

Identification of Susceptibility Loci in *IL6*, *RPS9/LILRB3*, and an Intergenic Locus on Chromosome 21q22 in Takayasu Arteritis in a Genome-Wide Association Study

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Objective. Takayasu arteritis is a rare large vessel vasculitis with incompletely understood etiology. This study was undertaken to perform the first unbiased genome-wide association analysis of Takayasu arteritis.

Methods. Two independent cohorts of patients with Takayasu arteritis from Turkey and North America were included in our study. The Turkish cohort consisted of 559 patients and 489 controls, and the North

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American cohort consisted of 134 patients and 1,047 controls of European ancestry. Genotyping was performed using the Omni1-Quad and Omni2.5 genotyping arrays. Genotyping data were subjected to rigorous quality control measures and subsequently analyzed to discover genetic susceptibility loci for Takayasu arteritis.

Results. We identified genetic susceptibility loci for Takayasu arteritis with a genome-wide level of significance in *IL6* (rs2069837) (odds ratio [OR] 2.07, $P = 6.70 \times 10^{-9}$), *RPS9/LILRB3* (rs11666543) (OR 1.65, $P = 2.34 \times 10^{-8}$), and an intergenic locus on chromosome 21q22 (rs2836878) (OR 1.79, $P = 3.62 \times 10^{-10}$). The genetic susceptibility locus in *RPS9/LILRB3* lies within the leukocyte receptor complex gene cluster on chromosome 19q13.4, and the disease risk variant in this locus correlates with reduced expression of multiple genes including the inhibitory leukocyte immunoglobulin-like receptor gene *LILRB3* ($P = 2.29 \times 10^{-8}$). In addition, we identified candidate susceptibility genes with suggestive levels of association ($P < 1 \times 10^{-5}$) with Takayasu arteritis, including *PCSK5*, *LILRA3*, *PPM1G/NRBP1*, and *PTK2B*.

Conclusion. Our findings indicate novel genetic susceptibility loci for Takayasu arteritis and uncover potentially important aspects of the pathophysiology of this form of vasculitis.

Takayasu arteritis is a rare inflammatory disease that typically involves the aorta and its major branches (1–3). The disease causes arterial stenosis, blood-vessel wall thickening, dilation, and progressive occlusion, leading to potentially life-threatening ischemia, aortic regurgitation, and absent or reduced pulses (1–3).

Takayasu arteritis can manifest with a broad range of nonspecific symptoms, including fever, fatigue, arthralgia, myalgia, and weight loss, and has a typical age at onset of between 20 and 40 years (4,5). The disease occurs worldwide and in all ethnicities, but the highest prevalence has been reported in East Asia, India, and Mexico. It is much more common in women, although the extent of this sex bias seems to be ethnicity dependent (4,6).

The etiology of Takayasu arteritis remains elusive. However, there is strong evidence of genetic contribution to the pathogenesis of the disease, supported by the repeatedly confirmed genetic association with *HLA-B*52* across multiple ethnicities (7–10). Recently, the genetic association between Takayasu arteritis and the HLA extended region was investigated using dense genotyping and imputation analysis (11). These data, which were derived by examining 2 sets of patients and controls from 2 different ethnicities, established the presence of 2 independent genetic associations within the HLA region in Takayasu arteritis (11). The strongest genetic association is in the *HLA-B/MICA* region, and the other is in the *HLA-DQB1/HLA-DRB1* locus in HLA class II. Outside the HLA region, we have previously established the association between Takayasu arteritis and genetic variants in *IL12B* (encoding the P40 regulatory subunit of interleukin-12 [IL-12] and IL-23 cytokines), and in the region encoding Fcγ receptors IIa and IIIa with a genome-wide level of significance (11). The association with the same genetic variants in *IL12B* was simultaneously described and confirmed in a Japanese cohort of patients with Takayasu arteritis (12). In this analysis, we performed the first unbiased genome-wide association study (GWAS) in Takayasu arteritis in 2 ethnically distinct cohorts of patients and controls.

PATIENTS AND METHODS

Patients and controls. We studied 2 ethnically distinct cohorts of patients with Takayasu arteritis and controls from Turkey and North America. The Turkish cohort included 559 patients enrolled by the Turkish Takayasu Study Group and 489 healthy controls, and the North American cohort included 134 European American patients enrolled in the Vasculitis Clinical Research Consortium Longitudinal Study of Takayasu Arteritis and 1,047 European American controls. All patients fulfilled the American College of Rheumatology 1990 classification criteria for Takayasu arteritis (13). Our sample size had ~90% power to detect a genetic effect with an odds ratio of 1.55 and with a genome-wide significant P value of 5×10^{-8} for variants with a minor allele frequency (MAF) of 0.35, with an estimated disease prevalence of 2 per million for Takayasu arteritis, using an additive genetic model. Genotyping data from the 1,047 European

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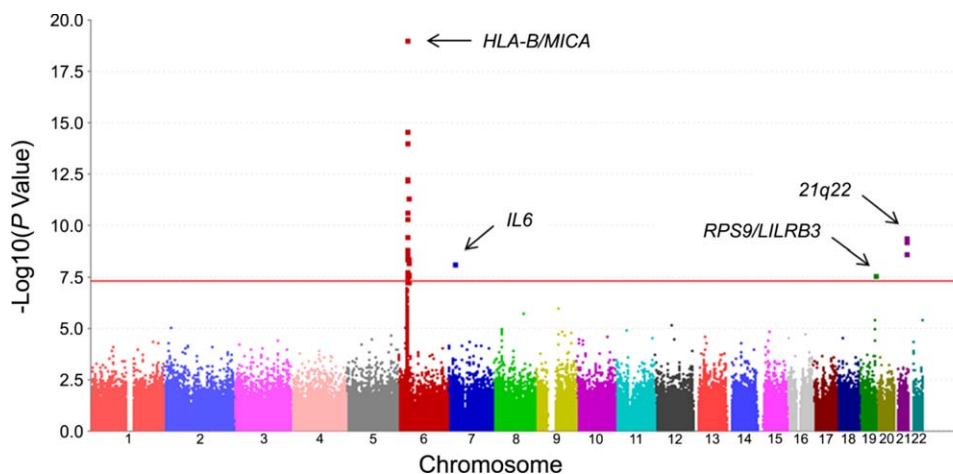


Figure 1. Manhattan plot showing the meta-analysis results for genotyped variants in the Turkish and North American cohorts of patients with Takayasu arteritis. The red line represents the threshold for genome-wide level of significance ($P = 5 \times 10^{-8}$).

American controls were derived from dbGaP (study accession no. phs000187.v1.p1). The study was approved by the institutional review boards and the ethics committees at all participating institutions, and written informed consent was obtained from all study participants.

Genotyping and data analysis. Genotyping of patients and controls was performed using the Omni1-Quad and Omni2.5 genotyping platforms (Illumina). Genotyping data from single-nucleotide polymorphisms (SNPs) included on both platforms were available for evaluation in both cohorts. After genotyping, we followed rigorous quality control measures as previously described (11,14). Briefly, samples were excluded from the analysis based on population stratification by principal components analysis (>4 SD), identity by descent (IBD) (>0.4), and autosomal heterozygosity (>2 SD around the mean). A 10-component principal components

analysis was performed using EigenStrat version 4.2 (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>) (15), and IBD and heterozygosity analyses were performed with Plink (16). Genotyped markers were filtered for MAF (>0.01), genotype success rate (>0.9), and Hardy-Weinberg equilibrium P value ($P > 0.01$ for controls; $P > 0.0001$ for cases). Markers with differential missingness between patients and controls ($P < 0.05$) were also excluded from the analysis.

After applying the quality control measures detailed above, a total of 474,442 variants were evaluated in the Turkish cohort and 547,389 in the North American cohort. A total of 516 patients and 462 controls in the Turkish cohort, and 119 patients and 993 controls in the North American cohort were included in the final analysis. Genomic control (GC)

Table 1. Genome-wide association analysis results showing genetic variants outside of the HLA region that were significantly associated ($P < 5 \times 10^{-8}$) with Takayasu arteritis in the Turkish and North American cohorts*

Locus/variant	Minor allele	Turkish cohort				North American cohort				Meta-analysis		
		Case MAF	Control MAF	OR (95% CI)	P	Case MAF	Control MAF	OR (95% CI)	P	OR	P	Q statistic P
<i>IL6</i>												
rs2069837	G	0.10	0.19	0.51 (0.39–0.66)	1.92×10^{-7}	0.03	0.09	0.32 (0.15–0.69)	2.32×10^{-3}	0.48	6.70×10^{-9}	0.274
<i>RPS9/LILRB3</i>												
rs11666543	A	0.19	0.30	0.56 (0.45–0.69)	3.55×10^{-8}	0.24	0.29	0.74 (0.54–1.02)	6.27×10^{-2}	0.61	2.34×10^{-8}	0.134
<i>21q22</i>												
rs2242944	A	0.30	0.40	0.65 (0.54–0.78)	4.98×10^{-6}	0.22	0.36	0.51 (0.37–0.70)	3.07×10^{-5}	0.61	1.93×10^{-9}	0.211
rs2836878	A	0.19	0.29	0.56 (0.46–0.70)	9.24×10^{-8}	0.17	0.27	0.55 (0.39–0.78)	7.23×10^{-4}	0.56	3.62×10^{-10}	0.912
rs2836881	T	0.19	0.29	0.57 (0.46–0.70)	1.40×10^{-7}	0.17	0.27	0.55 (0.39–0.78)	6.85×10^{-4}	0.56	5.16×10^{-10}	0.879

* The odds ratio (OR) for the minor allele is shown. MAF = minor allele frequency; 95% CI = 95% confidence interval.

was performed using filtered non-HLA variants with $MAF > 0.02$, and showed no to minimum evidence of population stratification in our cohorts ($\lambda_{GC_{Turkish}} = 1.05$, $\lambda_{GC_{EA}} = 1.00$). Genetic association analyses were performed using a basic allelic chi-square test with 1 degree of freedom, and the results were given as asymptotic P values. Meta-analysis was then performed using a fixed-effects model, and the results were filtered to exclude SNPs with a Cochran's Q statistic P value of less than 0.05. Meta-analysis was performed using Plink, and haplotype structure analysis was performed using Haploview 4.2 (17).

Additional genetic variants up to the 1000 Genomes Project density were imputed in the 3 non-HLA genetic loci that were detected with a genome-wide level of association with Takayasu arteritis. Imputation was performed using Impute2 (18) and a combined reference panel consisting of 1,092 individuals (19). We applied a posterior probability imputation threshold of 0.9, and filtered imputed variants based on $MAF (>1\%)$, imputation success rate ($>90\%$ of individuals), and Hardy-Weinberg P value (>0.0001) in controls prior to analysis, as previously described (11). Adjusted associations between SNPs were performed using conditional logistic regression in Plink. Regional linkage disequilibrium plots were generated using the programming language R version 3.1.1.

Expression quantitative trait loci (eQTL) analysis.

Expression quantitative trait loci analysis was performed to detect correlation between the presence or absence of the risk alleles in the identified Takayasu arteritis susceptibility loci and transcript expression levels in whole blood and lymphoblastoid cell lines. This was performed using the Genotype-Tissue Expression Project (20) and Gene Expression Variation eQTL databases (21).

RESULTS

We identified 4 association peaks that passed the level of genome-wide significance. In addition to the association with the HLA regions (rs12524487 [OR 3.29, $P = 8.17 \times 10^{-20}$]), 3 genetic associations in non-HLA loci were identified (Figure 1). We detected genetic associations between Takayasu arteritis and *IL6* (rs2069837 [OR 2.07, $P = 6.70 \times 10^{-9}$]), *RPS9/LILRB3* (rs11666543 [OR 1.65, $P = 2.34 \times 10^{-8}$]), and an intergenic locus on chromosome 21q22 that is closest to *PSMG1* (rs2836878 [OR 1.79, $P = 3.62 \times 10^{-10}$]) (Table 1).

Using the imputation approach described above, we found additional genetic variants within these loci that are associated with the risk of Takayasu arteritis (Figure 2) (see Supplementary Figures 2, 3, and 4 and Supplementary Tables 1 and 2, available on the *Arthritis & Rheumatology* web site at <http://online.library.wiley.com/doi/10.1002/art.39035/abstract>). There are a total of 10 and 11 genotyped or imputed SNPs with evidence of at least modest genetic association ($P < 0.05$) that are in linkage disequilibrium ($r^2 > 0.7$) with the

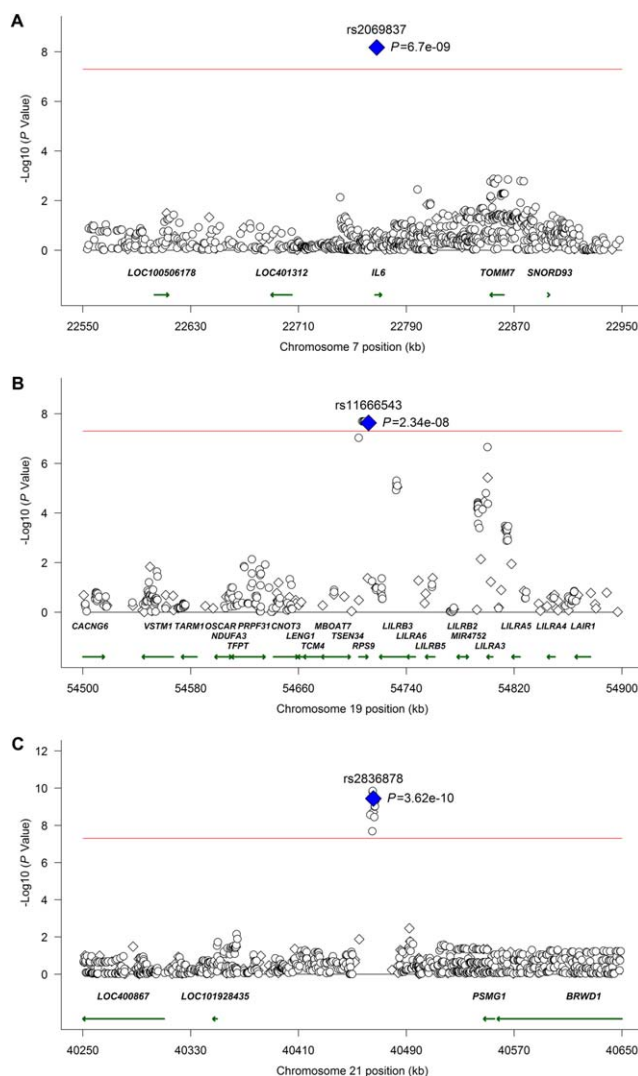


Figure 2. Regional meta-analysis results for genotyped and imputed variants in the Turkish and North American cohorts of patients with Takayasu arteritis. Association results are shown for the *IL6* (A), *RPS9/LILRB3* (B), and chromosome 21q22 (C) loci. Diamonds indicate genotyped variants; circles indicate imputed variants. The red line shows the threshold for a genome-wide level of significance ($P = 5 \times 10^{-8}$).

index SNP rs2836878 in the chromosome 21q22 region in the Turkish and North American cohorts, respectively. The high linkage disequilibrium in this locus precluded localization of this genetic effect to a single genetic variant using conditional regression analysis. However, the linkage disequilibrium structure in this locus, informed by transancestral data from the Turkish and North American cohorts, indicates that this association in chromosome 21q22 is explained by a relatively small region extending from 40,463,283 to 40,466,744 (HG19) located in the intergenic region between *PSMG1* and *LOC101928435* (see Supplementary

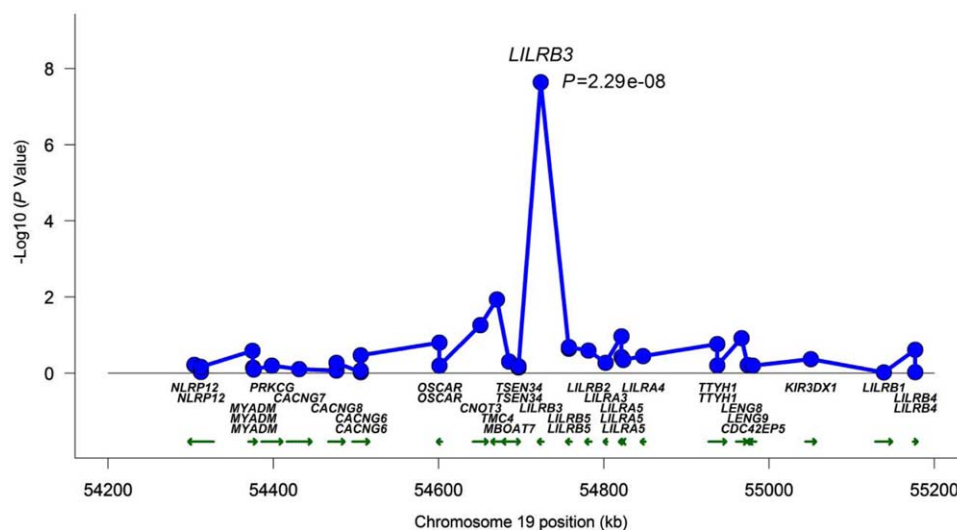


Figure 3. Expression quantitative trait loci association between rs11666543 and chromosome 19q13.4 genes in lymphoblastoid cell lines. The risk allele for Takayasu arteritis (G) was associated with a significant reduction in expression of mRNA for the leukocyte immunoglobulin-like receptor gene *LILRB3*.

Figure 2, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>).

A similar approach was attempted to further localize the novel genetic association we identified in Takayasu arteritis in the *RPS9/LILRB3* locus located on chromosome 19q13.4. This gene-rich locus includes multiple genes in the leukocyte immunoglobulin-like receptor family that are known to be expressed on antigen-presenting cells and other immunocompetent cells and interact with HLA class I. The linkage disequilibrium structure and genetic association results, using genotyped and densely imputed genetic variants in this region, localized the genetic effect tagged by the index SNP in this locus (rs11666543) to a region that includes *RPS9* and *LILRB3* (see Supplementary Figure 3, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>).

Similar to the genetic effect in chromosome 21q22, very high to complete linkage disequilibrium precluded further localization to a single genetic variant. Since this genetic effect is in a gene-rich region, it is possible that the functional effect of the identified genetic variants might extend to other genes on this same locus. Therefore, we performed eQTL analysis to determine if the index SNP in this locus (rs11666543) affects the expression levels of any of the genes or transcripts located within 1 million base pairs upstream and

downstream of this SNP. We detected a significant reduction in the expression of *LILRB3* in lymphoblastoid cell lines in the presence of the risk allele (G) in rs11666543 ($P = 2.29 \times 10^{-8}$) (Figure 3). The risk variant in rs11666543 was also associated with significant down-regulation of *RPS9* and with up-regulation of a long noncoding RNA (lncRNA) (CTB-83J4.1) and the pseudogene *LILRP1* in a whole blood eQTL database (Figure 4). CTB-83J4.1 and *LILRP1* are located ~16 kb and 500 kb from rs11666543, respectively. Taken together, these data suggest that the genetic risk variant tagged by the SNP rs11666543 is a putative functional variant that alters the expression of multiple transcripts within this gene-rich region on chromosome 19q13.4.

We also identified a novel genetic association between *IL6* and Takayasu arteritis (rs2069837) ($P = 1.92 \times 10^{-7}$ for the Turkish cohort, $P = 2.32 \times 10^{-3}$ for the North American cohort, and $P = 6.70 \times 10^{-9}$ for the meta-analysis). This genetic variant located within the second intron of *IL6* was not in linkage disequilibrium with any other variant that we genotyped or imputed in this locus. This is also consistent with the linkage disequilibrium data in HapMap and explains why only a single variant in this genetic locus was identified as a risk variant for Takayasu arteritis. We used ENCODE data to determine if this genetic variant in *IL6* localizes to a regulatory genetic region. We found that rs2069837 in *IL6* overlaps with an

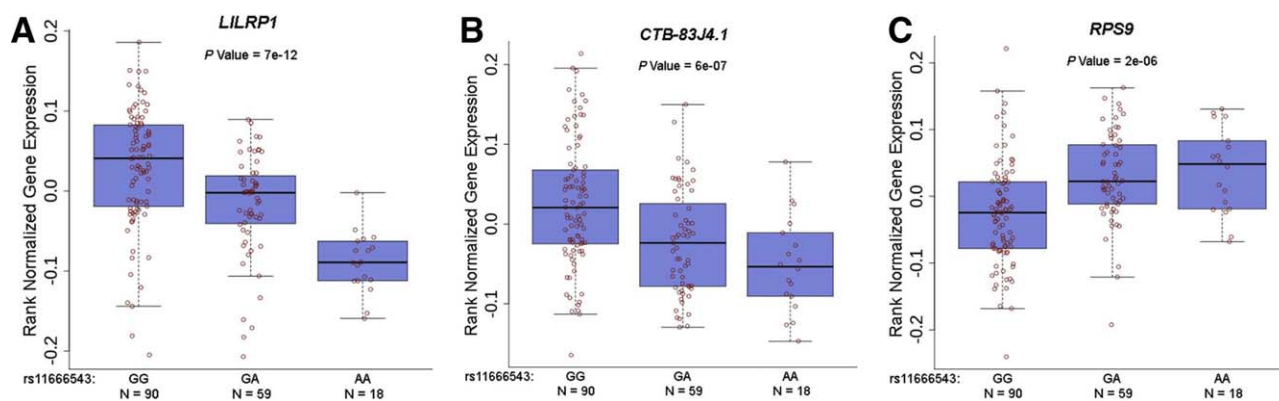


Figure 4. Expression quantitative trait loci associations between rs11666543 and several transcripts in 19q13.4 in whole blood. **A** and **B**, Correlation of the risk allele for Takayasu arteritis (G) in rs11666543 with increased expression of *LILRP1* (**A**) and the long noncoding RNA *CTB-83J4.1* (**B**). **C**, Correlation of the risk allele for Takayasu arteritis (G) in rs11666543 with decreased expression of *RPS9*.

H3K27 acetylated region, indicating that this genetic variant is located within an active enhancer.

In addition to identifying genetic associations in *IL6*, *RPS9/LILRB3*, and chromosome 21q22 with a genome-wide level of significance ($P < 5 \times 10^{-8}$), we identified several novel genetic susceptibility loci for Takayasu arteritis with suggestive evidence of association with the disease ($P < 1 \times 10^{-5}$). These include *PCSK5*, *ZFPM2*, *LOC100289420/FAM19A5*, *LILRA3*, *SLC16A7/LOC100289417*, *PPM1G/NRBP1*, and *PTK2B* (Table 2).

Genetic association results ($P < 1 \times 10^{-5}$) in the 2 independent cohorts are presented in Supplementary Tables 3 and 4, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>.

DISCUSSION

We performed the first unbiased GWAS in Takayasu arteritis and discovered and characterized novel genetic susceptibility loci that predispose to Takayasu arteritis in independent cohorts from Turkey

and North America. We established 3 risk loci for the disease outside of the HLA region with a genome-wide level of significance ($P < 5 \times 10^{-8}$). Two of these loci, *IL6* and *RPS9/LILRB3*, point to important immunoregulatory pathways that could further explain the underlying immunopathology of this large-vessel vasculitis. The third genetic locus we established with a genome-wide level of significance in Takayasu arteritis is located in a region on chromosome 21q22. This same genetic susceptibility locus confers risk for ulcerative colitis and ankylosing spondylitis (22,23), and the risk variant in this locus has recently been shown to increase the expression of 2 novel lncRNA transcripts in this intergenic region (24).

A role of IL-6 in the pathogenesis of Takayasu arteritis has been suspected from previous studies showing increased serum IL-6 levels in patients as compared to healthy controls (25,26). IL-6 plays an important role in regulating multiple aspects of the immune response, including the differentiation of T cells into Th17 cells and Treg cells (27). Previous candidate gene association studies have suggested a modest effect of genetic variants within the promoter region of *IL6* in

Table 2. Genetic variants with suggestive evidence of association with Takayasu arteritis (meta-analysis $P < 1 \times 10^{-5}$)*

SNP	Minor allele	Position	Gene symbol	Gene location	OR	P	Q statistic P
rs6560480	C	Chr9: 78599133	<i>PCSK5</i>	Intron	1.49	9.34×10^{-7}	0.676
rs1113601	G	Chr8: 106338217	<i>ZFPM2</i>	Intron	0.56	1.69×10^{-6}	0.834
rs9615754	T	Chr22: 48479166	<i>LOC100289420/FAM19A5</i>	Intergenic	0.58	3.70×10^{-6}	0.195
rs410852	G	Chr19: 54800371	<i>LILRA3</i>	Intron	1.47	3.74×10^{-6}	0.966
rs7956657	A	Chr12: 60228857	<i>SLC16A7/LOC100289417</i>	Intergenic	1.67	6.13×10^{-6}	0.188
rs11675428	C	Chr2: 27642734	<i>PPM1G/NRBP1</i>	Intergenic	0.54	8.06×10^{-6}	0.316
rs13260543	G	Chr8: 27251325	<i>PTK2B</i>	Intron	0.70	8.97×10^{-6}	0.156
rs7005183	G	Chr8: 27260484	<i>PTK2B</i>	Intron	0.70	9.01×10^{-6}	0.141

* The odds ratio (OR) for the minor allele is shown. SNP = single-nucleotide polymorphism.

Takayasu arteritis (28). While our data do not show evidence of associations with these 2 promoter region variants ($P > 0.05$), we identified a novel genetic association in Takayasu arteritis with a genetic variant located in a regulatory region within the second intron of *IL6*. This genetic variant lies within an experimentally identified active enhancer region, as suggested by the presence of a histone H3K27 acetylation mark within this locus and across multiple cell types. Multiple case reports have suggested successful treatment of refractory Takayasu arteritis with anti-IL-6 receptor monoclonal antibody (tocilizumab) (29).

Our discovery of a genetic risk locus for Takayasu arteritis on the leukocyte receptor complex immunoregulatory gene-rich region of chromosome 19q13.4 uncovers a potentially novel aspect of this disease. This genomic region includes genes encoding for killer cell immunoglobulin-like receptors, leukocyte immunoglobulin-like receptors, and leukocyte-associated immunoglobulin-like receptors (30). Using dense imputation and transancestral mapping, we localized the genetic susceptibility locus for Takayasu arteritis in this region to *RPS9/LILRB3*.

The leukocyte immunoglobulin-like receptor gene family encodes a set of cell surface receptor proteins possessing immunoglobulin domains. Inhibitory receptors in this gene family consist of 2 or 4 extracellular immunoglobulin domains, a transmembrane domain, and cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) (30). *LILRB3* binds to HLA class I antigens and generally provides a negative inhibitory signal to limit an immune response and prevent autoreactivity. Our data indicate that the index SNP in the *RPS9/LILRB3* locus tags a functional genetic variant that regulates multiple genes within this extended region. Specifically, the Takayasu arteritis risk allele in rs11666543 correlates with reduced messenger RNA (mRNA) expression of *RPS9* and *LILRB3*, and increased expression of the pseudogene *LILRP1* located ~500 kb from this SNP. In addition, the risk allele in this locus correlates with increased expression of an lncRNA (CTB-83J4.1) that is ~16 kb away. These data suggest a long-range interaction within this genomic region and a possible chromatin-looping configuration that brings multiple genes spread across this complex region into close proximity to this functional regulatory locus that includes rs11666543 and that confers risk of Takayasu arteritis.

Our eQTL analysis in the chromosome 19q13.4 locus that indicates significant reduction in *LILRB3* expression with the Takayasu arteritis risk allele suggests loss of inhibitory signaling that could result in

enhanced uncontrolled immune activation upon major histocompatibility complex (MHC) class I antigen presentation. It is intriguing that HLA class I is strongly associated with the risk of Takayasu arteritis. Our study was underpowered to establish epistatic interaction between the HLA class I risk locus in Takayasu arteritis (tagged by rs12524487 in *HLA-B/MICA*) and *RPS9/LILRB3* (data not shown). The variant tagging the *RPS9/LILRB3* genetic effect in Takayasu arteritis also alters the expression of mRNA for *RPS9*, which encodes for ribosomal protein S9 and is a component of the 40S ribosomal subunit.

We previously used the ImmunoChip custom-designed genotyping platform and found significant genetic associations with *IL12B* and *FCGR2A/FCGR3A* in Takayasu arteritis (14). The ImmunoChip platform included 196,524 genetic variants and allowed for very dense coverage and genotyping in ~200 genetic loci with a previously reported association in immune-mediated diseases. These same variants in *IL12B* and *FCGR2A/FCGR3A* were not included in the GWAS platform used in this study and could not be imputed and analyzed. The genetic association results with the genotyped variants in these 2 loci in this study are presented in Supplementary Figure 5, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>. Indeed, only one genetic variant analyzed in this study was in linkage disequilibrium with the previously reported risk variant in *IL12B*, and no variant was in linkage disequilibrium with the previously reported risk variant in *FCGR2A/FCGR3A* (see Supplementary Tables 5 and 6, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract>). Therefore, we predict that additional genetic susceptibility loci for Takayasu arteritis will be discovered in future studies when more comprehensive genotyping platforms or sequencing experiments are performed.

In summary, this multiethnic first unbiased GWAS study in Takayasu arteritis established 3 additional genetic susceptibility loci with a genome-wide level of significance for this disease. Our study revealed important novel aspects of the pathogenesis of Takayasu arteritis, and brings the total number of established genetic risk loci with a genome-wide level of significance in this disease to 7. These are the 2 independent MHC loci in HLA class I and class II, *FCGR2A/FCGR3A*, *IL12B*, *IL6*, *RPS9/LILRB3*, and the intergenic locus on chromosome 21q22 near *PSMG1*. Uncovering the genetic basis for Takayasu arteritis has the great potential to lead to a better understanding of the pathogenesis

of the disease and the discovery of novel therapeutic targets.

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.

Study conception and design. Saruhan-Direskeneli, Merkel, Direskeneli, Sawalha.

Acquisition of data. Renauer, Saruhan-Direskeneli, Coit, Adler, Aksu, Keser, Alibaz-Oner, Aydin, Kamali, Inanc, Carette, Cuthbertson, Hoffman, Akar, Onen, Akkoc, Khalidi, Koenig, Karadag, Kiraz, Langford, Maksimowicz-McKinnon, McAlear, Ozbalkan, Ates, Karaaslan, Duzgun, Monach, Ozer, Erken, Ozturk, Yazici, Cefle, Onat, Kisacik, Pagnoux, Kasifoglu, Seyahi, Fresko, Seo, Sreih, Warrington, Ytterberg, Cobankara, Cunnigham-Graham, Vyse, Pamuk, Tunc, Dalkilic, Bicakcigil, Yentur, Wren, Merkel, Direskeneli, Sawalha.

Analysis and interpretation of data. Renauer, Saruhan-Direskeneli, Sawalha.

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