the 16 SNPs used in our study was based on a multi-omics analysis. There may actually be many more SNPs than the 16 that have been identified until now. Although the vast majority of newly identified lncRNA SNPs showed no significant association with autoimmune disease, but with breast and prostate cancers, a thorough study of the association of the SNPs in the whole long noncoding region should be performed in the future to reinvestigate their association with autoimmune disease. Our study cannot be generalized for uveitis, since we only investigated VKH patients, and further studies including other uveitis entities are needed to address this issue. It is worth mentioning that all our experiments were performed with PBMCs, which consist of numerous types of immune cells. It is possible that ABL expression and proliferation might be relevant in one cell type such as T cells following CD3-CD28 antibody stimulation, while LPS might have influenced IL-10 production by monocytes. Therefore, the effects of rs3829794 on purified immune cells need to be further explored. It should also be noted that our study was limited to Han Chinese and our results also need to be confirmed in other ethnic groups.

In summary, we identified five new susceptibility loci for VKH disease at the lncRNA region and showed that a functional variant, lnc-TOR3A-1:1/rs3829794, might protect against VKH disease by modulating gene expression, proliferation of lymphocytes, and regulating anti-inflammatory cytokine production. Recent studies showed that gene therapy may provide a safe, effective, and long-term intervention for ocular diseases. Whether the novel molecular biomarker identified in this study could be used for gene therapy in VKH patients is expected to be addressed in a future study.

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References

1. Suttorp-Schulten MS, Rothova A. The possible impact of uveitis in blindness: a literature survey. *Br J Ophthalmol*. 1996;80:844–848.
2. Yang P, Zhang Z, Zhou H, et al. Clinical patterns and characteristics of uveitis in a tertiary center for uveitis in China. *Curr Eye Res*. 2005;30:943–948.
3. Greco A, Fusconi M, Gallo A, et al. Vogt-Koyanagi-Harada syndrome. *Autoimmun Rev*. 2013;12:1033–1038.
4. Sugita S, Takase H, Taguchi C, et al. Ocular infiltrating CD4+ T cells from patients with Vogt-Koyanagi-Harada disease recognize human melanocyte antigens. *Invest Ophthalmol Vis Sci*. 2006;47:2547–2554.
5. Yang P, Ren Y, Li B, Fang W, Meng Q, Kijlstra A. Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. *Ophthalmology*. 2007;114:606–614.
6. Tsai R. Vogt-Koyanagi-Harada disease (uveomeningitic syndrome). In: *Albert & Jakobiec's Principles and Practice of Ophthalmology*. Philadelpha, PA: Saunders/Elsevier; 2008: 1201–1209.
7. Qi J, Hou S, Zhang Q, et al. A functional variant of pre-miRNA-196a2 confers risk for Behcet's disease but not for Vogt-Koyanagi-Harada syndrome or AAU in ankylosing spondylitis. *Hum Genet*. 2013;132:1395–1404.
8. Kim YG, Park JH, Shaw MH, Franchi L, Inohara N, Nunez G. The cytosolic sensors Nod1 and Nod2 are critical for bacterial recognition and host defense after exposure to Toll-like receptor ligands. *Immunity*. 2008;28:246–257.
9. Deng B, Ye Z, Li L, et al. Higher expression of NOD1 and NOD2 is associated with Vogt-Koyanagi-Harada (VKH) syndrome but not Behcet's disease (BD). *Curr Mol Med*. 2016;16: 424–435.
10. Minoda H, Sakai J, Sugiura M, Imai S, Osato T, Usui M. High inducibility of Epstein-Barr virus replication in B lymphocytes in Vogt-Koyanagi-Harada disease [in Japanese]. *Nippon Ganka Gakkai Zasshi*. 1999;103:289–296.
11. Zhang C, Liu S, Hou S, et al. MIF gene polymorphisms confer susceptibility to Vogt-Koyanagi-Harada syndrome in a Han Chinese population. *Invest Ophthalmol Vis Sci*. 2013;54: 7734–7738.
12. Du L, Yang P, Hou S, et al. Association of the CTLA-4 gene with Vogt-Koyanagi-Harada syndrome. *Clin Immunol*. 2008;127: 43–48.
13. 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.
14. Attia S, Khochtali S, Kahloun R, Zaouali S, Khairallah M. Vogt–Koyanagi–Harada disease. *Expert Rev Ophthalmol*. 2012;7: 565–585.
15. Jin G, Sun J, Isaacs SD, et al. Human polymorphisms at long non-coding RNAs (lncRNAs) and association with prostate cancer risk. *Carcinogenesis*. 2011;32:1655–1659.
16. Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet*. 2015;47:199–208.
17. Satpathy AT, Chang HY. Long noncoding RNA in hematopoiesis and immunity. *Immunity*. 2015;42:792–804.
18. Engreitz JM, Haines JE, Perez EM, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing. *Nature*. 2016;539:452–455.
19. Yue Y, Zhang J, Yang L, et al. Association of long noncoding RNAs polymorphisms with ankylosing spondylitis, Vogt-Koyanagi-Harada disease, and Behcet's disease. *Invest Ophthalmol Vis Sci*. 2018;59:1158–1166.
20. Hou S, Du L, Lei B, et al. Genome-wide association analysis of Vogt-Koyanagi-Harada syndrome identifies two new susceptibility loci at 1p31.2 and 10q21.3. *Nat Genet*. 2014;46:1007–1011.
21. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. *Cancer Cell*. 2016;29:452–463.
22. Guo H, Ahmed M, Zhang F, et al. Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. *Nat Genet*. 2016;48:1142–1150.
23. Hrdlickova B, Kumar V, Kanduri K, et al. Expression profiles of long non-coding RNAs located in autoimmune disease-associated regions reveal immune cell-type specificity. *Genome Med*. 2014;6:88.
24. Sigdel KR, Cheng A, Wang Y, Duan L, Zhang Y. The emerging functions of long noncoding RNA in immune cells: autoimmune diseases. *J Immunol Res*. 2015;2015:848790.
25. Wu GC, Pan HF, Leng RX, et al. Emerging role of long noncoding RNAs in autoimmune diseases. *Autoimmun Rev*. 2015;14:798–805.
26. Farh KK, Marson A, Zhu J, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*. 2015;518:337–343.
27. Read RW, Holland GN, Rao NA, et al. Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. *Am J Ophthalmol*. 2001;131:647–652.
28. Gong J, Liu W, Zhang J, Miao X, Guo AY. lncRNASNP: a database of SNPs in lncRNAs and their potential functions in human and mouse. *Nucleic Acids Res*. 2015;43:D181–D186.
29. Ning S, Yue M, Wang P, et al. LincSNP 2.0: an updated database for linking disease-associated SNPs to human long non-coding RNAs and their TFBSs. *Nucleic Acids Res*. 2017; 45:D74–D78.
30. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. 2016;44:D877–D881.
31. GTEx Consortium. Human genomics: the Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348:648–660.
32. Zhou Q, Hou S, Liang L, et al. MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behcet's disease and Vogt-Koyanagi-Harada syndrome. *Ann Rheum Dis*. 2014;73:170–176.
33. Yang P, Ye Z, Du L, et al. Novel treatment regimen of Vogt-Koyanagi-Harada disease with a reduced dose of corticosteroids combined with immunosuppressive agents. *Curr Eye Res*. 2018;43:254–261.
34. Xie C, Yeo ZX, Wong M, et al. Fast and accurate HLA typing from short-read next-generation sequence data with xHLA. *Proc Natl Acad Sci U S A*. 2017;114:8059–8064.
35. Ovsyannikova IG, Schaid DJ, Larrabee BR, Haralambieva IH, Kennedy RB, Poland GA. A large population-based association

Investigative Ophthalmology & Visual Science

study between HLA and KIR genotypes and measles vaccine antibody responses. *PLoS One*. 2017;12:e0171261.

36. Gil-Henn H, Patsialou A, Wang Y, Warren MS, Condeelis JS, Koleske AJ. Arg/Abl2 promotes invasion and attenuates proliferation of breast cancer in vivo. *Oncogene*. 2013;32: 2622–2630.
37. Du L, Kijlstra A, Yang P. Vogt-Koyanagi-Harada disease: novel insights into pathophysiology, diagnosis and treatment. *Prog Retin Eye Res*. 2016;52:84–111.
38. Damico FM, Cunha-Neto E, Goldberg AC, et al. T-cell recognition and cytokine profile induced by melanocyte epitopes in patients with HLA-DRB1*0405-positive and -negative Vogt-Koyanagi-Harada uveitis. *Invest Ophthalmol Vis Sci*. 2005;46:2465–2471.
39. Horai R, Caspi RR. Cytokines in autoimmune uveitis. *J Interferon Cytokine Res*. 2011;31:733–744.
40. Xia M, Liu J, Liu S, et al. Ash1l and lnc-Smad3 coordinate Smad3 locus accessibility to modulate iTreg polarization and T cell autoimmunity. *Nat Commun*. 2017;8:15818.
41. Castellanos-Rubio A, Fernandez-Jimenez N, Kratchmarov R, et al. A long noncoding RNA associated with susceptibility to celiac disease. *Science*. 2016;352:91–95.
42. Elkon R, Agami R. Characterization of noncoding regulatory DNA in the human genome. *Nat Biotechnol*. 2017;35:732–746.
43. Siedlinski M, Cho MH, Bakke P, et al. Genome-wide association study of smoking behaviours in patients with COPD. *Thorax*. 2011;66:894–902.
44. Hirota T, Takahashi A, Kubo M, et al. Genome-wide association study identifies eight new susceptibility loci for atopic dermatitis in the Japanese population. *Nat Genet*. 2012;44:1222–1226.
45. Lauc G, Huffman JE, Pucic M, et al. Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. *PLoS Genet*. 2013;9:e1003225.
46. Shiraishi K, Kunitoh H, Daigo Y, et al. A genome-wide association study identifies two new susceptibility loci for lung adenocarcinoma in the Japanese population. *Nat Genet*. 2012;44:900–903.
47. Ramasamy A, Curjuric I, Coin LJ, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol*. 2011;128:996–1005.
48. Nilsson D, Henmyr V, Hallden C, et al. Replication of genomewide associations with allergic sensitization and allergic rhinitis. *Allergy*. 2014;69:1506–1514.
49. Jacobsen FA, Scherer AN, Mouritsen J, et al. A role for the non-receptor tyrosine kinase Abl2/Arg in experimental neuro-inflammation. *J Neuroimmune Pharmacol*. 2018;13:265–276.
50. Morita S, Villalta SA, Feldman HC, et al. Targeting ABL-IRE1alpha signaling spares ER-stressed pancreatic beta cells to reverse autoimmune diabetes. *Cell Metab*. 2017;25:883–897.
51. Zipfel PA, Zhang W, Quiroz M, Pendergast AM. Requirement for Abl kinases in T cell receptor signaling. *Curr Biol*. 2004; 14:1222–1231.
52. Weinstein JE, Pepple KL. Cytokines in uveitis. *Curr Opin Ophthalmol*. 2018;29:267–274.
53. Kazi JU, Rupar K, Marhall A, et al. ABL2 suppresses FLT3-ITD-induced cell proliferation through negative regulation of AKT signaling. *Oncotarget*. 2017;8:12194–12202.
54. Martin M, Schifferle RE, Cuesta N, Vogel SN, Katz J, Michalek SM. Role of the phosphatidylinositol 3 kinase-Akt pathway in the regulation of IL-10 and IL-12 by Porphyromonas gingivalis lipopolysaccharide. *J Immunol*. 2003;171:717–725.
55. Astier AL, Beriou G, Eisenhaure TM, Anderton SM, Hafler DA, Hacohen N. RNA interference screen in primary human T cells reveals FLT3 as a modulator of IL-10 levels. *J Immunol*. 2010;184:685–693.
56. Gu JJ, Ryu JR, Pendergast AM. Abl tyrosine kinases in T-cell signaling. *Immunol Rev*. 2009;228:170–183.

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