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


**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...
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 \(\text{IL6} (\text{rs2069837})\) (odds ratio [OR] 2.07, \(P = 6.70 \times 10^{-5}\)), \(\text{RSPS9/IL1RB3} (\text{rs11666543})\) (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 \(\text{RSPS9/IL1RB3}\) 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 \(\text{LILRB3}\) (\(P = 2.29 \times 10^{-5}\)). In addition, we identified candidate susceptibility genes with suggestive levels of association (\(P < 1 \times 10^{-5}\)) with Takayasu arteritis, including \(\text{PCSK5, LILRA3, PPM1G/NRBP1, and PTK2B}\).

**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).

---

**Patients and Methods.** 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 \(5 \times 10^{-8}\) 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
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)

![Figure 1](https://example.com/figure1.png)

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

<table>
<thead>
<tr>
<th>Locus/variant</th>
<th>Minor allele</th>
<th>Turkish cohort</th>
<th>North American cohort</th>
<th>Meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case MAF</td>
<td>Control MAF</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>IL6 rs2069837</td>
<td>G</td>
<td>0.10</td>
<td>0.19</td>
<td>0.51 (0.39–0.66)</td>
</tr>
<tr>
<td>RPS9/LILRB3</td>
<td>A</td>
<td>0.19</td>
<td>0.30</td>
<td>0.56 (0.45–0.69)</td>
</tr>
<tr>
<td>21q22 rs11666543</td>
<td>A</td>
<td>0.30</td>
<td>0.40</td>
<td>0.65 (0.54–0.78)</td>
</tr>
<tr>
<td>rs2836878</td>
<td>A</td>
<td>0.19</td>
<td>0.29</td>
<td>0.56 (0.46–0.70)</td>
</tr>
<tr>
<td>rs2836881</td>
<td>T</td>
<td>0.19</td>
<td>0.29</td>
<td>0.57 (0.46–0.70)</td>
</tr>
</tbody>
</table>

* 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 (\( \Delta G \)Turkish = 1.05, \( \Delta G \)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 Haplovie 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 programing 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, \( P = 3.62 \times 10^{-15} \)]) (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 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
A similar approach was attempted to further localize the novel genetic association we identified in Takayasu arteritis in the \textit{RPS9}/\textit{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 \textit{RPS9} and \textit{LILRB3} (see Supplementary Figure 3, available on the Arthritis \& Rheumatology web site at \url{http://onlinelibrary.wiley.com/doi/10.1002/art.39035/abstract}).

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 \textit{LILRB3}.

downstream of this SNP. We detected a significant reduction in the expression of \textit{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 \textit{RPS9} and with up-regulation of a long noncoding RNA (lncRNA) (CTB-83J4.1) and the pseudogene \textit{LILRP1} in a whole blood eQTL database (Figure 4). CTB-83J4.1 and \textit{LILRP1} are located \(\sim 16\) 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 \textit{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 \textit{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 \textit{IL6} localizes to a regulatory genetic region. We found that rs2069837 in \textit{IL6} overlaps with an
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 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 IncRNA 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 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>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>Position</th>
<th>Gene symbol</th>
<th>Gene location</th>
<th>OR</th>
<th>$P$</th>
<th>Q statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6560480</td>
<td>C</td>
<td>Chr9: 78599133</td>
<td>PCSK5</td>
<td>Intron</td>
<td>1.49</td>
<td>9.34 x 10^{-7}</td>
<td>0.676</td>
<td></td>
</tr>
<tr>
<td>rs1113601</td>
<td>G</td>
<td>Chr8: 106338217</td>
<td>ZFPM2</td>
<td>Intron</td>
<td>0.56</td>
<td>1.69 x 10^{-6}</td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>rs9615754</td>
<td>T</td>
<td>Chr22: 48479166</td>
<td>LOC100289420/FAM19A5</td>
<td>Intron</td>
<td>0.58</td>
<td>3.70 x 10^{-6}</td>
<td>0.195</td>
<td></td>
</tr>
<tr>
<td>rs410852</td>
<td>G</td>
<td>Chr19: 54800371</td>
<td>LILRA3</td>
<td>Intron</td>
<td>1.47</td>
<td>3.74 x 10^{-6}</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>rs7956657</td>
<td>A</td>
<td>Chr12: 60228857</td>
<td>SLC16A7/LOC100289417</td>
<td>Intergenic</td>
<td>1.67</td>
<td>6.13 x 10^{-6}</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td>rs11675428</td>
<td>C</td>
<td>Chr2: 27642734</td>
<td>PPM1G/NRBP1</td>
<td>Intergenic</td>
<td>0.54</td>
<td>8.06 x 10^{-6}</td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>rs13260543</td>
<td>G</td>
<td>Chr8: 27251325</td>
<td>PTK2B</td>
<td>Intron</td>
<td>0.70</td>
<td>8.97 x 10^{-6}</td>
<td>0.156</td>
<td></td>
</tr>
<tr>
<td>rs7005183</td>
<td>G</td>
<td>Chr8: 27260484</td>
<td>PTK2B</td>
<td>Intron</td>
<td>0.70</td>
<td>9.01 x 10^{-6}</td>
<td>0.141</td>
<td></td>
</tr>
</tbody>
</table>

* 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 autoactivity. 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 \(\sim 500\) kb from this SNP. In addition, the risk allele in this locus correlates with increased expression of an lncRNA (CTB-83J4.1) that is \(\sim 16\) 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 \(HLA–B\) or \(MICA\)) 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 \(\sim 200\) 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 multietnic 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.


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

REFERENCES