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Genetic Association of a Gain-of-Function *IFNGR1* Polymorphism and the Intergenic Region *LNCAROD/DKK1* With Behçet's Disease

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Objective. Behçet's disease is a complex systemic inflammatory vasculitis of incompletely understood etiology. This study was undertaken to investigate genetic associations with Behçet's disease in a diverse multiethnic population.

Methods. A total of 9,444 patients and controls from 7 different populations were included in this study. Genotyping was performed using an Infinium ImmunoArray-24 v.1.0 or v.2.0 BeadChip. Analysis of expression data from stimulated monocytes, and epigenetic and chromatin interaction analyses were performed.

Results. We identified 2 novel genetic susceptibility loci for Behçet's disease, including a risk locus in *IFNGR1* (rs4896243) (odds ratio [OR] 1.25; $P = 2.42 \times 10^{-9}$) and within the intergenic region *LNCAROD/DKK1* (rs1660760) (OR 0.78; $P = 2.75 \times 10^{-8}$). The risk variants in *IFNGR1* significantly increased *IFNGR1* messenger RNA expression in lipopolysaccharide-stimulated monocytes. In addition, our results replicated the association ($P < 5 \times 10^{-8}$) of 6 previously identified susceptibility loci in Behçet's disease: *IL10*, *IL23R*, *IL12A-AS1*, *CCR3*, *ADO*, and *LACC1*, reinforcing the notion that these loci are strong genetic factors in Behçet's disease shared across ancestries. We also identified >30 genetic susceptibility loci with a suggestive level of association ($P < 5 \times 10^{-5}$), which will require replication. Finally, functional annotation of genetic susceptibility loci in Behçet's disease revealed their possible regulatory roles and suggested potential causal genes and molecular mechanisms that could be further investigated.

Conclusion. We performed the largest genetic association study in Behçet's disease to date. Our findings reveal novel putative functional variants associated with the disease and replicate and extend the genetic associations in other loci across multiple ancestries.

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INTRODUCTION

Behçet's disease is a chronic relapsing–remitting inflammatory disorder characterized by recurrent oral and genital ulcers. It is a systemic vasculitis that can affect the eyes, skin, blood vessels, central nervous system, and gastrointestinal tract (1). Behçet's disease is also known as the "Silk Road disease," since its highest prevalence coincides with this ancient route, stretching from Japan to the Mediterranean region (2). However, patients worldwide have been diagnosed as having Behçet's disease (3). Although the etiology and pathogenesis of Behçet's disease remain incompletely understood, it is suspected that environmental factors, such as infectious agents and others, might trigger the onset of the disease in genetically predisposed individuals by propagating a dysregulated immune response (4).

The genetic studies performed to date in Behçet's disease have clearly established the HLA class I region as the most robust genetic susceptibility locus for the disease (5). Although the association with the classic HLA allele HLA–B*51 has been replicated in multiple ancestries, several additional loci within the HLA region, including a putative functional variant between *HLA–B* and *MICA*, have been reported (6–8). Outside the HLA region, at least 16 loci have been reported to be associated with Behçet's disease at a genome-wide level of significance (9–16). These genetic susceptibility loci, such as *IL10*, *IL23R-IL12RB2*, *STAT4*, and *FUT2*, among others, provided important insights into the pathogenic mechanisms that could be underlying the pathophysiology and immune dysregulation in Behçet's disease.

Despite the progress in understanding the genetic etiology of Behçet's disease, the majority of genome-wide association studies to date in this disease have presented data predominantly derived from 1 or 2 ancestral populations. In addition, currently available studies are limited by a relatively small sample size compared to genetic studies in other immune-mediated diseases, which is in part due to the low prevalence of Behçet's disease in many populations.

We performed a large genetic association study involving 9,444 individuals, including Behçet's disease patients and controls, from 7 diverse populations around the world. We identified 2 novel genetic associations in Behçet's disease, most notably including a susceptibility variant that increases the expression of the *IFNGR1* gene in monocytes. In addition, we extended the association of several previously reported genetic susceptibility loci to other populations and identified >30 loci with a suggestive association that provide additional insights into the pathogenesis of Behçet's disease.

PATIENTS AND METHODS

Study population. A total of 9,444 individuals (3,477 patients and 5,967 controls) were included in this study. All patients fulfilled the 1990 International Study Group classification criteria for Behçet's disease (17). Our study population consisted of the following 7 independent cohorts of diverse ancestries: Turkish (1,317 cases and 699 controls), Spanish (278 cases and 1,517 controls), Italian (144 cases and 1,270 controls), Korean (200 cases and 200 controls), Tunisian (136 cases and 186 controls), Japanese (120 cases and 218 controls), and Western European (67 cases and 599 controls). Genotyping was performed using Illumina ImmunoChip custom arrays (Infinium ImmunoArray-24 v.1.0 or v.2.0 BeadChip) according to the manufacturer's instructions. Additional genotyping data from 1,215 Turkish cases and 1,278 Turkish controls were obtained from dbGaP (accession no. phs000272.v1.p1) (9). A detailed description of the study population can be found in Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract. The study was approved by the institutional review boards and the ethics committees at all participating institutions, and all study participants signed a written informed consent.

Data quality assessment and measures. The same stringent quality control measures were applied separately in each population cohort, to maintain consistency across populations, using Plink v.1.9 (18). Single-nucleotide polymorphisms (SNPs) were removed if they had a genotyping call rate <98%, minor allele frequency (MAF) <1%, or deviation from Hardy-Weinberg equilibrium in either cases or controls ($P < 1 \times 10^{-3}$). SNPs on sex chromosomes were not analyzed. In addition, samples with a genotyping call rate <95% were filtered out. Relatedness was assessed, and 1 individual from each pair of duplicates and/or first-degree relatives (Pi-HAT > 0.4) was randomly excluded.

To control for possible population stratification, principal components analysis was performed using a set of linkage disequilibrium (LD)–pruned markers, pairwise $r^2 < 0.20$, with EigenSoft 6.1.4 software (19). Individuals >6 SD from the cluster centroids were considered outliers and were not included in the analyses. Dot plots showing the first 2 principal components were generated for each population using R 3.6 software (20) and are shown in Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract.

Imputation. Post–quality control genotyping data were used for the imputation of autosomal SNPs, which was conducted for each population independently with the Michigan Imputation Server using Minimac3 (21). The software SHAPEIT (22)

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No potential conflicts of interest relevant to this article were reported.

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was used for haplotype reconstruction with the Haplotype Reference Consortium r1.1 (23) as the reference population. Only SNPs with stringent correlation values ($r^2 > 0.9$) were maintained for further analyses. Finally, additional quality control measures were conducted, and variants with MAF <1% or Hardy-Weinberg equilibrium $P < 1 \times 10^{-3}$ were excluded.

Data analysis. Plink v.1.9 (18) was used to perform association analyses. First, logistic regression was assessed for each population independently. The 5 first principal components were used as covariates. Genomic inflation factor (λ) was calculated per cohort using a set of ~3,000 SNPs included in the ImmunoChip, known as "null" SNPs, that have not previously been associated with immune-mediated diseases. Quantile–quantile plots for the *P* values are shown in Supplementary Figure 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract. Then, we performed a multiethnic meta-analysis by means of the inverse variance method including the results of the logistic regressions for all populations. Heterogeneity of associations was tested using Cochran's Q test *P* value (Q) and heterogeneity index (I^2). A fixed-effects model was applied for those SNPs without evidence of heterogeneity ($Q > 0.1$ and $I^2 < 50\%$). $Q \leq 0.1$ and $I^2 \geq 50\%$ indicate evidence of heterogeneity, and a random-effects model was applied in that case. The commonly used genome-wide threshold of $P < 5 \times 10^{-8}$ was established for significant associations, and the SNP showing the lowest *P* value within each associated genomic region was reported as the lead SNP. In addition, a threshold of $P < 5 \times 10^{-5}$ was established for suggestive associations.

Next, we performed joint conditional analysis using GCTA software to determine if multiple independent associations exist within an associated genomic region (24,25). This method uses the summary statistic from the meta-analysis and corrects for LD. Genotyping data from the 7 populations were used to estimate the LD patterns used as reference, and the lead SNP was included as a covariate. We considered independent signals if a variant reached a conditional *P* value $< 5 \times 10^{-8}$. Both associated and suggestive genomic regions are named, in figures and tables, by the bounding genes except in the cases in which the literature repeatedly involves a specific gene. Finally, the qqman R package was used to generate the Manhattan and quantile–quantile plots.

To check if the overall risk allele frequencies identified in our study were different across our study populations, we first obtained the frequencies of the associated and suggestive variants ($P < 5 \times 10^{-5}$) for cases and controls independently using Plink v.1.9. Only variants that were present in ≥6 of the 7 populations after quality control were considered. One-way analysis of variance (ANOVA) was performed using GraphPad Prism version 8.1.1 (GraphPad Software). Results for each group are presented as the mean ± SD. *P* values less than 0.05 were considered significant.

Functional annotation. To better understand the statistical associations in the disease context, we evaluated the potential causalities of the identified associated variants by performing a comprehensive functional annotation. First, we explored RegulomeDB to annotate the SNPs with regulatory elements and get a probability score of how likely each variant plays a regulatory role (26). This score ranges from 0 to 1, with 1 being the most likely to be a regulatory variant. In addition, we queried HaploReg v4.1 for the epigenomic annotations (27) and the webtool Capture Hi-C plotter, https://www.chicp.org/chicp/, to evaluate chromatin interactions between SNPs and gene promoter regions (28). We also interrogated if our associated variants have been identified as acting as expression quantitative trait loci (eQTL) through the web tool FUMA GWAS (http://fuma.ctglab.nl) and through HaploReg v4.1.

We used expression and genotyping data from a previous study for the representation of the eQTL of *IFNGR1* (29,30). Briefly, expression profiling of primary CD14+ monocytes obtained from 260 European individuals and stimulated with lipopolysaccharide (LPS) for 2 hours was performed with a HumanHT-12v4 BeadChip (Illumina), and genotyping was performed using a HumanOmniExpress-12v1.0 BeadChip (Illumina) as previously described (29,30). These data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Finally, we explored the GWAS Catalog (https://www.ebi.ac.uk/gwas) to assess the pleiotropic effect of our associated signals.

RESULTS

After filtering with stringent quality controls, we analyzed a total of 8,982 individuals (3,197 patients) from 7 different populations: Turkish, Spanish, Italian, Korean, Tunisian, Japanese, and Western European. A summary of sample/variant quality control is shown in Supplementary Table 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract. Association testing was performed within each ancestry using a logistic regression model (Supplementary Figure 3, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract), and genomic control analysis showed no evidence of population stratification for any cohort (genomic inflation factor [λ] < 1.04) (Supplementary Table 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). Next, we undertook a multiethnic meta-analysis to combine the results of the 7 populations (Figure 1). Consistent with our current knowledge of the disease, the strongest association was observed within the HLA region, which has been characterized previously (6,12,13).

Excluding the well-known HLA region, our results revealed 62 variants at the genome-wide significance level ($P < 5 \times 10^{-8}$) that mapped onto 8 different genomic regions. Detailed results

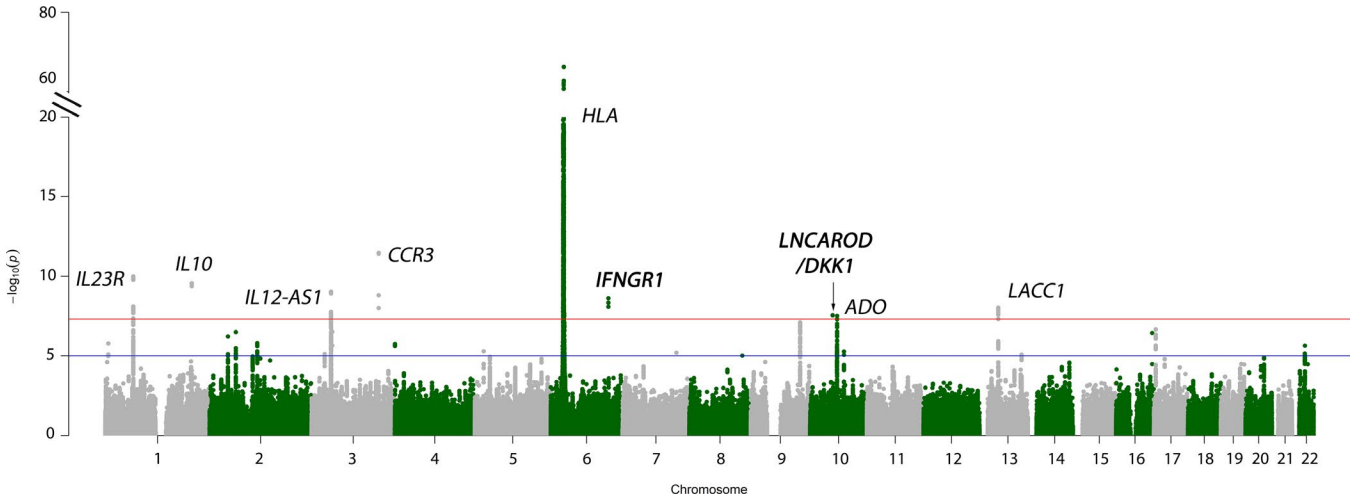


Figure 1. Manhattan plot showing the results of a meta-analysis of Behçet's disease cases and controls in the 7 populations included in this study (Turkish, Spanish, Italian, Korean, Tunisian, Japanese, and Western European). The $-\log_{10} P$ value for each genetic variant analyzed is plotted against its physical chromosomal position. The red line represents the genome-wide level of significance ($P < 5 \times 10^{-8}$), and the blue line represents the suggestive level of significance ($P < 5 \times 10^{-5}$).

of all these variants, including the association results for each population independently, are shown in Supplementary Table 3, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract. We performed joint conditional analysis to test if >1 variant within the associated genomic regions was independently associated with Behçet's disease. This approach did not identify any additional independent signals for any of the 8 loci; conditional *P* values are shown in Supplementary Table 4, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract. Therefore, we used the strongest associated variant within each locus as the lead SNP of the association. Results for the lead SNP of each locus are illustrated in Table 1. Two of these 8 loci (*IFNGR1* and the intergenic region *LNCAROD/DKK1*) are novel genetic associations in Behçet's disease, while the remaining 6 loci have been reported previously.

Of the 2 novel associated loci, the most strongly associated signal was located near *IFNGR1* (lead SNP rs4896243) (odds ratio [OR] 1.25; $P = 2.42 \times 10^{-9}$). This genetic region also harbored 2 additional genome-wide associated variants (rs4896242 [$P = 4.62 \times 10^{-9}$] and rs1327474 [$P = 8.35 \times 10^{-9}$]) representing the same signal of association. Consistent OR directions were observed across ancestries. These polymorphisms are in high LD, which was also reflected by the results of the conditional analysis (Supplementary Table 4). No additional markers in LD were included in the analyses, as illustrated in the regional plots (regional plots showing the results of this region in each population are shown in Supplementary Figure 4, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

In common with most genetic variants associated with immune-mediated diseases, the *IFNGR1* polymorphisms identified in our study reside in noncoding regions. Therefore, we carried out a comprehensive functional annotation to try to decipher the causal mechanisms of this association (Table 2 and Supplementary Table 5, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). Epigenetic annotation revealed colocalization of the 3 associated variants with enhancer histone marks. In addition, rs1327474 colocalizes with promoter histone marks and DNase hypersensitivity

Table 1. Results of the meta-analysis for the lead SNP of each genetic region associated with Behçet's disease at a GWAS level of significance*

Locus	Chromosome	Position (hg19)	SNP	Location	Minor allele	*P*	OR
IL10	1	206945311	rs3024490	Intronic	A	2.81×10^{-10}	1.26
Il23R	1	67744601	rs6660226	Downstream	A	1.01×10^{-10}	0.79
IL12A-AS1	3	159637678	rs76830965	Intronic	A	3.43×10^{-12}	1.66
CCR3	3	46208310	rs2087726	Intronic	G	9.33×10^{-10}	0.79
IFNGR1†	6	137514790	rs4896243	Downstream	C	2.42×10^{-9}	1.25
LNCAROD-DKK1†	10	54154620	rs1660760	Intergenic	T	2.75×10^{-8}	0.78
ADO	10	64561506	rs12220700	Upstream	G	3.07×10^{-8}	0.80
LACC1	13	44457925	rs2121034	Downstream	T	9.44×10^{-9}	0.79

* The genome-wide association study (GWAS) level of significance was set at $P < 5 \times 10^{-8}$. SNP = single-nucleotide polymorphism; hg19 = human reference genome; OR = odds ratio.
† Newly identified locus.

Table 2. Functional annotation of the 8 non-HLA loci associated with Behçet's disease at a GWAS level of significance*

Locus	SNP	RegulomeDB score	Promoter histone marks	Enhancer histone marks	DNase hypersensitivity	Proteins bound	eQTL in blood cells	eQTL in other tissues
IL10	rs1800872	0.609	Yes	Yes	No	Yes	*IL10*	*IL19, IL24, FAIM3*
Il23R	rs2019262	0.638	Yes	No	No		*IL23R*	*MIER1, IL12RB2, C1orf141*
IL12A-AS1	rs76830965	0.775	Yes	Yes	Yes	Yes	–	*IL12A, TRIM59, BTN3A1, STAT1, GBP1, IFI6, APOL3, IFI44L, HERC6, MX1, GBP2, SCHIP1*
CCR3	rs35678191	0.614	No	Yes	No	No	*CCR5, CCR3, CCR2, CCRL2, CCR9, LZTFL1, CCR1, LRRC2, CXCR6, SACM1L*	*CXCR6, CCR2, CCR1, SLC6A20, PRSS45, PRSS46, CCR5*
IFNGR1	rs4896243	0.805	No	Yes	No	No	*IFNGR1*	*IFNGR1*
LNCAROD-DKK1	rs1660760	0.184	No	No	No	No	–	*DKK1*
ADO	rs224106	0.154	No	No	No	No	*ADO*	*ADO, EGR2*
LACC1	rs2121033	0.922	No	No	No	No	*CCDC122, LACC1*	*CCDC122, LACC1, ENOX1*

* The variant showing the highest RegulomeDB score for each region is displayed. The genome-wide association study (GWAS) level of significance was set at $P < 5 \times 10^{-8}$. SNP = single-nucleotide polymorphism; eQTL = expression quantitative trait locus.

sites in multiple primary tissues and cell types, including blood cells. Finally, ChIP-Seq data revealed that rs1327474 is located within an RNA polymerase II binding site in a B cell line (GM12891). Because these data suggest a potential regulatory role of these polymorphisms in *IFNGR1*, we checked if these variants have been identified to act as eQTLs (Table 2 and Supplementary Table 6, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

Interestingly, a previous study found that the variants rs4896243 and rs1327474 act as eQTLs for *IFNGR1* expression in monocytes after a 2-hour LPS stimulation ($P = 2.07 \times 10^{-18}$ and 2.18×10^{-18}, respectively) (29). Our analysis of these data revealed that the Behçet's disease–associated risk alleles increased the expression level of *IFNGR1* (Figure 2). Finally, physical chromatin interactions between these polymorphisms and other genes, such as *TNFAIP3*, *IL22RA*, and *OLIG3*, have been detected in different blood cell types (Supplementary Table 7 and Supplementary Figure 5, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

We also reported 2 genome-wide associated SNPs located in an intergenic region between *LNCAROD* and *DKK1* (lead SNP rs1660760) (OR 0.78; $P = 2.75 \times 10^{-8}$), representing a newly identified signal associated with Behçet's disease. Only genetic data for the Turkish population were maintained in this locus after quality control, and none of these variants showed high LD with any other genotyped or imputed SNPs (Supplementary Figure 4, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). The lead SNP, rs1660760, has been reported to act as an eQLT in brain tissue (Table 2 and Supplementary Table 6, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

In addition to the 2 new susceptibility loci we report in this study, we replicated with a genome-wide level of significance 6 previously described loci in Behçet's disease: *IL10* (lead SNP rs3024490) (OR 1.26; $P = 2.81 \times 10^{-10}$), *IL23R* (lead SNP rs6660226) (OR 0.79; $P = 1.01 \times 10^{-10}$), *IL12A-AS1* (lead SNP rs76830965) (OR 1.66; $P = 3.43 \times 10^{-12}$), *CCR3* (lead SNP rs2087726) (OR 0.79; $P = 9.33 \times 10^{-10}$), *ADO* (lead SNP rs12220700) (OR 0.80; $P = 3.07 \times 10^{-8}$), and *LACC1* (lead SNP rs2121034) (OR 0.79; $P = 9.44 \times 10^{-9}$). Consistent OR directions were observed across ancestries for each locus. These data reinforce the strength of these associations and provide evidence of a shared genetic background across ancestries in Behçet's disease (Supplementary Table 3, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

We further evaluated the possible functional implications of the genetic variants identified in this study that are within the 6 loci previously identified with a genome-wide association study (GWAS) level of significance in Behçet's disease. We found overlap with epigenetic features for all 6 of these loci (Table 2 and Supplementary Table 5 and Supplementary Figure 6, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). Most of the polymorphisms identified as susceptibility variants for Behçet's disease within these loci may change regulatory motifs and potentially alter transcription factor binding, and a significant proportion of variants overlapped with promoter and/or enhancer histone marks in ≥1 tissue and/or cell type. In addition, most variants appear to act as eQTLs, thus modifying gene expression levels (Supplementary Table 6). Notably, the disease risk alleles in *IL10* are associated with reduced expression of *IL10* in whole blood. The Behçet's disease–associated variants in *IL23R* identified in our meta-analysis are also associated with altered expression levels in whole blood for the following genes: *PHKB*, *BATF2*, *CYB5R4*, *DRR1*, and *SLC35D1*. Finally, Hi-C data revealed that most of the genetic variants associated with Behçet's disease with a GWAS level of significance in our study showed physical chromatin interaction with gene promoter

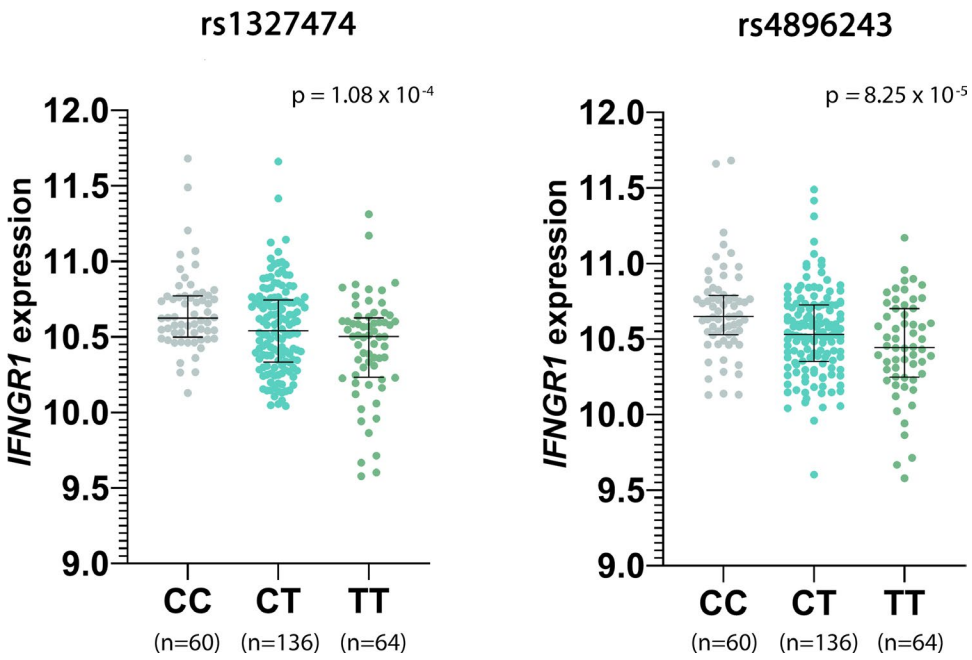


Figure 2. Expression quantitative trait locus associations between 2 *IFNGR1* variants (rs1327474 and rs4896243) and *IFNGR1* transcripts in monocytes stimulated with lipopolysaccharide for 2 hours. The risk alleles (C) for both single-nucleotide polymorphisms correlated with significantly higher expression levels of *IFNGR1*. *P* values shown were determined by one-way analysis of variance. Differences between genotypes were as follows: for rs1327474, $P = 4.40 \times 10^{-2}$ for CC versus CT, $P = 5.98 \times 10^{-5}$ for CC versus TT, and $P = 2.10 \times 10^{-2}$ for CT versus TT; and for rs4896243, $P = 6.15 \times 10^{-3}$ for CC versus CT, $P = 5.74 \times 10^{-5}$ for CC versus TT, and $P = 1.02 \times 10^{-1}$ for CT versus TT, by Tukey's multiple comparisons test. Symbols represent individual samples; bars show the mean ± SD.

regions (Supplementary Table 7). Of special note are those interactions between SNPs and the promoters of the genes whose expression levels were affected in the same cell type, such as the interactions between the Behçet's disease–associated *CCR3* variants and the promoters of *CCR1* and *CXCR6* in immune cells. These analyses support the idea that additional genes might be involved in the pathology of Behçet's disease and might represent potential targets that could be further investigated.

Our study also revealed evidence of a suggestive association ($P < 5 \times 10^{-5}$) in 752 additional SNPs corresponding to 39 genomic regions (including SNPs within *LACC1*, *CCR3,* and *IL23R*) (Supplementary Table 8, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). Among these loci, it is worth highlighting our findings in genes that have previously been found to be associated with Behçet's disease, such as *IL1A-IL1B* (lead SNP rs35145107; $P = 1.56 \times 10^{-6}$), *IRF8* (lead SNP rs6540239; $P = 3.61 \times 10^{-7}$), and *UBAC2* (lead SNP rs4771332; $P = 8.38 \times 10^{-6}$). Additional suggestive associations in our study include *IRF5* (lead SNP rs192829776; $P = 6.40 \times 10^{-6}$) and *LBP* (lead SNP rs139169382; $P = 4.36 \times 10^{-5}$), among others (Supplementary Table 8). Results from our data in genetic variants previously reported to be associated with Behçet's disease with a GWAS level of significance are shown in Supplementary Table 9, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract.

DISCUSSION

The present study is the largest genetic association study undertaken to date in Behçet's disease. Our results identified 2 novel genetic regions associated with Behçet's disease, a gain-of-function *IFNGR1* polymorphism and variants in the intergenic region *LNCAROD/DKK1*. In addition, our data replicated the association of 6 previously reported genetic susceptibility loci for this disease and extended those associations across ancestries.

We have demonstrated, for the first time, the involvement of *IFNGR1* as a susceptibility locus for Behçet's disease. *IFNGR1* encodes the binding subunit, α chain, of the interferon-γ (IFNγ) receptor. The binding of IFNγ stimulates the activation of the JAK/STAT signaling pathway, which is crucial for the activation of the immune system (31). Interestingly, Tulunay and colleagues observed an increase in JAK/STAT signaling in both CD14+ monocytes ($P = 9.55 \times 10^{-3}$) and CD4+ lymphocytes ($P = 8.13 \times 10^{-4}$) in patients with Behçet's disease compared with healthy individuals (32). Our functional annotation analysis strongly suggested a regulatory role of the *IFNGR1*-associated variants. Indeed, we demonstrated that the Behçet's disease risk alleles in this locus increase *IFNGR1* expression in monocytes after 2 hours of LPS stimulation. Few studies analyzing the involvement of monocytes in Behçet's disease have been published to date (32–35). Considering that the knowledge of the context and cell types that determine the strength of the eQTLs may help to identify molecular mechanisms

relevant to the disease (36), further research focused on elucidating the role of this genetic association in monocytes and its effect on Behçet's disease–related pathophysiology is warranted.

IFNγ has been shown to play a key role in multiple molecular processes that are essential for a normal immune response such as promoting macrophage activation, orchestrating activation of the innate immune system, regulating Th1/Th2 balance, enhancing antigen presentation, and mediating antiviral and antibacterial immunity (37,38). Notably, *IFNGR1* polymorphisms have been associated with susceptibility to several infectious agents, including *Helicobacter pylori*, *Mycobacterium tuberculosis*, and hepatitis B virus (39–41). All of this evidence supports the hypothesis that an infectious agent acts as a trigger for the onset of Behçet's disease in individuals with predisposing genetic background, and highlight monocytes as a relevant cell type in the pathophysiology of Behçet's disease. In addition, these data support a potential role for JAK/STAT inhibitors as therapeutic consideration for clinical trials in Behçet's disease (42).

Interestingly, an intronic variant in *IFNGR1*, rs7749390, has recently been identified as a genetic factor for mouth ulcers, albeit with a modest effect (OR 1.08 [95% confidence interval 1.07–1.08]) (43). This variant only passed the quality control measures in the Tunisian population in our study, which limited the statistical power to detect a genome-wide level association. However, our results showed a nominal association for this SNP with Behçet's disease (OR 1.53 [95% confidence interval 1.05–2.21]; $P = 1.36 \times 10^{-2}$). In addition, the *IFNGR1*-associated variants identified in our study and rs7749390 are in LD, suggesting that they might correspond with the same signal (Supplementary Table 10, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract).

The results of our study also revealed a new genetic signal within an intergenic region between *LNCAROD* and *DKK1*. *LNCARDOD* encodes a long intergenic non–protein-coding RNA that acts as an activating regulator of *DKK1*. *DKK1* inhibits β-catenin–dependent Wnt signaling by binding to the co-receptor *LRP6* (44). Wnt signaling has been shown to play a crucial role in several biologic processes, including cellular proliferation, angiogenesis, and development of the immune system (45,46). In addition, recent evidence suggested the pathogenic involvement of *DKK1* through the Wnt signaling pathway in immune-mediated diseases such as rheumatoid arthritis, psoriasis, systemic sclerosis, systemic lupus erythematosus, and ankylosing spondylitis (45,47). Therefore, Wnt signaling has gained increasing attention as a possible therapeutic target in immune-mediated diseases (47). However, considering that these polymorphisms were identified only in the Turkish cohort in our study, replication as well as functional studies are needed.

Our results replicated the association of *IL10*, *IL23R*, *IL12A-AS1, CCR3, ADO*, and *LACC1* in Behçet's disease. In addition, several of the associated variants in these loci have been reported to be associated with other immune-mediated disorders and/or infectious agents, which indicates a pleiotropic effect of these genetic variants (Supplementary Table 11, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). However, the causal mechanisms of these genetic associations remain unclear. Functional annotation analysis can reveal predicted functional effects and generate testable hypotheses. For example, associated SNPs in *IL10* and *IL12A-AS1* loci colocalize with promoter and enhancer histone marks in a multitude of cell types. *IL23R* variants have been identified to modify the expression levels of 10 different genes. It is worth highlighting *CCR3*-associated polymorphisms which act as eQTLs for *CCR1* and *CXCR6* and show evidence of chromatin interactions with the promoters of these genes in blood cells. These predicted functional effects expand the genomic associations to several target genes that could be further investigated to decipher the exact molecular mechanisms involved in the pathophysiology of Behçet's disease.

Finally, we observed significant differences in the risk allele frequencies of the variants identified in our study ($P < 5 \times 10^{-5}$) across populations for both cases and controls (Supplementary Table 12, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41637/abstract). Overall, the results suggest that the frequency of genetic variants identified in this study are consistent with the prevalence data for Behçet's disease, showing the highest mean frequencies in the Tunisian, Turkish, and Asian populations (2,3).

In conclusion, we present the results of a large, multinational collaborative effort and dense genotyping in immune-related genetic loci to understand the genetic basis of Behçet's disease. We identified novel genetic susceptibility loci for the disease, including a genetic association with a gain-of-function variant in *IFNGR1* and genetic variants in the intergenic region *LNCAROD/DKK1*. We replicated a number of previously identified genetic susceptibility loci for Behçet's disease and extended them across diverse populations and ancestries. In addition, our functional and epigenetic annotation analysis revealed potential new candidate genes involved in Behçet's disease. Furthermore, >30 additional loci with a suggestive level of association were identified, which will require further validation.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Sawalha had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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