Global Transcriptomic Profiling Identifies Differential Gene Expression Signatures Between Inflammatory and Noninflammatory Aortic Aneurysms

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Objective. To identify hallmark genes and biomolecular processes in aortitis using high-throughput gene expression profiling, and to provide a range of potentially new drug targets (genes) and therapeutics from a pharmacogenomic network analysis.

Methods. Bulk RNA sequencing was performed on surgically resected ascending aortic tissues from inflammatory aneurysms (giant cell arteritis [GCA] with or without polymyalgia rheumatica, n = 8; clinically isolated aortitis [CIA], n = 17) and noninflammatory aneurysms (n = 25) undergoing surgical aortic repair. Differentially expressed genes (DEGs) between the 2 patient groups were identified while controlling for clinical covariates. A protein–protein interaction model, drug–gene target information, and the DEGs were used to construct a pharmacogenomic network for identifying promising drug targets and potentially new treatment strategies in aortitis.

Results. Overall, tissue gene expression patterns were the most associated with disease state than with any other clinical characteristic. We identified 159 and 93 genes that were significantly up-regulated and down-regulated, respectively, in inflammatory aortic aneurysms compared to noninflammatory aortic aneurysms. We found that the up-regulated genes were enriched in immune-related functions, whereas the down-regulated genes were enriched in neuronal processes. Notably, gene expression profiles of inflammatory aortic aneurysms from patients with GCA were no different than those from patients with CIA. Finally, our pharmacogenomic network analysis identified genes that could potentially be targeted by immunosuppressive drugs currently approved for other inflammatory diseases.

Conclusion. We performed the first global transcriptomics analysis in inflammatory aortic aneurysms from surgically resected aortic tissues. We identified signature genes and biomolecular processes, while finding that CIA may be a limited presentation of GCA. Moreover, our computational network analysis revealed potential novel strategies for pharmacologic interventions and suggests future biomarker discovery directions for the precise diagnosis and treatment of aortitis.

INTRODUCTION

The etiology and pathogenic mechanisms leading to noninfectious inflammation of the aortic wall (aortitis) remain largely unknown. In clinical practice, distinguishing patients with inflammatory aortic aneurysms from those with noninflammatory aortic aneurysms may be difficult, since aortitis may be asymptomatic or associated with nonspecific symptoms (1,2). Moreover, there are currently no laboratory diagnostic markers specifically for aortitis or for 2 of its most common underlying conditions, i.e., giant cell arteritis (GCA) and clinically isolated aortitis (CIA) (1,3,4). Therefore, the unmet need for patients with aortitis include the discovery of novel biomolecular features that stratify inflammatory and noninflammatory aortic aneurysms and thereby complement current diagnostic approaches and improve long-term treatment outcomes.
Previously, Quimson et al investigated the histopathologic and radiologic differences between inflammatory and noninflammatory aortic aneurysms in patients who underwent open aortic aneurysm repair (2). Their study uncovered 5 factors (i.e., age at the time of surgery, sex, absence of coronary artery disease, diameter of the aneurysms, arterial wall thickening) that were associated with aortitis. Moreover, the investigators found that, among patients who underwent open surgical repair of aortic aneurysms (inflammatory or noninflammatory), elderly women with no history of coronary artery disease and aortic wall thickening were more likely to have histologic evidence of aortitis.

Despite the significance of previous observations by others, our understanding of aortitis can be advanced further by identifying disease-associated biomolecular processes using high-throughput technologies. To this point, genome-wide expression analyses with RNA sequencing (RNA-seq)—which have yet to be performed in aortitis—provide a promising avenue for subsequent studies (5).

In this study, we performed, for the first time, global transcriptomic profiling using RNA-seq on surgically resected inflammatory and noninflammatory aortic aneurysms in order to reveal differences in their tissue gene expression. Our approach demonstrates the utility of bulk transcriptomic sequencing for the discovery of not only signature genes and cellular functions of aortitis, but also potentially novel therapeutic targets.

PATIENTS AND METHODS

Subject and aortic specimen identification. Subjects in whom thoracic aortic replacement was performed between January 1, 2012 and December 31, 2019 were identified retrospectively through the use of current procedural terminology (CPT) coding. All aortic specimens had been previously reviewed by a vascular histopathologist. Inclusion criteria for aortitis samples was a description of “active giant cell aortitis” in the resected ascending aortic tissue. Charts were manually reviewed, and patients with features suggestive of localized or systemic infection were excluded. Age- and sex-matched comparators were identified among patients with noninflammatory aortic aneurysm resection during the same study period. Clinical parameters, including erythrocyte sedimentation rate, C-reactive protein, historical use of glucocorticoids, glucocorticoid dose at time of surgery, presence of systemic symptoms, history of other rheumatic diseases, smoking history, and use of aspirin, statin and angiotensin-converting enzyme (ACE)/angiotensin II receptor blocker (ARB) medications, were abstracted for both inflammatory aortic aneurysm (aortitis) and noninflammatory aortic aneurysm comparators. The clinical and demographic characteristics of the study participants are summarized in Table 1.

This study was approved by the Mayo Clinic Institutional Review Board (no. 17-010612MJK) in accordance with the Declaration of Helsinki. All methods and procedures were performed in accordance with the Mayo Clinic Institutional Review Board guidelines and regulations.

**Formalin-fixed paraffin-embedded (FFPE) block sectioning and aortic tissue preparation.** FFPE blocks containing ascending aortic aneurysm tissues were cut into 10-μm thick sections. Prior to cutting, the microtomes and workstations were cleaned to prevent DNase and RNase contamination. New blades were used between blocks. The flotation bath contained Milli-Q water (DNase- and RNase-free) and was cleaned between blocks. CitriSolv was used to remove paraffin. Tissues were then washed with absolute ethanol and dried with a heat block at 37°C.

**RNA purification, library preparation, and sequencing.** Once dry, the tissues were added with buffer PKD and proteinase K, and placed in a QIAcube for the RNA purification (extraction). On the instrument, the wash buffers used were red blood cell, RPE, and ethanol. RNA was then eluted in
RNase-free water. The quality control of the total RNA was performed by the Qubit and Agilent 2100 Bioanalyzer. DV200 values (the percentage of RNA fragments >200 nucleotides) were determined by 2100 expert software. Samples with DV200 values above 30% were used for library preparation. Libraries were prepared using a TruSeq RNA Exome Capture kit (Illumina) following the manufacturer’s protocol with minor modifications. Briefly, 500 ng of FFPE RNA was used for synthesizing the first-strand complementary DNA (cDNA) at 42°C, and the second-strand cDNA was generated at 16°C for 1 hour with a second-strand marking buffer. Double-stranded cDNA was A-tailed, ligated with index adapters, and amplified over 15 cycles. The cDNA library was quantified using Qbit (ThermoFisher Scientific) and Agilent TapeStation D1000, and 200 ng of each library was pooled for exome enrichment and capture. The pooled library was amplified over 10 cycles after finishing the second enrichment. The final libraries were quantified using an Agilent TapeStation D1000 and Qubit double-stranded DNA broad range assay kit. Finally, the 101-bp, paired-end reads were sequenced on an Illumina HiSeq4000 platform. Importantly, samples of inflammatory and noninflammatory aortic aneurysms were not sequenced separately, thus negating the need for batch correction protocols.

Pre-processing and aligning RNA-seq data. FASTQC was used to estimate the quality of the generated paired-end reads (.fastq files). No files were reported to have a flag of poor sequence quality. Paired-end raw reads were trimmed by trimomatic (version 0.38) (6) with the following parameter: ILLUMINA-CLIP:TruSeq3-PE:2:30:10. Next, STAR (version 2.5.4b) (7) was used to align the trimmed paired-end reads on the human reference genome (hg38). RSEM (version 1.3.1; -star-sjidboverhang 100) (8) was used to calculate transcripts per million (TPM) from the .bam files generated by STAR. Gene annotations for hg38 were retrieved from the UCSC Genome Browser.

Investigation of global transcriptome variance. Transcriptomes composed of log2-transformed TPM (with a pseudocount addition of 0.001) values of 26,475 genes from 50 samples (25 with inflammatory aortic aneurysms and 25 with noninflammatory aortic aneurysms) were projected onto a principal component analysis (PCA) ordination plot. Agglomerative hierarchical clustering (Euclidean distance, complete-linkage) was performed on the gene expression profiles (n = 50) to observe clusters associated with clinical and demographic characteristics (i.e., inflammatory status of aortitis, ACE/ARB use, aspirin use, sex, history of other rheumatic diseases, smoking history, and statin use).

Identification of clinical covariates. A logistic linear regression model was used to identify clinical covariates associated with inflammatory and noninflammatory aortic aneurysms. The inflammatory status (inflammatory or noninflammatory) was used as the response variable, while the predictors (i.e., sex, smoking history, age, aspirin use, statin use, ACE/ARB use, history of other rheumatic diseases) were individually assessed. P values were retrieved for the corresponding regression coefficient of the predictor variables. Predictors with P values less than 0.1 were considered as potential confounders and were adjusted for during the identification of differentially expressed genes (DEGs).

Identification of DEGs. DESeq2 (version 1.26.0) (9) was used to identify DEGs between patient groups with inflammatory aortic aneurysms (n = 25) and noninflammatory aortic aneurysms (n = 25), those with aortitis with GCA/polymyalgia rheumatica (PMR) (documented diagnosis or clinical features compatible with GCA and/or PMR, n = 8), and those with CIA (inflammatory aortitis without diagnosis of, or clinical features compatible with, either GCA or PMR, n = 17). Of note, statin use, ACE/ARB use, and history of other rheumatic diseases were considered as potential confounders (P < 0.1, coefficient of the logistic regression model) and were adjusted for during the identification of up-regulated DEGs (log2[fold change] >2; Benjamini-Hochberg-adjusted P < 0.01) and down-regulated DEGs (log2[fold change] <−2; Benjamini-Hochberg-adjusted P < 0.01). All DEG analysis results are summarized in Supplementary Table 1, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138.

Functional annotation and gene set enrichment analysis. The up-regulated and down-regulated DEGs were analyzed using PANTHER (version 16.0) (10) for functional annotations and DAVID (version 6.8) (11) for gene set enrichment analysis. PANTHER was used to obtain the gene protein class and biological pathway annotation, and DAVID was used to obtain statistically enriched Gene Ontology (GO) terms (12) from the following categories: Biological Process (GOTERM_BP_FAT), Cellular Component (GOTERM_CC_FAT), and Molecular Function (GOTERM_MF_FAT). GO terms with Expression Analysis Systematic Explorer scores (i.e., a P value from a modified Fisher’s exact test [11]) less than 0.05 were considered to be statistically enriched.

Construction of the pharmacogenomic network. A pharmacogenomic network was constructed with human protein–protein interaction (PPI) information from the STRING database (version 11) (13) and drug–gene interaction information from the Drug Gene Interaction Database (version 4.0) (14). First, a DEG-specific interactome was constructed by mapping the aforementioned up-regulated and down-regulated DEGs to the “high-confidence” (combined score >0.7 in STRING) PPI network. From this high-confidence PPI network, the following nodes and edges were discarded during network construction: nodes (genes and proteins were considered as equals) that were...
not mapped by DEGs, and edges (interaction between 2 different nodes) that do not connect 2 different DEGs. As a result, an interactome comprising 71 nodes (DEGs) and 122 edges was constructed (Supplementary Figure 1, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138). From this, the largest connected component (LCC) of the interactome was identified, leaving 36 nodes and 92 edges. Finally, the Drug Gene Interaction Database was used to create a pharmacogenomic network by linking US Food and Drug Administration (FDA)–approved drugs that are known to target (directly or indirectly) any of the corresponding nodes of the LCC.

Data availability. Source codes and data sets that were used in this study are available at: https://github.com/jaeyunsung/Aortitis_2022.

RESULTS

Genome-wide expression profiles of inflammatory and noninflammatory aortic aneurysms. Figure 1A illustrates our analysis strategy to identify differential gene expression signatures between inflammatory and noninflammatory aortic aneurysms from 50 surgically resected aortic tissue samples. We first investigated whether the clinical characteristics (i.e., disease condition, ACE/ARB use, aspirin use, statin use, sex, history of other rheumatic diseases, smoking history) cluster according to the gene expression profiles acquired from the FFPE tissues. Using PCA (Figure 1B) and hierarchical clustering (Figure 1C), we observed that gene expression profiles of 26,475 genes were the most distinguishable based upon disease condition (inflammatory or noninflammatory aortic aneurysm) compared to other clinical characteristics.

Next, we examined whether there were any statistical associations between disease condition and other clinical characteristics to identify potential confounders in our study. Using logistic regression, we identified ACE/ARB use ($P = 0.048$), statin use ($P = 0.093$), and the history of other rheumatic diseases ($P = 0.099$) as being associated with disease condition. Henceforth, these clinical characteristics were considered as confounding variables while investigating the relationship between inflammatory/noninflammatory aortic aneurysm and global gene expression. In contrast, aspirin use ($P = 0.396$), sex ($P = 0.556$), and smoking history ($P = 0.260$) were considered not to be associated with disease condition.

Figure 1. Data analysis pipeline and unsupervised clustering results on genome-wide expression (transcriptome) profiles of inflammatory and noninflammatory aortic aneurysms. A, Study design to investigate transcriptomic differences between inflammatory and noninflammatory aortic aneurysms. B, Principal components analysis (PCA) of gene expression profiles (26,475 total genes) from 50 surgically resected ascending aortic tissue samples across 2 patient groups (inflammatory aortic aneurysm, n = 25; noninflammatory aortic aneurysm, n = 25). C, Hierarchical clustering on all 50 gene expression profiles, showing samples clustering together by disease condition (inflammatory/noninflammatory aortic aneurysms) more than by any other clinical characteristic (i.e., angiotensin-converting enzyme [ACE]/angiotensin II receptor blocker [ARB] use, aspirin use, sex, history of rheumatic disease [RD], smoking history, and statin use). Heatmap of gene expression profiles is not shown due to space constraints.
Identification of DEGs between inflammatory and noninflammatory aortic aneurysms. We found transcriptomic differences between inflammatory and noninflammatory aortic aneurysms while adjusting for the aforementioned clinical covariates. From 26,475 total genes, we identified 159 up-regulated genes in inflammatory aortic aneurysms, including CXCL9, TEX28, CLEC5A, and OR8B2, and 93 down-regulated genes, including PLD5, SFRP1, CARTPT, and FAR2P1 (Figure 2A). Among the 159 up-regulated genes, 99 mapped onto PANTHER protein classes, including “metabolite interconversion enzyme” (24 of 99 [24.2%]), “defense/immunity protein” (13 of 99 [13.1%]), and “intercellular signal molecule” (12 of 99 [12.1%]) (Figure 2B). Additionally, 29 up-regulated genes mapped onto PANTHER biological pathways, of which “inflammation mediated by chemokine and cytokine signaling pathway” (6 of 29 [20.7%]) was the most abundant annotation (Figure 2C). Alternatively, among the 93 down-regulated genes, 50 genes mapped onto PANTHER protein classes, including “transporter” (13 of 50 [26%]), “transmembrane signal receptor” (7 of 50 [14%]), and “cell adhesion molecule” (5 of 50 [10%]) (Figure 2D). Finally, 56 of the down-regulated genes mapped onto PANTHER biological pathways, of which “Wnt signaling pathway” (6 of 56 [10.7%]) was the most abundant annotation (Figure 2E). P values and fold change for all genes are listed in Supplementary Table 1. Full details of our functional classification results are summarized in Supplementary Tables 2–5, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138.

No significant differences in tissue gene expression displayed between GCA/PMR and CIA. GCA is typically associated with various clinical presentations, such as headache, temporal artery abnormalities, elevated markers of inflammation, and PMR (4,15). Meanwhile, patients with CIA are generally asymptomatic, and aortitis is often incidentally identified within histopathology (16). Interestingly, however, inflammatory aortic aneurysms of GCA and CIA are radiographically and histopathologically indistinguishable. Moreover, it remains unclear whether CIA is truly isolated to the aorta or represents a subclinical systemic vasculitis. As there has not been any investigation into the gene expression differences between GCA and CIA, we sought to compare transcriptomes of aortic tissue resections between these 2 clinical phenotypes. Strikingly, there were no significant differences in gene expression profiles between GCA/PMR and CIA (Benjamini-Hochberg-adjusted P < 0.1) (Supplementary Table 6, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138), indicating that CIA may be pathophysiologically closely related to GCA.

Functional enrichment of DEGs between inflammatory and noninflammatory aortic aneurysms. Having identified DEGs and their protein class and biological pathway annotations, we next investigated whether the up- and down-regulated genes display statistically significant enrichment in GO terms. Among the 159 up-regulated genes, we identified 228 enriched GO terms (Supplementary Table 7, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138).
wiley.com/doi/10.1002/art.42138), the top 10 of which were all associated with immune response (Figure 3A), as can be expected given the nature of the disease being studied. Interestingly, several GO terms were of the response to microbial agents (e.g., “cellular response to interferon-gamma,” “response to molecule of bacterial origin,” and “defense response to bacterium”) (Supplementary Table 7).

The top 10 GO terms shared a considerable number of genes which could be driving the robust immune signature (Figure 3B). In particular, we identified 19 genes common to the top 3 GO terms (i.e., “immune response,” “defense response,” “inflammatory response”) enriched in the up-regulated DEGs (Supplementary Figures 2A–C, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138). Several of these genes were previously reported to be associated with autoimmune or inflammatory diseases. One example is IL23R, which encodes for the receptor of a key proinflammatory cytokine (interleukin-23 [IL-23]) that stimulates the proliferation of Th17 cells in inflammatory diseases (17). IL23R is located upstream of the JAK/STAT signaling pathway, which has been implicated in the pathogenesis of several inflammatory and autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis (15).

Similarly, we identified an up-regulation of IL1A, which encodes for a proinflammatory cytokine (IL-1α) that can cause severe acute or chronic inflammation when dysregulated (18). The role of IL1A in human inflammatory aortic aneurysms is not fully understood; however, it has been shown in a mouse model that Il1a deficiency can be protective against the formation of Kawasaki disease-associated abdominal aortic aneurysm (19).

In addition, we identified an up-regulation of CCR6, which encodes for a receptor that can mediate the recruitment of immature/mature dendritic cells and other antigen-presenting cells (20). Immune cells that express CCR6 (such as CCR6+ T cells) have been reported to populate the wall infiltrate in GCA patients and can cause injury to vascular smooth muscle cells (21).

Meanwhile, from the 93 genes down-regulated in inflammatory aortic aneurysm, we identified 206 enriched GO terms (Supplementary Table 8, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138). The top 10 of these were mostly associated with neuronal activities (Figure 3C and Supplementary Figures 2D–F), which may possibly reflect an elevated presence of damaged neurons in the aorta resulting from sustained levels of inflammation. Notably, we observed that these top GO terms shared far fewer genes among each other than the top GO terms enriched in the

![Figure 3](image-url)

**Figure 3.** Gene Ontology (GO) enrichment analysis revealed strong up-regulation of immune response and down-regulation of neuronal activity in inflammatory aortic aneurysms. A and B, Top 10 enriched GO terms of the up-regulated DEGs (n = 159) (A) and a network of their gene set similarities (B). C and D, Top 10 enriched GO terms of the down-regulated DEGs (n = 93) (C) and a network of their gene set similarities (D). The size of nodes in the network corresponds to the number of genes of each GO term. The width of the edge represents the number of genes common to both GO terms. Colors of nodes (pink to red) signify the statistical significance of GO enrichment. Gene set enrichment analysis of GO terms was performed in DAVID (version 6.8).
up-regulated genes (Figure 3D). Moreover, we only identified 1 common gene \((\text{ATP1A2})\) across the top 3 GO terms (i.e., “integral component of plasma membrane,” “neurotransmitter transport,” “synapse”), which encodes an \(\alpha_2\)-subunit of the sodium/potassium pump primarily found in glial cells (22). To date, the role of \(\text{ATP1A2}\) in inflammatory diseases remains unclear, although it has been suspected that this gene may be involved in neuroinflammatory processes (23).

**Differential expression of genes targeted by aortitis treatment drugs.** We next investigated known drug targets (i.e., genes) in aortitis. Specifically, we focused on the expression of genes known to be targeted by JAK inhibitors, IL-6 inhibitors, Th1/Th17 inhibitors, prednisone, and methotrexate (Figure 4). Of note, none of these drugs except for prednisone \((n = 1)\) were administered to our study population.

Baricitinib, tofacitinib, and ruxolitinib are JAK inhibitors that can target members of the JAK/STAT signaling pathway. Drugs that inhibit the JAK/STAT signaling pathway can potentially suppress vascular inflammation by reducing the activity of vascular dendritic cells and T cells (15,24–26). Interestingly, we identified \(\text{JAK1}\) and \(\text{JAK3}\) to be up-regulated in inflammatory aortic aneurysms, which further supports the utility of JAK inhibitors for the treatment of aortitis. However, \(\text{JAK2}\) was not identified to have differential expression between inflammatory and noninflammatory aortic aneurysms.

The IL-6 inhibitors sirukumab and tocilizumab target the proinflammatory cytokine IL-6 and its receptor (IL-6R), respectively. This blocks the binding of IL-6 to IL-6R and thereby reduces the recruitment of new macrophages (27). The up-regulation of \(\text{IL6R}\) found in our study supports the use of tocilizumab for aortitis treatment (27). On the other hand, we did not identify differences in the expression of \(\text{IL6}\) in resected tissues between inflammatory and noninflammatory aortic aneurysms.

Ustekinumab and abatacept are Th1/Th17 inhibitors that suppress the activation of T cells by blocking proinflammatory cytokines (i.e., IL-12, IL-23) and Th1/Th17 membrane receptors (CD80, CD86). Our study identified genes that encode for the targets of abatacept (CD80 and CD86) to be up-regulated in inflammatory aortic aneurysms. This finding is consistent with the use of abatacept to reduce the activity of T cells by interrupting the communication between Th1/Th17 and antigen-presenting cells in aortitis (28). Likewise, among the proinflammatory cytokines targeted by ustekinumab, \(\text{IL23A}\) was found to be up-regulated in inflammatory aortic aneurysms. Ustekinumab has been evaluated in patients with GCA and appears to be of limited benefit (29).

Last, the known gene targets of prednisone and methotrexate were not found to be differentially expressed in inflammatory aortic aneurysms. This result may help to confirm the relatively limited efficacy of these two drugs reported for aortitis (30,31).

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**Figure 4.** Transcriptomics analysis confirmed higher expression of a subset of known target genes in aortitis. Among the known targets of aortitis immunosuppressive drugs (e.g., JAK inhibitors, interleukin-6 inhibitors, Th1/Th17 inhibitors, prednisone, and methotrexate), 6 genes \((\text{JAK1, JAK3, IL6R, IL23A, CD80, and CD86})\) were found to display differential expression between inflammatory and noninflammatory aortic aneurysms. Square and circle nodes indicate drug names and gene symbols, respectively. Interactions (edges) with a hammerhead indicate inhibition. The color (blue to red) of each node represents gene expression fold change between inflammatory and noninflammatory aortic aneurysms. BH = Benjamini-Hochberg.
Identification of potential drug targets through pharmacogenomic network analysis. Global transcriptomic profiling has been well demonstrated for drug target discovery (32), and several studies have coupled gene expression profiles with various systematic approaches for drug repurposing or biomarker discovery (32,33). Among various strategies for novel drug target identification, the network-based framework uses curated network topology (e.g., PPI network, metabolic network) to investigate the association across genes (or their products), diseases, and drugs (34). In this sense, we constructed a pharmacogenomic network composed of 36 genes and 92 interactions (representing the LCC; see Supplementary Figure 1, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138, for the full interactome network) by integrating DEGs, a high-confidence PPI network, and drug–gene interactions. Our pharmacogenomic network identified 10 potentially new druggable gene targets in aortitis: BLK, CNR2, CR2, GZMB, IDO1, IFNG, IL1A, CXCL10, CXCL13, and S1PR5 (Figure 5).

Near the hub of the pharmacogenomic network, we identified up-regulated genes for cytokines (IL1A, IFNG) and chemokines (CXCL5, CXCL9, CXCL10, CXCL11, CXCL13, CCL1, CCL7, CCL20) that can be targeted by drugs already known for the treatment of aortitis or other inflammatory diseases. For example, our results suggest that drugs targeting IFNG (e.g., methylprednisolone, prednisone, cisplatin) and CXCL10 (e.g., atropine, zidovudine, atorvastatin) can be potentially used for the treatment of aortitis. Notably, interferon-γ (IFNγ) (product of IFNG), which is produced by effector Th1 cells, is considered...
as one of the key cytokines involved in the pathogenesis of GCA (35). In addition, CXCL10, which is an IFN-stimulated gene that responds to different types of IFNs (i.e., IFNa, IFNβ, IFNγ, IFNδ), encodes a chemokine that promotes the recruitment of CD8+ T cells and contributes to the production of antibodies (40).

Of note, Corbera-Bellalta et al performed an ex vivo experiment in cultured GCA arteries showing reduced expression of CXCL9, CXCL10, and CXCL11 by blocking endogenous IFNγ with A6-abrogated STAT1 phosphorylation (35). Furthermore, CXCL13, which is a chemokine that attracts B cells and contributes to the production of antibodies (37), may be potentially targeted with rituximab in aortitis. Rituximab is an anti-CD20 monoclonal antibody designed to target the surface of B cells and block interactions with effector T cells (38); preliminary studies have demonstrated promising results for rituximab in GCA (39). In summary, our pharmacogenomic network analysis identified promising drug targets for potentially new treatment strategies in aortitis. We summarize all gene–gene and drug–gene interactions found within our pharmacogenomic network in Supplementary Table 9, available on the Arthritis & Rheumatology website at https://onlinelibrary.wiley.com/doi/10.1002/art.42138. In addition, a comprehensive summary of the enriched functions (GO terms) of all genes in the pharmacogenomic network is provided in Supplementary Table 10.

**DISCUSSION**

In this study, we performed a genome-wide expression analysis to identify signature genes and biomolecular processes underlying aortitis. First, we compared gene expression profiles between inflammatory and noninflammatory aortic aneurysms. Within inflammatory aortic aneurysms, we compared profiles between GCA/PMR and CIA. Notably, this study revealed for the first time that the transcriptomic signature of CIA is not different from that of aortitis related to GCA/PMR, suggesting that these 2 disease states largely share common pathophysiologic mechanisms. Next, we investigated the functional annotations (i.e., protein class, biological pathway) and enriched GO terms of the DEGs. We found that the up-regulated genes were generally enriched in immune processes, and in particular those in response to microbial agents. In contrast, the down-regulated genes were enriched in neuronal processes. Finally, a pharmacogenomic network-based approach revealed a range of potentially new drug targets (genes) and therapeutics for the multimodal treatment of aortitis. The findings described herein motivate future research using multiomics data or peripheral blood to investigate the broad landscape of biomolecular pathways and networks in aortitis, as well as advancing biomarker discovery (40), as we have demonstrated in another autoimmune disease (41,42).

Interestingly, a subset of the DEGs (Benjamini–Hochberg-adjusted \( P < 0.05 \)) were associated with receptors for pathogen-associated molecular patterns. It is yet unclear whether infectious agents play a causal role in blood vessel inflammation, and there has been no compelling evidence showing that abating aortitis symptoms are linked to the clearance of infections (43). Nevertheless, our study identified up-regulation of the following: viral single-stranded RNA–specific endosomal pattern recognition receptors (TLR7, TLR8); other Toll-like receptors (TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8) and nucleotide-binding oligomerization domain–like receptors (NOD1, NOD2, NLRC4); pattern recognition receptor pathways that stimulate type 1 IFN production (MYD88, IRAK4, Traf3, IkBkB, IFR5); members of the IFN/JAK/STAT pathway (IFNAR1, IFNAR2, IL10RB, IFNGR1, IFNGR2, JAK1, TYK2, STAT1, STAT2, IRF9); IFN regulatory factors (IRF1, IRF2, IRF4, IRF5, IRF8, IRF9); mature dendritic cell markers (CD80, CD83, CD86); and cell surface markers of plasmacytoid dendritic cells (CLEC4C, CCR7, LILRB4, NRP1), which are a subtype of dendritic cells that specifically sense viral RNA and DNA (44).

By providing a system-wide view of mechanistic gene (protein) interactions, our pharmacogenomic network analysis can facilitate the design of novel pharmacologic intervention strategies. For example, our analysis identified CD79A (immunoglobulin-associated alpha), which encodes a component of B cell antigen receptors, as a putative target in aortitis treatment. The product of CD79A is a highly reliable marker for B cells that is present on the cell surface throughout their life cycle (45). Although the efficacy of targeting B cells in aortitis has yet to be clearly and convincingly demonstrated, the role of B cells in aortitis is gradually being revealed (15). For example, van der Geest et al reported changes in the distribution and homeostasis of B cells in GCA (46). Additionally, a B cell–activating factor in GCA patients was shown to directly correlate with disease activity (47). In consideration of these findings, we can hypothesize that B cell suppressors may be beneficial for aortitis patients.

During the preparation of this manuscript, a transcriptomics study in large-vessel GCA was published by Vieira et al (48). The investigators used microarray technology on aortic tissues from patients with GCA (\( n = 10 \)) and controls (\( n = 9 \)). Like our results above, up-regulated gene sets for pathways involving interferons, JAK/STAT signaling, and proinflammatory cytokines and chemokines in GCA (compared to controls) were found. The authors also identified higher expression of members of the JAK/STAT signaling pathway (e.g., STAT1, STAT2) and type I–specific IFN response genes (e.g., Epsilont11) in inflamed aortic aneurysms in GCA. Therefore, our study using high-resolution transcriptome profiling by RNA-seq demonstrated favorable reproducibility of previous findings by others and enabled novel insights including the discovery of other possible DEGs, the finding that GCA and CIA are not different at the gene expression level, and new (albeit putative) targets of current aortitis treatment drugs (e.g., JAK1 inhibitors, Th1/Th17 inhibitors) and FDA-approved off-label drugs.
We note a few limitations of this study. First, we acknowledge that 25 cases (inflammatory aortic aneurysms) and 25 controls (noninflammatory aortic aneurysms) are a relatively small number of samples. Nevertheless, we were still able to identify a large number of statistically significant genes even after multiple hypothesis correction; this indicates that despite the limited sample sizes, global gene expression differences between inflammatory and noninflammatory aortic aneurysms are sufficiently robust. Second, RNA-seq only allows us to observe the biomolecular processes within inflammatory aortic aneurysms at the gene expression level. Integrating our current findings with laboratory tests (e.g., real-time quantitative polymerase chain reaction, immunohistochemistry staining) or other omics data, such as metabolomics, proteomics, and single-cell RNA-seq or mass cytometry (CyTOF), in future studies can confirm our results or elucidate additional details of the inherent biological processes in aortitis.

Third, the current protein interactome network may not fully reflect the associations between the identified DEGs during the construction of the pharmacogenomic network. The stringent cutoff used to obtain the high-confidence network may lead to a loss in interactions between genes and therefore missed opportunities to identify additional potential targets of known drugs. Nevertheless, the constructed pharmacogenomic network still identified genes that can be targeted by drugs conventionally prescribed for aortitis (e.g., prednisone) or other inflammatory diseases (e.g., rituximab). Last, our study used only FFPE samples from tissue biopsies but did not investigate immune cells in circulation. Future immunophenotyping studies conducted with patient blood samples are needed to explore how the peripheral immune system is altered during disease onset and progression (49,50).

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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. Sung 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. Warrington, Sung.

Acquisition of data. Koster, Jang, Weyand, Warrington, Sung.

Analysis and interpretation of data. Hur, Koster, Jang, Weyand, Warrington, Sung.

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