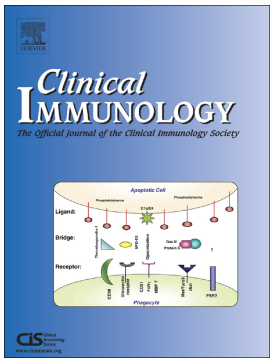

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Clinical trait-specific genetic analysis in Behçet's disease identifies novel loci associated with ocular and neurological involvement

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Running title: Genetics of clinical manifestations in Behçet's disease

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Abbreviations: GRS, genetic risk score; GWAS, genome-wide association study; HWE, Hardy-Weinberg equilibrium; IBD, inflammatory bowel disease; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

ABSTRACT

Behçet's disease is a complex inflammatory vasculitis with a broad spectrum of clinical manifestations. The purpose of this study was to investigate the genetics underlying specific clinical features of Behçet's disease in a group of patients with > 20 years of follow up. A total of 436 patients with Behçet's disease from Turkey were studied. Genotyping was performed using the Infinium ImmunoArray-24 BeadChip. After imputation and quality control measures, logistic regressions adjusting for sex and the first five principal components were performed for each clinical trait using a case-case genetic analysis approach. A weighted genetic risk score was calculated for each clinical feature. Genetic association analyses of previously identified susceptibility loci in Behçet's disease revealed a genetic association between ocular lesions and *HLA-B*/*MICA* (rs116799036: OR=1.85, 95% CI=1.35-2.52, p-value=$1.1x10^{-4}$). The genetic risk score was significantly higher in Behçet's disease patients with ocular lesions compared with those without ocular involvement, and is explained by the genetic variation in the HLA region. New genetic loci predisposing to specific clinical features in Behçet's disease were suggested when genome-wide variants were evaluated. The most significant associations were observed in ocular involvement with *SLCO4A* (rs6062789: OR=0.41 (95% CI=0.30-0.58), p-value=$1.92x10^{-7}$), and neurological involvement with *DDX60L* (rs62334264: OR= 4.12 (95% CI 2.34 to 7.24), p-value = $8.85x10^{-7}$). Our results emphasize the role of genetic factors in predisposing to specific clinical manifestations in Behçet's disease, and might shed additional light into disease heterogeneity, pathogenesis, and variability of Behçet's disease presentation across populations.

1. INTRODUCTION

Behçet's disease is a chronic inflammatory illness with multiorgan involvement, and is characterized by recurrent oral and genital ulcers, among other clinical symptoms. This disease has a worldwide distribution with the highest prevalence described in East Asia and the Mediterranean region [1]. Behçet's disease affects both genders and it is typically diagnosed in the third and fourth decade of life [1]. Despite this, it has been described that the disease shows higher prevalence of severe manifestations in men, including ocular, vascular, and neurological involvement, possibly related to a higher genetic risk [2].

The prevalence of clinical features in Behçet's disease can vary among different geographical locations, and patients can show heterogeneity in their manifestations [1]. This, together with the absence of specific diagnostic tests, makes the identification and collection of a broad spectrum of clinical features necessary in the diagnosis process [3].

Although the pathogenesis of Behçet's disease remains unclear, environmental factors and genetic predisposition have been hypothesized to contribute to the disease evolution [4]. Regarding the genetic component of Behçet's disease, the development of new genotyping techniques has led to more powerful genetic studies [5], allowing the validation of genetic associations and the identification of new ones. This strongly supports a role for a genetic component in the disease etiology [6, 7]. In this sense, the most robust genetic association described in Behçet's disease resides in the HLA class I region, consistent with the inflammatory nature of the disease [1, 7]. Moreover, the development of genome-wide association studies (GWAS) has contributed to the identification of disease-associated genetic variation outside the HLA region, such as the *IFNGR1* and *LNCAROD/DKK1* genes, among many others [1, 8].

Considering the clinical heterogeneity and the strong genetic background of Behçet's disease, we performed case-case genetic association analyses to determine the role of genetics in the clinical heterogeneity of this disease.

2. METHODS

2.1. Study population

Samples from 436 Behçet's disease patients from Turkey were included in this study. These included 270 men and 166 women. The age at disease diagnosis was 30.32±9.34 (yrs, mean±SD), and the patients had a disease duration of 20.05±7.43 (yrs, mean±SD). All patients fulfilled the 1990 International Study Group classification criteria for Behçet's disease [9]. For each patient, ten clinical features were evaluated, comprising genital aphthosis, pseudo-folliculitis, erythema nodosum, ocular lesions, gastrointestinal involvement, neurological involvement, arthritis, and vascular involvement, with the last divided into arterial and venous disease (**Table 1**). This study was approved by the institutional review boards and the ethics committees at the participating institutions, and all study participants signed a written informed consent.

Table 1. Demographic and clinical characteristics of patients included in this study.

Clinical feature	Number of patients with/without the clinical feature	Sex (% male patients with the clinical feature)
Genital aphthosis	367/68	231 (63%)
Pseudo-folliculitis	318/113	203 (64%)
Erythema nodosum	233/203	136 (58%)
Ocular lesions	191/245	122 (61%)
Gastrointestinal involvement	11/425	8 (73%)
Arthritis	180/255	106 (59%)
Neurological involvement	52/383	32 (62%)
Vascular involvement	124/311	95 (77%)
Arterial involvement	21/409	16 (76%)
Venous involvement	107/322	85 (79%)

2.2. Genotyping, quality control, and imputation

Genotyping was performed using the Illumina Infinium ImmunoArray-24 V.1.0 and V.2.0 BeadChip arrays. Quality control assessment for individual and genotype data were described

elsewhere [8]. Briefly, single nucleotide polymorphisms (SNPs) with genotyping call rate < 98%, minor allele frequency (MAF) < 1%, and Hardy-Weinberg equilibrium (HWE) P < 0.001 were removed. Individual samples with genotyping call rate < 95% were removed. When samples had a pairwise identity by descent proportion (Pi-HAT) of > 0.4 only one randomly selected sample was retained in the analysis. As previously described [8], principal component analysis was performed using the Eigensoft 6.1.4 software [10] and those samples that were more than 6 standard deviations from the center of the cluster were filtered out. Genotype data were imputed with the Michigan Imputation Server using Minimac2 and the TOPMed Imputation Reference panel [11-13]. Assembly GRCh38 hg38 was used for SNP annotation. Imputed variants with an imputation quality metric rsq > 0.3, MAF > 1% and HWE P > 0.001 were included in further analyses [8, 14]. A total of 1,416,102 SNPs were included in the analyses.

In order to assess the association of previously reported Behçet's disease susceptibility loci with the different clinical manifestations, twenty susceptibility SNPs were considered for this approach [7]. These SNPs tag nineteen loci, with each locus being represented by one SNP except for *IRF8*, where two independent tag SNPs were included (**Supplemental Table 1**).

2.3. Data analysis

Case-case logistic regressions adjusting for sex and the first five principal components were performed using PLINK [15] for each clinical feature. When previously reported Behçet's disease susceptibility loci were evaluated (**Supplemental Table 1**), the significance threshold was established at a p-value $<2.5x10^{-3}$ according to the Bonferroni multiple testing correction.

Regarding the GWAS case-case analysis approach, the genome-wide significance threshold was established at a p-value $<5\times10^{-8}$, while those association with a p-value $<1\times10^{-5}$

were considered as suggestive. For each region that showed significant or suggestive association in the GWAS, the SNP with the lowest p-value was reported as the lead SNP. Genomic inflation factors (λ) were estimated for each trait considering around 3,000 SNPs that have not been previously associated with immune-mediated diseases. For these, quantile-quantile plots (QQ-plots) were estimated for the clinical-specific p-values (**Supplemental Figure 1**). The genomic inflation factors and QQ-plots were generated with the package "gap", R version 4.2.0.

To evaluate the overall genetic risk contribution of previously reported susceptibility loci on each clinical trait in Behçet's disease, a cumulative genetic risk score (GRS) was calculated. For this, all individuals included in the analysis were expected to have a 100% genotyping rate for the selected SNPs, and if a particular marker was not genotyped, imputed genotype data were used for the calculation. Odds ratios (OR) used in GRS estimation at each selected locus corresponded to those reported in previous case-control association analyses in the disease [7]. Individual genotypes were coded as 0, 1, or 2 indicating the number of Behçet's disease risk alleles present for each SNP. Cumulative GRSs were calculated multiplying the natural logarithm of their corresponding OR by the number of effect alleles ($\sum_{k=1}^{i} \ln(OR_i)n_i$) [16] followed by a Welch's t-test to identify significant differences between the clinical groups.

3. RESULTS

Case-case association analyses were performed in order to test the relationship between previously reported genetic susceptibility loci in Behçet's disease and the different clinical manifestations of the disease. A significant genetic difference was detected between patients with and without ocular involvement (rs116799036: OR = 1.85 [95% CI 1.35 to 2.52], p-value =$1.1x10^{-4}$) (**Table 2**). This genetic variant is in the *HLA-B*/*MICA* region, indicating the influence of this genetic susceptibility locus on the development of eye involvement in patients with Behçet's disease. These analyses also showed nominal associations with other clinical traits in Behçet's disease such as arthritis (*ADO*, rs224127: OR = 0.67 [95% CI 0.52 to 0.88], p-value = $4.0x10^{-3}$), arterial involvement (*IL10*, rs1518111: OR = 0.29 [95% CI 0.12 to 0.68], p-value = $4.6x10^{-3}$) and erythema nodosum (*HLA-C*, rs12525170: OR = 1.52 [95% CI 1.10 to 2.12], p-value = $1.3x10^{-2}$). Interestingly, the presence of ocular lesions was also nominally associated with two other SNPs located in the *HLA-A* and *IL1A* gene regions (rs3783550: OR = 1.46 [95% CI 1.10 to 1.92], p-value = $7.9x10^{-3}$; rs114854070: OR = 1.46 [95% CI 1.08 to 1.97], p-value = $1.4x10^{-2}$). We then performed a subset analysis to examine these genetic associations in patients who only have one organ involvement compared to patients with only mucocutaneous disease. As expected, this subset analysis was underpowered. However, the association between rs116799036 in the *HLA-B*/*MICA* region with ocular involvement remained significant (OR = 2.26 [95% CI 1.23 to 4.15], p-value = $8.46x10^{-3}$).

Table 2. Nominally significant associations of previously identified genetic risk loci for Behçet's disease in patients with different clinical features. A case-case genetic association analysis in patients with compared to without individual clinical features was performed.

Clinical feature	Chr	Bp	SNP ID	Gene	Effect allele	OR	P-value
Ocular lesions	6	31381371	rs116799036	*HLA-B*/MICA	A	1.85	**1.10x10^{-4}**
	2	112775308	rs3783550	*IL1A*	T	1.46	7.91x10^{-3}
	6	29750443	rs114854070	*HLA-A*	A	1.46	1.40x10^{-2}
Arthritis	10	62701513	rs224127	*ADO*	A	0.67	4.01x10^{-3}
Arterial involvement	1	206771300	rs1518111	*IL10*	T	0.29	4.61x10^{-3}
	6	31131984	rs12525170	*HLA-C*	A	0.29	1.65x10^{-2}
Erythema nodosum	6	31131984	rs12525170	*HLA-C*	A	1.52	1.32x10^{-2}
	6	31381371	rs116799036	*HLA-B*	A	1.36	4.51x10^{-2}
Pseudo-folliculitis	10	62701513	rs224127	*ADO*	A	0.68	1.51x10^{-2}
	3	46164194	rs7616215	*CCR3*	C	0.68	3.88x10^{-2}
Neurological involvement	1	67294457	rs924080	*IL12RB2*	C	0.55	1.78x10^{-2}
	3	159929885	rs17753641	*IL12A*	G	0.21	3.41x10^{-2}
Gastrointestinal involvement	6	137193653	rs4896243	*IFNGR1*	C	2.79	3.16x10^{-2}
Vascular involvement	1	206771300	rs1518111	*IL10*	T	0.72	4.78x10^{-2}
	3	159929885	rs17753641	*IL12A*	G	0.50	4.96x10^{-2}

Abbreviations: Bp, base pair; chr chromosome; OR odds ratio.
All positions are in GRCh38 build. The analyses were performed using logistic regression adjusting for sex and the first five genetic principal components.

In order to assess the overall contribution of these susceptibility loci to the sub-phenotype genetic risk, a cumulative GRS for each clinical feature was calculated. Since sex-specific differences in genetic risk had previously been reported [17], sex was added as a covariate in the analysis. Significant differences in the risk contribution were identified when patients were classified according to the presence of ocular lesions (**Figure 1**). Expectedly, differences observed

in ocular lesions were reduced upon removal of rs114854070 or rs12525170 (*HLA-A* and *HLA-C*; P= 0.027 and 0.0095, respectively), while they disappeared completely when only rs116799036 (*HLA-B*/*MICA*) was removed (P= 0.33). Similarly, the difference in GRS between patients with compared to patients without ocular involvement disappears when all SNPs in the HLA region are removed (P= 0.41). This indicates that these results are driven by the genetic risk within the HLA region.

We have previously reported the association between male sex and rs116799036 (*HLA-B*/*MICA*) in Behçet's disease [2]. Because ocular involvement is more frequent in men compared to women with Behçet's disease, we performed a sex-stratified analysis to examine the association between rs116799036 (*HLA-B*/*MICA*) and ocular involvement in men and women separately. Our results revealed a stronger association in men (OR = 2.02 [95% CI 1.37 to 2.98], p-value=$4.30x10^{-4}$) compared to women (OR=1.67 [95% CI 0.99 to 2.84], p-value=$5.66x10^{-2}$). Our study includes significantly fewer women than men with eye involvement, which results in lower statistical power in the analysis performed in women. However, these data suggest that both male sex and ocular involvement are independently associated with rs116799036 in Behçet's disease.

To identify novel genetic variation associated with the presence of different clinical traits in Behçet's disease, a GWAS approach was applied for each clinical feature. After QC and imputation, 1,416,102 variants were assessed adjusting for sex and the first five principal components. No genomic inflation was observed in any of the logistic regressions performed (**Supplemental Figure 1**). A total of 61 suggestive signals (**Supplemental Table 2**) were identified for some of the clinical features assessed (**Figure 2**). These signals represent 13 independent loci, being the most significant variants within each locus shown in **Table 3**. Several of these associations stand out for their significance in comparison to the rest of the associations

observed, and their close location to genes of special relevance. The most significant results were observed in ocular lesions (rs6062789: OR = 0.41 (95% CI 0.30 to 0.58), p-value = $1.92x10^{-7}$) and neurological involvement (rs62334264: OR= 4.12 (95% CI 2.34 to 7.24), p-value = $8.85x10^{-7}$), located near the genes *SLCO4A1* and *DDX60L*, respectively (**Figure 2**). We also observed a suggestive association of a SNP located near the *HLA-C* gene with the presence of erythema nodosum (rs2524099: OR = 0.51 (95% CI 0.38 to 0.69), p-value = $9.69x10^{-6}$). We then performed a subset analysis to re-examine the genetic associations between rs6062789 and rs62334264 with ocular and neurological involvement, respectively. Patients with only ocular lesions and no other organ involvement, or only neurological involvement and no other organ involvement, were compared to patients with only mucocutaneous manifestations. Despite a significantly reduced statistical power in such analysis due to low sample size, we were still able to detect consistent genetic associations and with a trend for statistical significance in ocular lesions (rs6062789, OR = 0.60 [95% CI 0.38 to 0.93], p-value = 0.058) and neurological involvement (rs62334264, OR=5.11 [95% CI 1.26 to 20.82], p-value = 0.056).

Table 3. Genome-wide associations in clinical sub-phenotypes in Behçet's disease with P-value < 1×10^{-5}. Only the most significant genetic variant in each locus for each sub-phenotype is shown.

Clinical feature	Chr	BP (GRCh38)	SNP ID	Gene	Effect allele	Allele freq	OR (95% CI)	P-value
Ocular lesions	20	62675341	rs6062789	*SLCO4A1*	G	0.27	0.41 (0.30-0.58)	1.92×10^{-7}
Neurological involvement	4	168762849	rs62334264	*DDX60L*	T	0.09	4.12 (2.34-7.24)	8.85×10^{-7}
Genital aphthosis	10	93215254	rs12220128	*CYP26A1*	C	0.07	0.22 (0.12-0.34)	1.14×10^{-6}
Arterial involvement	19	29421622	rs892076	*VSTM2B*	A	0.06	13.06 (4.63-36.85)	1.20×10^{-6}
Arterial involvement	12	47920979	rs73111983	*VDR*	T	0.02	32.95 (7.86-138.10)	1.75×10^{-6}
Neurological involvement	20	19577919	rs6061007	*RIN2*	G	0.02	16.86 (5.29-53.74)	1.77×10^{-6}
Genital aphthosis	15	87564501	rs11073721	*NTRK3*	C	0.12	0.27 (0.16-0.46)	1.87×10^{-6}
Gastrointestinal involvement	5	56220145	rs75274358	*ANKRD55*	A	0.01	72.22 (12.03-433.50)	2.86×10^{-6}
Ocular lesions	3	116475009	rs592391	*SEMA6A*	G	0.31	0.49 (0.36-0.66)	4.39×10^{-6}
Arthritis	22	50679152	rs9616915	*SHANK3*	C	0.44	0.50 (0.37-0.67)	5.02×10^{-6}
Neurological involvement	6	28121455	rs960872412	*ZSCAN16*	A	0.11	3.25 (1.95-5.42)	6.18×10^{-6}
Genital aphthosis	11	3150587	rs75048411	*OSBPL5*	T	0.01	0.08 (0.03-0.23)	6.73×10^{-6}
Erythema nodosum	6	31268274	rs2524099	*HLA-C*	G	0.35	0.51 (0.38-0.69)	9.69×10^{-6}

Genetic associations with P-value < 1×10^{-5} were considered suggestive.

Abbreviations: Allele Freq, allele frequency; bp, base pair; chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

4. DISCUSSION

In this study we assessed clinical trait-specific genetic differences in patients with Behçet's disease. Our analysis highlights the genetic contribution of the *HLA-B*/*MICA* genetic region to the risk of ocular involvement in Behçet's disease. Further, using a GWAS approach, we identified possible genetic contribution for loci in *SLCO4A1* and *DDX60L* in ocular and neurological involvements in Behçet's disease, respectively. A locus near the *HLA-C* gene appears to be associated with the development of erythema nodosum in Behçet's disease.

The most robust genetic susceptibility locus previously reported in Behçet's disease is within the *HLA-B*/*MICA* region [18]. We have previously reported the association between this genetic locus (tagged by rs116799036) with male sex in patients with Behçet's disease [17]. The same genetic variant within the *HLA-B*/*MICA* region was found to be associated with ocular involvement in Behçet's disease, which is more common and more severe in male patients [19, 20]. Indeed, a previous meta-analysis suggested an increased risk for eye involvement in Behçet's disease patients with HLA-B*51 [20]. HLA-B*51 is tagged by rs116799036, and our previous work suggested that the genetic association with HLA-B*51 in Behçet's disease might be explained by rs116799036 located between *HLA-B* and *MICA* [18]. In addition, our genetic risk assessment findings highlighted the *HLA-B*/*MICA* locus as the driver of the contribution to the genetic risk observed in Behçet's disease patients with ocular lesions. These results highlight the importance of the *HLA-B*/*MICA* genetic region not only in the overall pathogenesis of Behçet's disease, but also in ocular involvement.

The GWAS approach allowed the identification of new suggestive loci associated with different clinical manifestations of Behçet's disease. The most significant association was again identified with ocular lesions close to the *SLCO4A1* gene, which encodes for a solute carrier

organic anion transporter, expressed in numerous epithelial and cancer tissues [21]. Although other members of the solute carrier transporter family have been previously related to Behçet's disease [22, 23], this is the first time that genetic variation close to *SLCO4A1* shows association with the disease. *SLCO4A1* gene has been identified as a marker of photoreceptor cell death during retinal detachment, which is an uncommon condition in patients with Behçet's disease [24, 25]. In addition, previous data reported upregulation of *SLCO4A1* expression in 3D corneal tissue in a study where drug transporter genes were evaluated, suggesting a role of this gene in ocular tissue [26].

The second most significant signal was identified close to the *DDX60L* in association with neurological involvement in Behçet's disease. *DDX60L* encodes a helicase family member that has been described as an interferon-stimulated gene due to its role during hepatitis C virus infection [27]. Interferon alpha (IFN-α) was proposed for the treatment of Behçet's disease [28], showing good efficacy in cases of Behçet's disease with neurological involvement and ocular lesions [29].

Finally, the suggestive association near the *HLA-C* with the presence of erythema nodosum is noteworthy, given the role of this gene in the immune response [30]. The relatedness of this gene with Behçet's disease has been previously reported [18]. The erythema nodosum-associated variant identified in our analysis showed association in PheWAS reference data [31, 32] with psoriasis, an immune-mediated disease that mainly affects the skin. In fact, several studies have linked different *HLA-C* alleles as well as changes in their expression patterns to the immune response in psoriasis patients [33, 34]. A recent population-based study reported an increased risk of psoriasis in patients with Behçet's disease, which was more pronounced in men [35]. A shared feature of Behçet's disease with spondyloarthropathies as MHC-1 associated disorders (MHC-I-opathies) has been suggested [36].

A limitation of our study is the relatively small sample size of the clinical sub-groups included in the analysis. In addition, effects influenced by other co-morbidities could not be assessed. However, as a major strength, our study has a long patient follow-up duration. Most manifestations of Behçet's disease are observed in the first 5 years of disease onset. Therefore, we believe we have captured most of the possible clinical manifestations in our study group for phenotype analysis.

5. CONCLUSION

Our results suggest that genetic variability plays a role in the heterogeneity and sub-phenotype penetrance in Behçet's disease. This is particularly relevant considering the variability of clinical traits among populations in this disease [37]. Our genetic data might provide new insights into the risk and pathogenesis of specific clinical manifestations of Behçet's disease, particularly ocular and neurological involvement. How these data and future extension of similar findings can be used to determine risk and personalize follow up in patients with Behçet's disease remains to be seen. With the aim of confirming the suggestive associations we identified, further genetic studies in larger cohorts of well-phenotyped patients will be necessary. In addition, it will be important to confirm our results in other populations.

DECLARATION OF COMPETING INTEREST

All authors declare that they have no conflicts of interest regarding this article.

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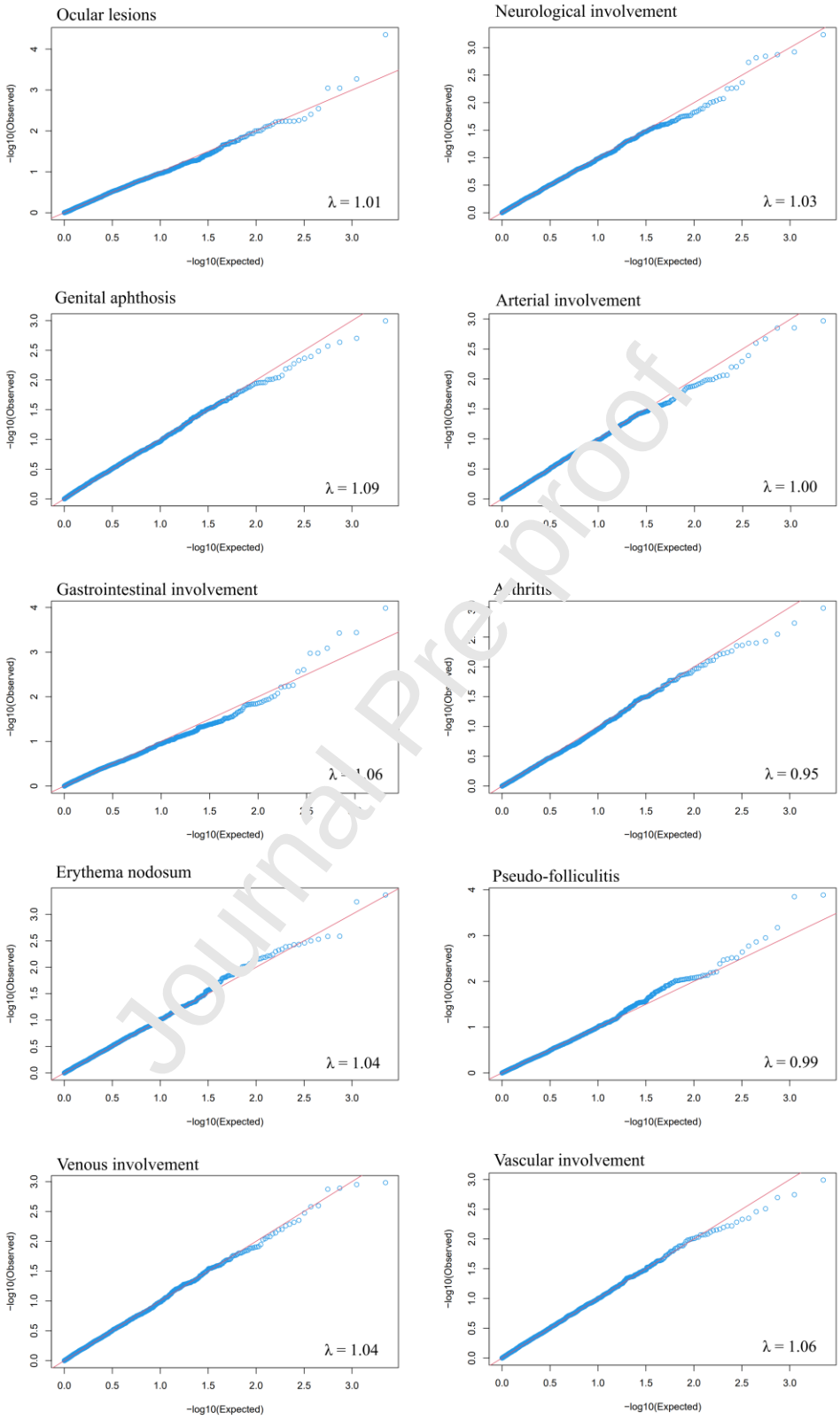
Figure Legends

Figure 1. Genetic risk score (GRS) distribution in Behçet's disease patients with (blue) and without (orange) ocular lesions. Distribution curves of GRS were calculated with Behçet's disease susceptibility alleles (A) including and (B) excluding all SNPs in the HLA region.

Figure 2. Manhattan plots depicting the genetic association results for each clinical trait in Behçet's disease. Y and X axes refer the $-\log_{10}$ P-values and chromosome positions, respectively. The red horizontal line indicates the genome-wide association threshold (P-value < 5×10^{-8}) and the blue line refers to the suggestive threshold (P-value < 1×10^{-5}).

Supplementary material

Supplemental Figure 1. Quantile–quantile plots (QQ-plots) for the p-values of each clinical trait in patients with Behçet's disease. The x-axis indicates the expected distribution of - log10 (p-values) and the y-axis indicates the observed distribution of -log10 (p-values).



Supplemental Table 1. Previously reported genetic susceptibility loci associated with Behçet's disease analyzed in this study.

Chr	Bp	SNP ID	Reported gene	Effect allele	OR	References
1	67294457	rs924080	*IL12RB2*	T	1.28	[38]
1	206771300	rs1518111	*IL10*	T	1.45	[38]
2	112775308	rs3783550	*IL1A*	G	1.33	[39]
2	191145762	rs7574070	*STAT4*	A	1.27	[40]
3	46164194	rs7616215	*CCR3*	T	0.72	[41]
3	159929885	rs17753641	*IL12A*	G	1.90	[39]
5	96783162	rs17482078	*ERAP1*	T	3.08	[41]
6	29750443	rs114854070	*HLA-A*	A	1.95	[18]
6	31131984	rs12525170	*HLA-C*	A	3.01	[18]
6	31381371	rs116799036	*HLA-B/MICA*	A	3.88	[18]
6	137514790	rs4896243	*IFNGR1*	C	1.25	[8]
10	52394860	rs1660760	*Intergenic LNCAROD/DKK1*	T	0.78	[8]
10	62701513	rs224127	*ADO*	A	1.27	[39]
11	98216871	rs2848479	*Intergenic JRKL/CNTN5*	T	1.66	[42]
12	10408358	rs2617170	*KLRC4*	C	0.78	[41]
13	43900916	rs2121033	*LACC1*	G	0.76	[39]
16	85983088	rs7203487	*IRF8*	C	1.39	[39]
16	85985910	rs11117433	*IRF8*	C	0.63	[39]
19	48703205	rs6[illegible]45	*FUT2*	T	1.30	[43]
20	50338887	rs9[illegible]8	*Intergenic CEBPB/PTPN1*	C	1.33	[39]

Abbreviations: Bp, base pair; Chr chromosome; OR odds ratio.
All positions are in GRCh38 build.

Supplemental Table 2. List of suggestive associated signals ($P < 1 X 10^{-5}$) of the individual logistic regression for each clinical feature in Behçet's disease patients.

Clinical feature	Chr	Bp (GRCh38)	SNP ID	Gene	Effect Allele	MAF	OR	95% CI		P-value
Ocular lesions	20	62675341	rs6062789	*SLCO4A1*	G	0.27	0.41	0.3	0.58	1.92 E-7
Neurological involvement	4	168768097	rs968694	*DDX60L*	T	0.1	4.15	2.37	7.26	6.57 E-7
Neurological involvement	4	168762849	rs62334264	*DDX60L*	T	0.09	4.12	2.34	7.24	8.85 E-7
Genital aphthosis	10	93215254	rs12220128	*CYP26A1*	C	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93215428	rs12217975	*CYP26A1*	T	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93217199	rs4918924	*CYP26A1*	G	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93217426	rs4918925	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93217881	rs6719212 8	*CYP26A1*	T	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93218250	rs1225283	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93219341	rs1968218 6	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93226276	rs2243218 8	*CYP26A1*	C	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93221141	rs1927470	*CYP26A1*	T	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93232273	rs4119682	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93232345	rs1927468	*CYP26A1*	C	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93232759	rs10786079	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93236569	rs1927466	*CYP26A1*	C	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93255256	rs56850410	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6
Genital aphthosis	10	93258687	rs7091054	*CYP26A1*	A	0.07	0.22	0.12	0.4	1.14 E-6

Neurological involvement	4	168763461	rs62334265	*DDX60L*	A	0.09	4.04	2.3	7.09	1.18E-6
Neurological involvement	4	168763885	rs17542654	*DDX60L*	T	0.09	4.04	2.3	7.09	1.18E-6
Neurological involvement	4	168765644	rs17614553	*DDX60L*	A	0.09	4.04	2.3	7.09	1.18E-6
Neurological involvement	4	168766405	-	*PALLD*	C	0.09	4.04	2.3	7.09	1.18E-6
Arterial involvement	19	29421622	rs892076	*VSTM2B*	A	0.06	13.06	4.63	36.9	1.20E-6
Arterial involvement	12	47920979	rs73111983	*VDR*	T	0.07	32.95	7.86	138	1.75E-6
Neurological involvement	20	19577919	rs6106107	*RIN2*	G	0.02	16.86	5.29	53.7	1.77E-6
Genital aphthosis	15	87564501	rs11073721	*NTRK3*	C	0.12	0.27	0.16	0.46	1.87E-6
Arterial involvement	19	29421589	rs892075	*VSTM2B*	C	0.07	12.52	4.43	35.4	1.91E-6
Ocular lesions	20	62673801	rs57279839	*SLCO4A1*	T	0.21	0.42	0.29	0.6	2.38E-6
Gastrointestinal involvement	5	56220143	rs75274358	*ANKRD55*	A	0.01	72.22	12.03	434	2.86E-6
Arterial involvement	19	29417604	rs1042682 0	*VSTM2B*	G	0.07	9.98	3.75	26.5	3.99E-6
Genital aphthosis	15	87557046	rs11636066	*NTRK3*	G	0.18	0.31	0.18	0.51	4.01E-6
Neurological involvement	4	168774456	rs62334291	*CBR4*	A	0.12	3.35	2	5.6	4.14E-6
Neurological involvement	4	168777657	rs72701835	*CBR4*	A	0.12	3.35	2	5.6	4.14E-6
Ocular lesions	5	116475009	rs592391	*SEMA6A*	G	0.31	0.49	0.36	0.66	4.39E-6
Ocular lesions	20	62685035	rs60294026	*NTSR1*	A	0.24	0.46	0.33	0.64	5.00E-6
Arthritis	22	50679152	rs9616915	*SHANK3*	C	0.44	0.5	0.37	0.67	5.02E-6
Arthritis	22	50676090	rs9616911	*SHANK3*	T	0.44	0.5	0.37	0.68	6.04E-6
Neurological involvement	6	28121455	rs960872412	*ZSCAN16*	A	0.11	3.25	1.95	5.42	6.18E-6
Neurological involvement	6	28184935	-	*ZKSCAN8P1*	T	0.12	3.22	1.94	5.34	6.54E-6

Neurological involvement	6	28188698	rs1150685	*ZKSCAN8P1*	C	0.12	3.22	1.94	5.34	6.54E-6
Genital aphthosis	11	3150587	rs75048411	*OSBPL5*	T	0.01	0.08	0.02	0.23	6.73E-6
Genital aphthosis	10	93248499	rs7098478	*CYP26A1*	C	0.08	0.25	0.14	0.46	7.10E-6
Genital aphthosis	10	93248988	rs1984996	*CYP26A1*	A	0.08	0.25	0.14	0.46	7.10E-6
Genital aphthosis	10	93251222	rs12219795	*CYP26A1*	C	0.08	0.25	0.14	0.46	7.10E-6
Genital aphthosis	10	93257752	rs4917708	*CYP26A1*	A	0.08	0.25	0.14	0.46	7.10E-6
Neurological involvement	6	28077538	-	*ZNF165*	G	0.11	3.22	1.93	5.36	7.32E-6
Neurological involvement	6	28087717	rs10484404	*ZNF165*	T	0.11	3.22	1.93	5.36	7.32E-6
Neurological involvement	6	28113609	rs75889632	*ZSCAN16*	A	0.11	3.22	1.93	5.36	7.32E-6
Neurological involvement	4	168777515	-	*PALLD*	C	0.12	3.23	1.94	5.4	7.45E-6
Neurological involvement	4	168778292	rs72701840	*CBR4*	T	0.12	3.23	1.94	5.4	7.45E-6
Arthritis	22	50661446	rs8138300	*SHANK3*	G	0.44	0.5	0.37	0.68	8.63E-6
Ocular lesions	20	62675278	rs4803477	*SLCO4A1*	T	0.24	0.47	0.34	0.66	8.68E-6
Neurological involvement	4	168775510	rs9233429 3	*CBR4*	T	0.12	3.2	1.92	5.35	8.97E-6
Neurological involvement	4	168771693	rs7270182 8	*DDX60L*	G	0.12	3.19	1.91	5.34	9.14E-6
Neurological involvement	4	168773335	rs1392752	*DDX60L*	G	0.12	3.19	1.91	5.34	9.14E-6
Genital aphthosis	15	87510042	rs6416541	*NTRK3*	G	0.11	0.29	0.16	0.5	9.14E-6
Genital aphthosis	15	87534722	rs3900605	*NTRK3*	G	0.12	0.29	0.16	0.5	9.14E-6
Genital aphthosis	15	87545216	rs4887298	*NTRK3*	G	0.12	0.29	0.16	0.5	9.14E-6
Ocular lesions	5	116475524	rs423834	*SEMA6A*	C	0.31	0.5	0.37	0.68	9.49E-6
Ocular lesions	5	116475823	rs3213797	*SEMA6A*	G	0.31	0.5	0.37	0.68	9.49E-6
Erythema nodosum	6	31268274	rs2524099	*HLA-C*	G	0.35	0.51	0.38	0.69	9.69E-6

Abbreviations: Bp, base pair; chr, chromosome; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
All positions are in GRCh38 build.

Highlights

- Genetic contributions to clinical manifestations in Behçet's disease were comprehensively evaluated.
- *HLA-B*/*MICA* locus predisposes to ocular involvement (odds ratio ~2)
- Novel loci in *SLCO4A* (odds ratio ~ 2) and *DDX60L* (odds ratio ~ 4) predispose to ocular and neurological involvement, respectively.
- Genetics play a role in disease heterogeneity in Behçet's disease.

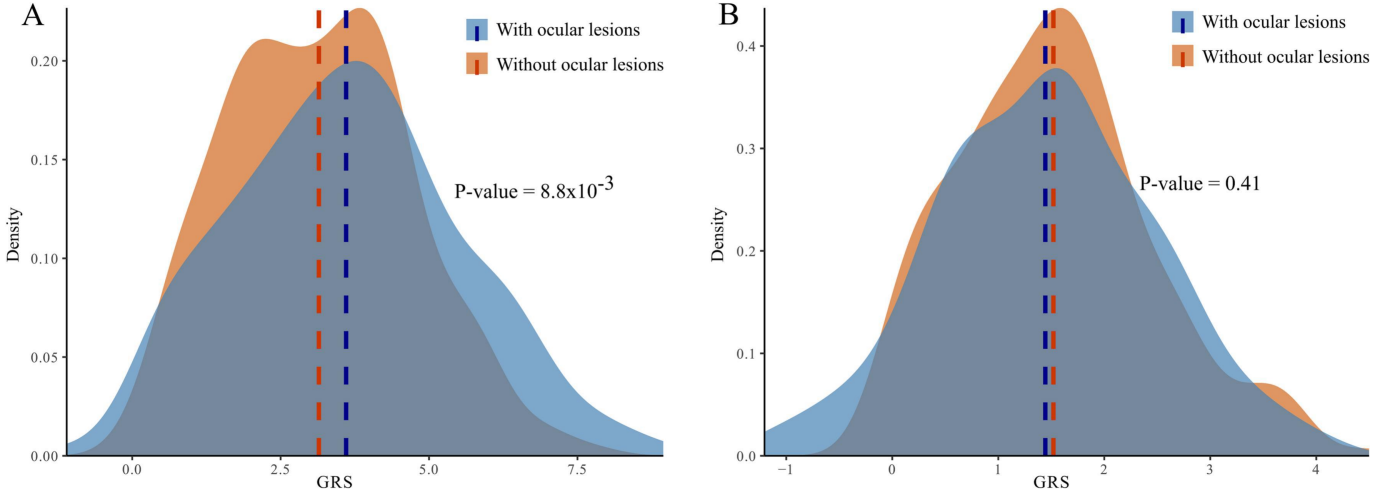

A
With ocular lesions
Without ocular lesions
P-value = 8.8x10-3
Density
GRS
0.00
0.05
0.10
0.15
0.20
0.0
2.5
5.0
7.5
B
With ocular lesions
Without ocular lesions
P-value = 0.41
Density
GRS
0.0
0.1
0.2
0.3
0.4
−1
0
1
2
3
4

Figure 1

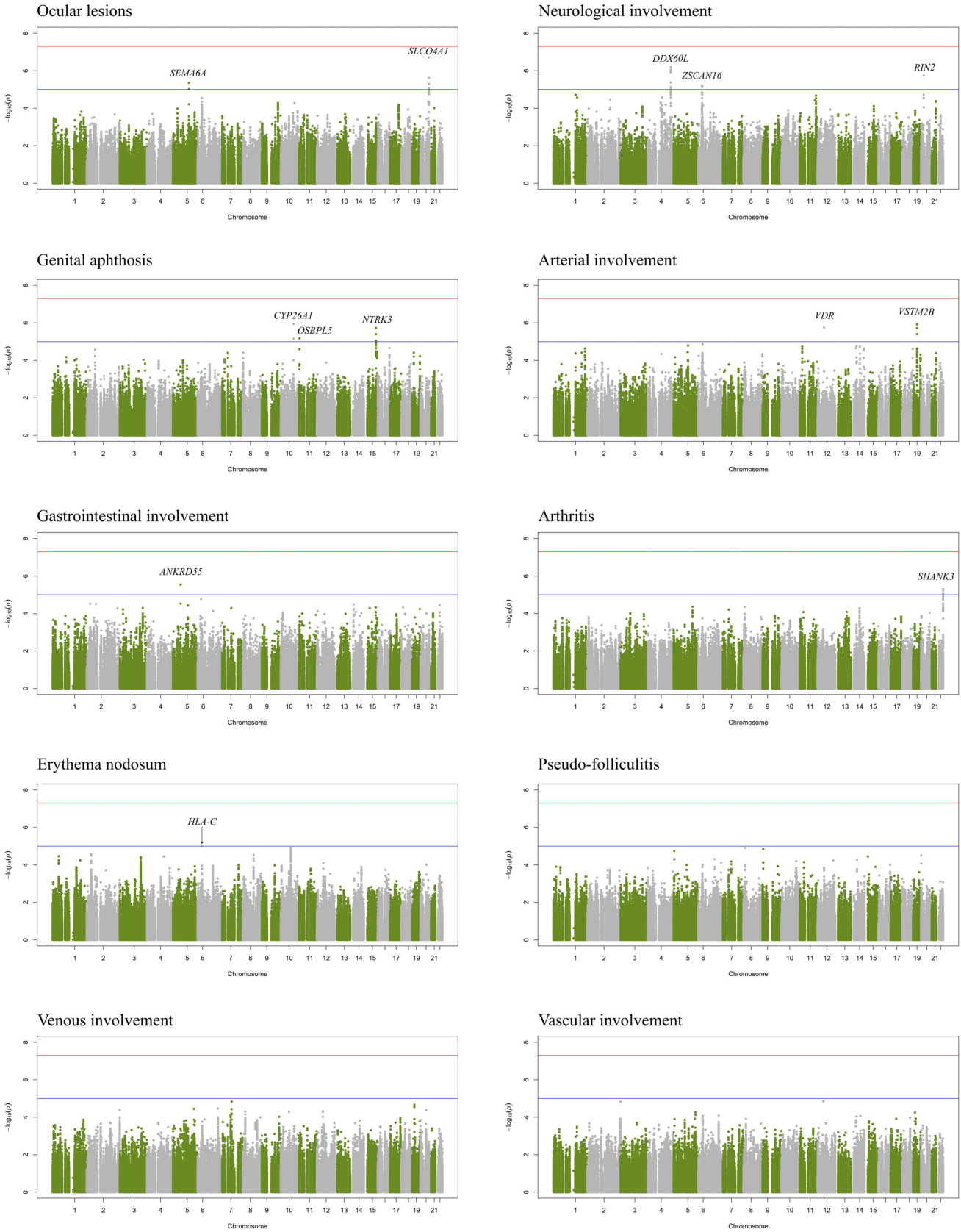

Ocular lesions
SEMA6A
SLCO4A1
Neurological involvement
DDX60L
ZSCAN16
RIN2
Genital aphthosis
CYP26A1
OSBPL5
NTRK3
Arterial involvement
VDR
VSTM2B
Gastrointestinal involvement
ANKRD55
Arthritis
SHANK3
Erythema nodosum
HLA-C
Pseudo-folliculitis
Venous involvement
Vascular involvement
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21
0 2 4 6 8

Figure 2