American Academy of Ophthalmology
The Eye M.D. Association

Genetic Variations of *IL17F* and *IL23A* Show Associations with Behçet's Disease and Vogt-Koyanagi-Harada Syndrome

Shengping Hou, PhD,[1,2] Dan Liao, MD,[1,2] Jun Zhang, MD,[1,2] Jing Fang, PhD,[1,2] Lu Chen, MD,[1,2] Jian Qi, PhD,[1,2] Qi Zhang, PhD,[1,2] Yunjia Liu, MD,[1,2] Lin Bai, MD,[1,2] Yan Zhou, MD,[1,2] Aize Kijlstra, PhD,[3] Peizeng Yang, PhD[1,2]

Purpose: To investigate the associations of *IL17A*, *IL17F*, *IL23A*, and *IL23R* copy number variants (CNVs) with Vogt-Koyanagi-Harada (VKH) syndrome and Behçet's disease (BD) and the possible mechanisms involved.

Design: Two-stage case-control and functional studies.

Participants: A total of 1159 VKH patients, 1036 BD patients, and 2050 controls were enrolled.

Methods: TaqMan real-time polymerase chain reaction assay was used for genotyping of copy number variant. Cell proliferation was measured by colorimetric assay.

Main Outcome Measures: Association of CNVs in *IL17A*, *IL17F*, *IL23A*, and *IL23R* with BD and VKH syndrome and the functional roles of *IL17F* CNVs.

Results: Increased frequencies of more than 2 copies of *IL17F* and *IL23A* were found in BD patients as compared with controls (*IL17F*: $P = 4.17 \times 10^{-8}$; odds ratio [OR], 2.2; *IL23A*: $P = 2.86 \times 10^{-11}$; OR, 2.8, respectively). A similar result was found for VKH syndrome (*IL17F*: $P = 2.84 \times 10^{-13}$; OR, 2.7; *IL23A*: $P = 4.46 \times 10^{-17}$; OR, 3.4, respectively). Interestingly, the association of *IL17F* and *IL23A* with BD was found only in male patients (*IL17F*: $P = 1.06 \times 10^{-6}$; OR, 2.3; IL23A, $P = 3.81 \times 10^{-8}$; OR, 2.8, respectively), but not in female patients. No association of CNVs in *IL17A* and *IL23R* was found for BD and VKH syndrome. IL17F protein levels were correlated positively with gene copy numbers ($P = 3.43 \times 10^{-7}$). Individuals with high *IL17F* copies showed enhanced peripheral blood mononuclear cells (PBMC) proliferation ($P = 5.67 \times 10^{-3}$).

Conclusions: High gene copy numbers of *IL17F* and *IL23A* were associated with BD and VKH syndrome. Enhanced IL17F protein production and PBMC proliferation were associated with high *IL17F* copy numbers. *Ophthalmology 2015;122:518-523 © 2015 by the American Academy of Ophthalmology.*

Uveitis is one of the leading causes of blindness for middle-aged people in the world.[1] It is estimated to cause 10% to 15% of all cases of blindness in the United States and 3% to 7% in Europe.[1] Behçet's disease (BD) and Vogt-Koyanagi-Harada (VKH) syndrome are 2 commonly seen uveitis entities in Asia, especially in China.[2–4] Behçet's disease is a chronic multisystem immune-mediated disorder characterized by nongranulomatous uveitis, genital ulcers, recurrent oral ulcers, and skin lesions.[5] Vogt-Koyanagi-Harada syndrome is a multisystemic autoimmune disorder characterized by bilateral granulomatous panuveitis frequently associated with systemic involvement including vitiligo, poliosis, alopecia, auditory, and central nervous system signs.[6] The different clinical symptoms suggest that uveitis in which symptoms are confined to the eye and systemic uveitis may be caused by different pathogenetic pathways.

Usually, autoreactive effector CD4+ T cells have been implicated as the mediators in the pathogenesis of immune diseases. Until recently, a new effector T-cell subset, the IL17-producing Th17 cell, has emerged as a major player in autoimmune or autoinflammatory diseases. Accumulating evidences showed that Th17 cells are associated with VKH syndrome[7,8] and BD.[9,10] Recently, studies indicated that the numbers of IL17-producing T cells are elevated in VKH patients and BD patients.[7,11] These studies suggest that the Th17 cell or Th17 cell–related genes may be responsible for the pathogenesis of uveitis.

Copy number variation (CNV) recently was identified as a major cause of structural variation in the genome, involving both duplications and deletions of sequences. Evidence is accumulating that CNVs have phenotypic consequences and play important roles in human diseases, including BD,[12,13] systemic lupus erythematosus (SLE),[14] and leprosy.[15] The association of CNV with Th17 cell-related genes recently was addressed in patients with SLE.[14] As yet, no studies have been reported concerning the role of Th17-related gene copy numbers in uveitis, and therefore this was the subject of the study presented herein.

This study aimed to investigate the association between the copy number variants of *IL17A*, *IL17F*, *IL23A*, and *IL23R* and various uveitis entities including BD and VKH syndrome. Our findings provide further support for important roles of Th17 cell–related genes in the pathogenesis of intraocular inflammation.

http://dx.doi.org/10.1016/j.ophtha.2014.09.025
ISSN 0161-6420/14

Table 1. Clinical Features of Behçet's Disease Patients Enrolled in the Present Study

Clinical Features	Behçet's Disease Patients	
	No. (n = 1036)	%
Age at onset (yrs), mean ± standard deviation	33.4±8.7	
Male	876	84.6
Female	160	15.4
Uveitis	1036	100
Oral ulcer	1036	100
Skin lesions	752	72.6
Genital ulcer	562	54.2
Arthritis	158	15.3
Positive pathergy test results	207	20.0
Hypopyon	257	24.8

Methods

Uveitis Patient and Normal Control Recruitment

A total of 1036 BD patients, 1159 VKH patients, and 2050 healthy individuals were recruited from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) or the Zhongshan Ophthalmic Center, Sun Yat-sen University (Guangzhou, China; Tables 1 and 2). The diagnoses of VKH syndrome and BD were based strictly on the revised diagnostic criteria 2001 for VKH syndrome[16] and the International Study Group for BD,[17] respectively. If there was any doubt about the diagnosis, the patients were excluded from the study. The controls used in this study were unrelated healthy individuals without any intraocular inflammation. They were age- and ethnicity-matched with the patients. All participants were Chinese Han and gave written informed consent. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (permit no., 20092-01008) and adhered to the tenets of the Declaration of Helsinki. This project was registered in the Chinese Clinical Trial Registry (registration no., ChiCTR-CCC-12002184).

Table 2. Clinical Features of the Vogt-Koyanagi-Harada Syndrome Patients in the Study

Clinical Features	Patients with Vogt-Koyanagi-Harada Syndrome	
	No. (n = 1159)	%
Age at onset of disease (yrs), mean ± standard deviation	39.0±13.8	
Male	635	54.8
Female	524	45.2
Uveitis	1159	100
Nuchal rigidity	129	8.8
Headache	497	42.9
Scalp allergy	176	15.2
Tinnitus	525	45.3
Dysacusia	385	33.2
Alopecia	449	38.7
Poliosis	427	36.8
Vitiligo	206	17.8

Genomic DNA Extraction and Analysis of *IL17A*, *IL17F*, *IL23A*, and *IL23R* Gene Copy Number Variation

Genomic DNA was extracted from peripheral blood using the QIAGEN QIAamp DNA Mini Blood Kit (Hilden, Germany) according to the manufacturers' recommendations. Gene CNVs of *IL17A*, *IL17F*, *IL23A*, and *IL23R* were determined by TaqMan real-time polymerase chain reaction and were performed in 96-well optical plates on a 7500 real-time polymerase chain reaction system following the manufacturer's protocols (Applied Biosystems, Foster City, CA). TaqMan assays labeled with FAM (6-carboxy-fluorescein) were used to detect *IL17A* (Hs00859511_cn), *IL17F* (Hs00345894_cn), *IL23A* (Hs00323081_cn), and *IL23R* (Hs02945077_cn; all Applied Biosystems), respectively. TaqMan RNaseP assay labeled with VIC (Applied Biosystems green fluorescent dye) was used as an internal copy number reference (Applied Biosystems). Copy number was calculated according to the manufacturer's instructions. We also evaluated the accuracy of the 4 CNVs using 60 randomly selected samples with the AccuCopy technique (Shanghai Geneskies Biotech, Shanghai, China).[18]

Cell Proliferation Assay

Peripheral blood mononuclear cells (PBMCs) were prepared from venous blood by Ficoll-Hypaque density-gradient centrifugation. The PBMCs were treated with anti-CD3 (OKT3, 0.5 μg/ml)/anti-CD28 antibodies (15E8, 0.1 μg/ml; Miltenyi Biotec, Palo Alto, CA) for 72 hours. Cell proliferation was measured using the Cell Counting kit-8 (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. The absorbance was determined at 450 nm using an ELISA reader (SpectraMax M2^e; Molecular Devices, Beijing, China).

Statistical Analysis

The real-time polymerase chain reaction data were analyzed by 7500 software version 2.0.6 (Applied Biosystems). Relative gene copy numbers were examined by the comparative C_T method using CopyCaller version 2.0 (Applied Biosystems). The differences in total *IL17A*, *IL17F*, *IL23A*, and *IL23R* were compared between patients and controls by the chi-square test using SPSS software version 17.0 (SPSS, Inc, Chicago, IL). The expression of IL17F was analyzed by 1-way ANOVA analysis using SPSS version 17.0. To account for multiple testing, the Bonferroni correction was applied.

Results

Clinical Findings of Uveitis Patients and Controls

The clinical characteristics of the uveitis patients were assessed at the time of diagnosis and are summarized in Tables 1 and 2. The average age ± standard deviation of normal controls was 39.5 ± 11.0 years.

High Copy Number of *IL17F* and *IL23A* Confer Susceptibility to Behçet's Disease and Vogt-Koyanagi-Harada Syndrome in Chinese Han Individuals

The association of CNVs in *IL17A*, *IL17F*, and *IL23A* and its receptor, including *IL23R*, in patients with BD and VKH syndrome was examined. The results showed that increased

Table 3. Summary of the Association of *IL17A*, *IL17F*, *IL23A*, and *IL23R* Copy Number Variants with Behçet's Disease in the Chinese Han Population

		Men				Women				Total			
Gene	Gene Copy No.	Behçet's Disease	NC	P Value	Odds Ratio (95% Confidence Interval)	Behçet's Disease	NC	P Value	Odds Ratio (95% Confidence Interval)	Behçet's Disease	NC	P Value	Odds Ratio (95% Confidence Interval)
IL17A	<2	6	4	0.28	2.0 (0.6–7.0)	0	3	—		6	7	0.34	1.7 (0.6–5.0)
	2	778	1057	0.03	0.7 (0.5–1.0)	141	811	0.17	0.7 (0.4–1.2)	919	1868	7.84×10^{-3}	0.7 (0.6–0.9)
	>2	92	91	0.04	1.4 (1.0–1.9)	19	72	0.12	1.5 (0.9–2.6)	111	163	0.012	1.4 (1.1–1.8)
IL17F	<2	15	17	0.66	1.2 (0.6–2.4)	2	9	0.79	1.2 (0.3–5.8)	17	26	0.48	1.3 (0.7–2.3)
	2	771	1086	3.41×10^{-6}	0.5 (0.4–0.7)	147	838	0.22	0.7 (0.4–1.3)	918	1924	8.08×10^{-8}	0.5 (0.4–0.6)
	>2	90	54	1.06×10^{-6}	2.3 (1.6–3.3)	11	41	0.23	1.5 (0.8–3.0)	101	95	4.17×10^{-8}	2.2 (1.7–2.9)
IL23A	<2	34	35	0.29	1.3 (0.8–2.1)	6	33	0.98	1.0 (0.4–2.5)	40	68	0.44	1.2 (0.8–1.7)
	2	756	1080	2.27×10^{-7}	0.5 (0.3–0.6)	144	830	0.16	0.7 (0.4–1.2)	900	1910	7.05×10^{-9}	0.5 (0.4–0.6)
	>2	86	44	3.81×10^{-8}	2.8 (1.9–4.0)	10	28	0.05	2.1 (1.0–4.3)	96	72	2.86×10^{-11}	2.8 (2.0–3.8)
IL23R	<2	1	1	0.843	1.3 (0.1–21.2)	0	1	—		1	2	1.00	1.0 (0.09–10.9)
	2	839	1115	0.558	0.9 (0.6–1.4)	156	867	0.95	1.0 (0.4–3.0)	995	1982	0.29	0.8 (0.5–1.2)
	>2	36	42	0.575	1.1 (0.7–1.8)	4	22	0.98	1.0 (0.3–3.0)	40	64	0.29	1.3 (0.8–1.9)

$P<0.05/12 = 4.2 \times 10^{-3}$ was considered significant.

frequencies of more than 2 copies of *IL17F* and *IL23A* were found in BD patients as compared with controls (*IL17F*: $P = 4.17 \times 10^{-8}$; odds ratio [OR], 2.2; *IL23A*: $P = 2.86 \times 10^{-11}$; OR, 2.8, respectively; Table 3). More than 2 copies of *IL17F* and *IL23A* also showed a similar association with VKH syndrome (*IL17F*: $P = 2.84 \times 10^{-13}$; OR, 2.7; *IL23A*: $P = 4.46 \times 10^{-17}$; OR, 3.4, respectively; Table 4). Because there is quite a marked disparity between the male-to-female ratio (876 men vs. 160 women) in the BD patients and in view of the worse ocular disease in male patients, a separate comparison was performed according to gender. The results showed that increased frequencies of high copies of *IL17F* and *IL23A* were found only in male BD patients (*IL17F*: $P = 1.06 \times 10^{-6}$; *IL23A*: $P = 3.81 \times 10^{-8}$), but not in female BD patients (Table 3). No association of CNVs in *IL17A* and *IL23R* was found for BD and VKH syndrome.

The genotyping accuracy of the 4 CNVS also was assessed with the AccuCopy technique.[18] The results showed that the concordant call (percent) of the genotyping between the TaqMan and AccuCopy techniques was more than 96.7%.

Correlation between Protein Levels of IL17F and Their Copy Numbers

We subsequently investigated whether the protein levels of IL17F positively were correlated with its copy numbers. The results showed that the expression of IL17F was increased in individuals with more than 2 copies of *IL17F* as compared with those with 2 copies of *IL17F* ($P = 3.43 \times 10^{-7}$; Fig 1). As with the low frequency of high copies of *IL23A* (CNV > 2: less than 3.6% in controls), fewer than 3 samples with high copies of *IL23A* were found in 82 controls. We therefore did not test the association between the expression of *IL23A* and its copy numbers.

Peripheral Blood Mononuclear Cell Proliferation in Individuals with Different Copy Numbers of *IL17F*

Because the expression of *IL17F* showed an association with its CNVs, we therefore focused on its function. *IL17* can stimulate the proliferation of human mesenchymal stem cells,[19] and in view of a lack of evidence regarding whether this is also true for PBMCs, we

Table 4. Summary of the Association of *IL17A*, *IL17F*, *IL23A*, and *IL23R* Copy Number Variants with Vogt-Koyanagi-Harada Syndrome in the Chinese Han Population

Gene	Gene Copy No.	Vogt-Koyanagi-Harada Syndrome	NC	P Value	Odds Ratio (95% Confidence Interval)
IL17A	<2	7	7	0.28	1.8 (0.6–5.0)
	2	1027	1868	4.62×10^{-3}	0.7 (0.6–0.9)
	>2	125	163	8.14×10^{-3}	1.4 (1.1–1.8)
IL17F	<2	14	26	0.79	0.9 (0.5–1.8)
	2	1011	1924	1.81×10^{-11}	0.4 (0.3–0.6)
	>2	134	95	2.84×10^{-13}	2.7 (2.0–3.5)
IL23A	<2	35	68	0.65	0.9 (0.6–1.4)
	2	997	1910	2.71×10^{-11}	0.5 (0.4–0.6)
	>2	127	72	4.46×10^{-17}	3.4 (2.5–4.6)
IL23R	<2	5	2	0.11	4.5 (0.9–22.9)
	2	1091	1982	2.00×10^{-3}	0.6 (0.4–0.8)
	>2	58	64	6.98×10^{-3}	1.6 (1.1–2.4)

$P<0.05/12 = 4.2 \times 10^{-3}$ was considered significant.

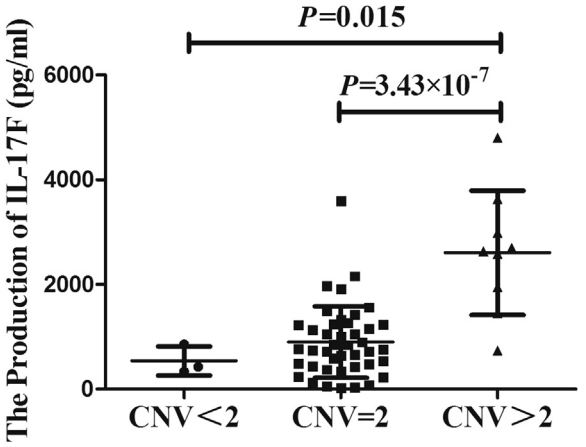

Figure 1. Graph showing the relationship between the expression of IL17F and *IL17F* copy number variant (CNV). Supernatants of stimulated peripheral blood mononuclear cell samples from 3 normal controls with fewer than 2 copies of *IL17F*, 43 normal controls with 2 copies of *IL17F*, and 9 normal controls with more than 2 copies of *IL17F* were collected to detect the production of IL17F using an enzyme-linked immunosorbent assay. Data are expressed as mean ± standard deviation. Statistical analysis was performed using the 1-way analysis of variance.

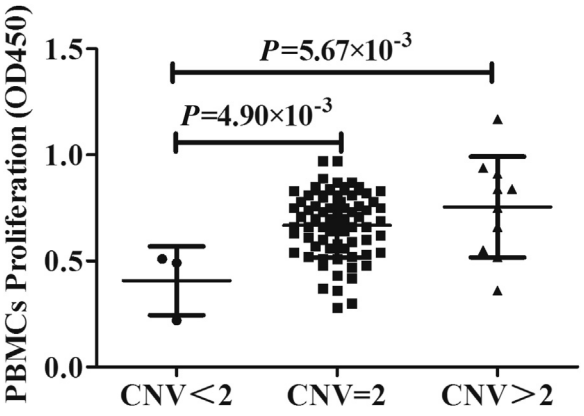

Figure 2. Graph showing the influence of *IL17F* copy number variants (CNVs) on the proliferation of peripheral blood mononuclear cells (PBMCs). Proliferation of PBMCs stimulated by anti-CD3/CD28 antibodies was tested in normal controls carrying different copy numbers of *IL17F* (CNV < 2, n = 3; CNV = 2, n = 70; CNV > 2, n = 10). Data are expressed as mean ± standard deviation.

examined whether the different CNVs of *IL17F* affected the proliferation of PBMCs. The results revealed that an increased tendency of PBMC proliferation was found in individuals with high copies of *IL17F*. Having high copies of *IL17F* (CNV > 2) resulted in an increased PBMC proliferation after stimulation with anti-CD3/CD28 antibodies as compared with individuals having a low copy number of *IL17F* (CNV < 2; $P = 5.67 \times 10^{-3}$; Fig 2).

Discussion

Previous studies have shown that Th17 cells and Th17 cell-related genes were implicated as the critical agents in the pathogenesis of uveitis in such settings as VKH syndrome and BD.[7,20,21] To our knowledge, this is the first study addressing copy number variants of Th17 cell-related genes with ocular disease. We identified 2 common risk CNVs of *IL17F* and *IL23A* for VKH syndrome and BD. More importantly, our study showed that high copies of *IL17F* were related positively with the expression of *IL17F* and may enhance PBMC proliferation.

Accumulating evidence suggested that a variety of human diseases were caused by alterations in DNA sequence or dosage of gene expression. Th17 cell-related genes show an upregulated expression in uveitis patients and play an important role in the pathogenesis of uveitis.[7,20] DNA sequence variants such as CNVs clearly have been shown to have a potential influence on the dosage of disease-related genes.[22] Our findings were consistent with an increased risk of high *IL17F* CNVs reported recently in a systemic autoimmune disease such as SLE.[14] More generally, our results, together with the report on SLE,[14] illustrated that *IL17F* CNVs may be a shared risk for systemic disorders including SLE, VKH syndrome, and BD.

Further functional studies were designed to investigate the relationship between *IL17F* CNV and its expression. The present study showed that an increased copy number of *IL17F* was correlated with elevated levels of IL17F. This finding was consistent with data shown earlier in SLE patients.[14] Previous studies showed that *IL17* was elevated in uveitis patients as in BD and VKH syndrome,[7,11] but also in animals with experimental autoimmune uveoretinitis,[21] supporting the important role of Th17 cells in the pathogenesis of intraocular inflammation. Combining the association of *IL17F* CNVs with systemic uveitis, the results suggested that *IL17F* CNV may be involved in uveitis via the upregulated expression of *IL17F*.

Further functional studies were performed to assess whether *IL17F* CNVs had an effect on proliferation of PBMCs. In view of the marked clinical heterogeneity of uveitis and the fact that these patients often were receiving a treatment regimen that included immunosuppressive drugs, we examined these issues in healthy controls. Our results showed that individuals with a high copy number of *IL17F* showed enhanced PBMC proliferation when these cells were stimulated by anti-CD3/CD28 antibodies. To our knowledge, the role of *IL17F* on PBMC proliferation has not yet been shown. Earlier reports showed that *IL17* induced proliferation of human bone marrow–derived mesenchymal stem cells.[19] This study did not show an association of *IL17A* and *IL23R* CNVs with BD and VKH syndrome; however, our previous study showed the association of *IL23R* polymorphisms, other common genetic variants, with these 2 types of uveitis in Chinese Han persons.[23,24] These results suggest that specific genetic variants of *IL23R*, gene polymorphisms but not CNVs, are involved in the development of VKH syndrome and BD.

It is worthwhile to point out that a large number of controls also have more than 2 copies of *IL17F* or *IL23A*, but do not have immune-mediated disease such as uveitis. Moreover, we observed a relatively low OR value for *IL17F* and *IL23A* CNVs. This evidence suggests that *IL17F* and *IL23A*, although important, are not sufficient to explain their genetic role in uveitis and indicates indirectly the existence

of other risk factors. In addition to the association of *IL17F* and *IL23A* with uveitis, many other genes are shown to be associated with uveitis, such as CNVs in complement component 4[13,25] and polymorphisms in *HLA*,[23,26,27] *IL23R*,[23] *STAT4*,[27,28] *CCR1-CCR3*,[29] *UBAC2*,[30] *SUMO4*,[31,32] and *PDGFRL*.[33] Additionally, although the actual number of BD patients in our study is barely higher than the number of controls in the more than 2 copies group of *IL17F*, the significant difference of the frequency of having more than 2 copies for *IL17F* and the relatively low frequency of *IL17F* cases with more than 2 copies between BD patients and controls (9.75% vs. 4.65%) led to the high *P* value. Further studies are needed to allow an extrapolation of our findings to larger samples and other ethnic populations.

In conclusion, our results identified 2 common risk CNVs of *IL17F* and *IL23A* for BD and VKH syndrome. We also found a positive association of *IL17F* copy number with the expression of *IL17F* and observed an enhanced PBMC proliferation in individuals carrying a high copy number of *IL17F*.

Acknowledgments. The authors thank Meifen Zhang, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing, China; Xiuyun Zheng, Jinan Mingshui Eye Hospital, Jinan, China; and Minglian Zhang, Xingtai Eye Hospital, Xingtai, China, for their assistance in sample collection.

References

1. Suttorp-Schulten MS, Rothova A. The possible impact of uveitis in blindness: a literature survey. Br J Ophthalmol 1996;80:844–8.
2. Chang JH, Wakefield D. Uveitis: a global perspective. Ocul Immunol Inflamm 2002;10:263–79.
3. Goto H, Mochizuki M, Yamaki K, et al. Epidemiological survey of intraocular inflammation in Japan. Jpn J Ophthalmol 2007;51:41–4.
4. 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;30:943–8.
5. Yang P, Fang W, Meng Q, et al. Clinical features of chinese patients with Behçet's disease. Ophthalmology 2008;115: 312–318.e4.
6. Yang P, Ren Y, Li B, et al. Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. Ophthalmology 2007;114:606–14.
7. Chi W, Yang P, Li B, et al. IL-23 promotes CD4+ T cells to produce IL-17 in Vogt-Koyanagi-Harada disease. J Allergy Clin Immunol 2007;119:1218–24.
8. Commodaro AG, Bombardieri CR, Peron JP, et al. p38{alpha} MAP kinase controls IL-17 synthesis in Vogt-Koyanagi-Harada syndrome and experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 2010;51:3567–74.
9. Kim J, Park JA, Lee EY, et al. Imbalance of Th17 to Th1 cells in Behçet's disease. Clin Exp Rheumatol 2010;28(suppl):S16–9.
10. Hamzaoui K. Th17 cells in Behçet's disease: a new immunoregulatory axis. Clin Exp Rheumatol 2011;29(suppl):S71–6.
11. Chi W, Zhu X, Yang P, et al. Upregulated IL-23 and IL-17 in Behçet patients with active uveitis. Invest Ophthalmol Vis Sci 2008;49:3058–64.
12. Park JJ, Oh BR, Kim JY, et al. Copy number variation of beta-defensin genes in Behçet's disease. Clin Exp Rheumatol 2011;29:S20–3.
13. Hou S, Qi J, Liao D, et al. Copy number variations of complement component C4 are associated with Behçet's disease but not with ankylosing spondylitis associated with acute anterior uveitis. Arthritis Rheum 2013;65: 2963–70.
14. Yu B, Guan M, Peng Y, et al. Copy number variations of interleukin-17F, interleukin-21, and interleukin-22 are associated with systemic lupus erythematosus. Arthritis Rheum 2011;63:3487–92.
15. Ali S, Srivastava AK, Chopra R, et al. IL12B SNPs and copy number variation in IL23R gene associated with susceptibility to leprosy. J Med Genet 2013;50:34–42.
16. 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;131:647–52.
17. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990;335: 1078–80.
18. Du R, Lu C, Jiang Z, et al. Efficient typing of copy number variations in a segmental duplication-mediated rearrangement hotspot using multiplex competitive amplification. J Hum Genet 2012;57:545–51.
19. Huang H, Kim HJ, Chang EJ, et al. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death Differ 2009;16:1332–43.
20. Zhou ZY, Chen SL, Shen N, Lu Y. Cytokines and Behçet's disease. Autoimmun Rev 2012;11:699–704.
21. Amadi-Obi A, Yu CR, Liu X, et al. TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. Nat Med 2007;13:711–8.
22. McKinney C, Merriman ME, Chapman PT, et al. Evidence for an influence of chemokine ligand 3-like 1 (CCL3L1) gene copy number on susceptibility to rheumatoid arthritis. Ann Rheum Dis 2008;67:409–13.
23. 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: 1007–11.
24. Jiang Z, Yang P, Hou S, et al. IL-23R gene confers susceptibility to Behçet's disease in a Chinese Han population. Ann Rheum Dis 2010;69:1325–8.
25. Hou S, Qi J, Liao D, et al. High C4 gene copy numbers protects against Vogt-Koyanagi-Harada syndrome in Chinese Han. Br J Ophthalmol 2014;98:1733–7.
26. Hughes T, Coit P, Adler A, et al. Identification of multiple independent susceptibility loci in the HLA region in Behçet's disease. Nat Genet 2013;45:319–24.
27. Kirino Y, Bertsias G, Ishigatsubo Y, et al. Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B*51 and ERAP1. Nat Genet 2013;45:202–7.
28. Hou S, Yang Z, Du L, et al. Identification of a susceptibility locus in STAT4 for Behçet's disease in Han Chinese in a genome-wide association study. Arthritis Rheum 2012;64: 4104–13.
29. Hou S, Xiao X, Li F, et al. Two-stage association study in Chinese Han identifies two independent associations in CCR1/CCR3 locus as candidate for Behçet's disease susceptibility. Hum Genet 2012;131:1841–50.

30. Hou S, Shu Q, Jiang Z, et al. Replication study confirms the association between UBAC2 and Behçet's disease in two independent Chinese sets of patients and controls. Arthritis Res Ther 2012;14:R70.
31. Hou S, Yang P, Du L, et al. SUMO4 gene polymorphisms in Chinese Han patients with Behçet's disease. Clin Immunol 2008;129:170–5.
32. Hou S, Yang P, Du L, et al. Small ubiquitin-like modifier 4 (SUMO4) polymorphisms and Vogt-Koyanagi-Harada (VKH) syndrome in the Chinese Han population. Mol Vis 2008;14:2597–603.
33. Hou S, Xiao X, Zhou Y, et al. Genetic variant on PDGFRL associated with Behçet disease in Chinese Han populations. Hum Mutat 2013;34:74–8.

Footnotes and Financial Disclosures

Originally received: August 7, 2014.
Final revision: September 16, 2014.
Accepted: September 16, 2014.
Available online: November 14, 2014. Manuscript no. 2014-1266.

[1] Department of Ophthalmology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

[2] Chongqing Eye Institute and Chongqing Key Laboratory of Ophthalmology, Chongqing, China.

[3] University Eye Clinic Maastricht, Maastricht, the Netherlands.

Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Supported by the Natural Science Foundation (Major International [Regional] Joint Research Project of China (grant no.: 81320108009 [P.Y.]); National Basic Research Program of China (973 Program; grant no.: 2011CB510200 [P.Y.]); Key Project of Natural Science Foundation (grant no.: 81130019 [P.Y.]); National Natural Science Foundation Project (grant nos.: 31370893 [P.Y.] and 81270990 [S.H.]); Basic Research program of Chongqing (grant no.: cstc2013jcyjC10001 [P.Y., S.H.]); Chongqing Key Laboratory of Ophthalmology (grant no.: CSTC 2008CA5003 [P.Y.]); National Key Clinical Specialties Construction Program of China (P.Y.); Key Project of Health Bureau of Chongqing (2012-1-003) (P.Y), Fund for PAR-EU Scholars Program (P.Y.); and the Youth Talent Support Plan of Chongqing (S.H.).

Abbreviations and Acronyms:
BD = Behçet's disease; **CNV** = copy number variant; **OR** = odds ratio; **PBMC** = peripheral blood mononuclear cell; **SLE** = systemic lupus erythematosus; **VKH** = Vogt-Koyanagi-Harada.

Correspondence:
Peizeng Yang, PhD, or Shengping Hou, PhD, The First Affiliated Hospital of Chongqing Medical University, Youyi Road 1, Chongqing, 400016, China. E-mail: peizengycmu@126.com or sphou828@163.com.