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# Genome-Wide DNA Methylation Study Reveals Specific Signatures in the Affected Arterial Tissue of Patients With Giant Cell Arteritis

Gonzalo Borrego-Yaniz,[1] Ana Márquez,[1] Elkyn Estupiñán-Moreno,[2] Laura C. Terrón-Camero,[3] Miguel A. González-Gay,[4,5] Santos Castañeda,[6] Giuliana Guggino,[7] David Saadoun,[8] Pietro Lio,[9] Simona Fontana,[10] Martina Bonacini,[11] Alessandro Rossi,[11] Alberto Cavazza,[12] Francesco Muratore,[13,14] Carlo Salvarani,[13,14] Nicolo Pipitone,[14] Javier Martin,[1] Stefania Croci,[11] and Lourdes Ortiz-Fernández[1]

**Objective.** Giant cell arteritis (GCA) is a large-vessel vasculitis, potentially causing complications such as blindness and strokes. This study aims to gain insights into the pathogenesis of GCA by identifying specific DNA methylation signatures in the arterial tissue of patients with this vasculitis.

**Methods.** DNA methylation profiling was analyzed in 79 temporal artery biopsy samples (69 patients with GCA and 10 controls) by performing an epigenome-wide association study (EWAS). Differential analysis was performed to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs). Lastly, we compared our findings with previous transcriptomics and epigenomics studies on GCA-affected arteries.

**Results.** EWAS identified 3,644 DMPs ($P_{adj} < 0.05$, $|\Delta\beta| > 0.3$), indicating a profound alteration within GCA-affected arterial tissue. These DMPs were annotated to 1,517 potentially dysregulated genes. A total of 282 additional genes were identified by annotation of significant DMRs. Pathway enrichment analysis revealed a significant alteration of inflammatory mechanisms, such as interleukin-2 and interleukin-7, as well as pathways related to vascular remodeling. Omics study comparison revealed 37 genes consistently affected across datasets, many of them linked to immune signaling and T cell regulation. Notably, markers of exhausted T cells, including *SLAMF6* and *HAVCR2*, were present among them.

**Conclusion.** Our study identified GCA-specific DNA methylation signatures in arterial tissue, revealing disrupted inflammatory and vascular pathways and suggesting the involvement of exhausted T cells in this condition. These findings offer new insights into GCA pathogenesis and provide new potential targets for the treatment of this debilitating disease.

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[1]Gonzalo Borrego-Yaniz, MSc, Ana Márquez, PhD, Javier Martin, MD, PhD, Lourdes Ortiz-Fernández, PhD: Institute of Parasitology and Biomedicine López-Neyra, Consejo Superior de Investigaciones Cientificas (CSIC), Granada, Spain; [2]Elkyn Estupiñán-Moreno, PhD: Josep Carreras Leukaemia Research Institute (IJC), Epigenetics and Immune Disease Group, Badalona, Barcelona, Spain; [3]Laura C. Terrón-Camero, PhD: Bioinformatics Unit, Institute of Parasitology and Biomedicine López-Neyra, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain; [4]Miguel A. González-Gay, MD, PhD: Division of Rheumatology, Instituto de Investigación Sanitaria (IIS)-Fundación Jiménez Díaz, Madrid, Spain;[5]Miguel A. González-Gay, MD, PhD: Department of Medicine, University of Cantabria, Santander, Spain; [6]Santos Castañeda, MD, PhD: Division of Rheumatology, Hospital de la Princesa, Instituto de Investigación Sanitaria (IIS)-Princesa, Madrid, Spain; [7]Giuliana Guggino, MD, PhD: PROMISE Department Rheumatology, University of Palermo, Palermo, Italy; [8]David Saadoun, MD, PhD: Hôpital Pitié-Salpêtrière-Charles Foix Hospital, Paris, France; [9]Pietro Lio, PhD: Department of Computer Science and Technology, University of Cambridge, Cambridge, United Kingdom; [10]Simona Fontana, PhD: Dipartimento di Biomedicina, Neuroscienze e Diagnostica Avanzata, University of Palermo, Palermo, Italy; [11]Martina Bonacini, PhD, Alessandro Rossi, MSc, Stefania Croci, PhD: Clinical Immunology, Allergy and Advanced Biotechnologies Unit, Azienda Unità Sanitaria Locale (AUSL)- Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) di Reggio Emilia, Reggio Emilia, Italy; [12]Alberto Cavazza, MD: Unit of Pathology, Azienda Unità Sanitaria Locale (AUSL)- Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) di Reggio Emilia, Reggio Emilia, Italy; [13]Francesco Muratore, MD, Carlo Salvarani, MD: University of Modena and Reggio Emilia, Modena, Italy; [14]Francesco Muratore, MD, Carlo Salvarani, MD, Nicolo Pipitone, MD: Unit of Rheumatology, Azienda Unità Sanitaria Locale (AUSL)- Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS)di Reggio Emilia, Reggio Emilia, Italy.

Drs Martin, Croci, and Ortiz-Fernández contributed equally to this work.

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Address correspondence via email to Lourdes Ortiz-Fernández, PhD, at lourdes@ipb.csic.es.

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## INTRODUCTION

Giant cell arteritis (GCA) is the most common form of vasculitis in individuals aged >50 years old, characterized by chronic inflammation of the aorta and its branches. This condition carries significant morbidity and mortality, with complications such as irreversible vision loss and stroke.[1] Despite its clinical impact, the molecular mechanisms underlying GCA pathogenesis remain insufficiently understood, limiting the development of improved treatments and management strategies.[2,3]

Pathologic hallmarks of GCA are the loss of immune privilege in the arterial tissue, sustained inflammatory damage leading to severe vascular remodeling, and the neovascularization of the extracellular matrix.[4] These processes create a chronic inflammatory microenvironment that sustains the malfunctioning immune response in the vascular tissue. Different studies have shown genetic risk factors and reprogrammed gene regulation associated with this condition,[5–8] showing a complex contribution of genetic and epigenetic drivers to GCA pathogenesis.

Epigenetics is a dynamic mechanism to regulate gene expression, affecting development, tissue differentiation, and cellular responsiveness.[9] DNA methylation is the most studied epigenetic marker, having been associated with pathogenic conditions such as immune-mediated inflammatory diseases (IMIDs).[10] Epigenetic signatures associated with disease progression hold great potential for the discovery of new therapeutic targets because of their reversible nature and potential to address disease mechanisms influenced by genetics, lifestyle, and environmental factors.[11] In GCA, a previous study revealed thousands of methylation changes present in temporal artery biopsies (TABs)[12]; however, because of limited sample size and CpGs coverage, there is an incomplete understanding of the role of this mechanism in GCA pathogenesis.

In this study, we conducted a comprehensive DNA methylation analysis of GCA-affected arteries. By leveraging genome-wide profiling in the largest GCA cohort studied so far, we sought to provide foundational insights into GCA pathogenesis and identify potential therapeutic targets to address the significant clinical burden of this condition.

## MATERIALS AND METHODS

**Study cohort.** TABs from 69 patients with GCA and 10 controls were collected in hospitals from Italy, France, and Spain. All patients with GCA had a positive TAB, with transmural inflammation, and fulfilled the 1990 American College of Rheumatology classification criteria for this disease.[13] Clinical criteria for both inclusion or exclusion from the study is listed in Supplementary Materials section 1. Control samples were obtained from individuals with suspected GCA whose TABs were negative. Moreover, the diagnosis of GCA was discarded based on additional negative large-vessel imaging results (temporal artery color Doppler ultrasonography was performed in 9 of 10 patients, and positron emission tomography and computed tomography was performed in 6 of 10 patients) and a definitive alternative diagnosis deriving from a clinical follow-up of at least one year. The main demographic and clinical characteristics of the study cohort are described in Table 1. All participants signed an informed consent form in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The protocol adhered to all ethical regulations and obtained approval from the ethics committee Area Vasta Emilia Nord of the principal investigator (protocol 777/2018/OSS/AUSLRE) and from those of the participating institutions involved in this study.

**Table 1.** Clinical and demographic characteristics of the study cohort

| Characteristics | Total | Cases | Controls* |
|---|---|---|---|
| Cohort size, n | 79 | 69 | 10 |
| Age, mean ± SD | 75.54 ± 7.04 | 74.87 ± 6.72 | 80.20 ± 7.84 |
| Women, n (%) | 62 (70) | 58 (84) | 6 (60) |
| GC treatment at TAB, n (%)[a] | 43 (53) | 41 (61) | 2 (20) |
| Treatment during follow-up period, GC/GC + TCZ | – | 64/5 | – |
| Responders to treatment, n (%)[a] | – | 32 (48) | – |
| Any cranial symptoms, n (%)[a,b] | 69 (90) | 61 (91) | 8 (80) |
| Any visual symptoms, n (%)[a,c] | 25 (32) | 22 (33) | 3 (30) |
| Any systemic symptoms, n (%)[a,d] | 38 (49) | 38 (57) | 0 (0) |
| Polymyalgia rheumatica, n (%)[a] | 30 (48) | 27 (40) | 3 (30) |

* Control samples were obtained from individuals with suspected giant cell arteritis whose TABs were negative, in addition to further clinical criteria and follow-up of at least one year. The final diagnoses for controls were the following: nonarteritic anterior ischemic optic neuropathy (n = 3), polymyalgia rheumatica (n = 3), Raynaud disease (n = 1), abdominal aortic aneurysm of atherosclerotic genesis (n = 1), temporomandibular joint dysfunction (n = 1), and crowned dens syndrome (n = 1). GC, glucocorticoid; TAB, temporal artery biopsy; TCZ, tocilizumab.
[a] Information about clinical manifestations and response to treatment was not available for two patients with giant cell arteritis.
[b] Any cranial symptoms of the following: headache, scalp dysesthesia, or jaw claudication.
[c] Any systemic symptoms of the following: fever, fatigue, or weight loss ≥4 kg.
[d] Any visual symptoms of the following: permanent visual loss, amaurosis fugax, or diplopia.

**DNA methylation profiling.** To preserve the in situ cellular and molecular composition, TABs samples were snap frozen in liquid nitrogen and stored at –80°C within 30 minutes of the withdrawal. DNA from TABs was isolated with the DNA/RNA/Protein Purification Plus Kit (Norgen) following the manufacturer's instructions and quantified with Qubit Fluorometric Quantification (Thermo Fisher Scientific). DNA methylation profiling was performed using 500 ng of bisulfite-converted DNA hybridized to the Infinium MethylationEPIC BeadChip array (Illumina) in accordance with the manufacturer's protocol. This array, designed to target 850,000 CpG sites, provides coverage of 99% of annotated RefSeq genes. Probe fluorescence was measured using a BeadArray Reader (Illumina).

**Quality controls and normalization.** DNA methylation raw data were processed using minfi R package[14] (version 1.55) under R version 4.4. First, we checked for samples showing poor bisulfite conversion (detection $P > 0.05$) and those with discrepancies between recorded sex information and sex inferred from the data. Next, probes showing poor quality (detection $P > 0.01$) were removed. We also filtered out CpGs from chromosomes X and Y as well as those probes containing either a single nucleotide polymorphism at the CpG interrogation site or at the single base extension site. Finally, cross-reactive probes reported in the literature were also removed.[15] Probes were annotated using IlluminaHumanMethylationEPICanno (version 10b5.hg38). Subsequently, we normalized raw methylation values using the stratified quantile normalization method.

Methylation levels were measured as β and M values. β values, representing the fraction of methylated probes on a scale from 0 to 1, were used for visual representation and biologic interpretation. M values (log2-transformed β values) were calculated for statistical purposes, to approximate a normal distribution of methylation values, more adequate for linear modeling. To evaluate potential confounding effects, principal component analysis (PCA) was conducted (Supplementary Materials section 2). In addition, PCA was also used to estimate potential outliers in the cohort. Samples exceeding a threshold of 4 SDs from the cluster centroids were excluded from further analyses.

**Genome-wide differential methylation analysis.** We compared methylation levels of patients with GCA versus controls of all CpGs that passed quality controls using an empirical Bayes moderated *t*-statistics test from limma R package.[16] For this comparison, we conducted independent sensitivity tests to examine the contribution of all potential confounders (sex, age, treatment at TAB collection, sample plate, slide, and well) to the analysis. Pearson correlation or Wilcoxon signed-rank test was applied depending on whether the variable of interest was continuous or categorical. Variables showing an false discovery rate (FDR) value ($P_{adj}$) < 0.05 were considered to significantly contribute to DNA methylation profiles (Supplementary Table 1). Accordingly, sample plate and sample slide were included in the final regression model as covariates. To further support that sex was not notably influencing the methylation changes observed in our cohort, we also performed the differential methylation analysis including sex as a covariate in addition to a disease–sex interaction model. These complementary analyses are described in detail in Supplementary Materials sections 3-5. For multiple-testing correction, we applied a FDR of 0.05. To assess the potential influence of unknown confounding factors on our findings, we performed a permutation test in which case and control labels were randomly reassigned within our cohort across 100 iterations, and the resulting differential analyses were compared with those obtained in the original dataset (Supplementary Materials section 6). The differential of β values (Δβ) between the groups considered in each comparison were estimated as the difference in the median values. Those CpG sites with an $P_{adj} < 0.05$ and $|\Delta\beta| > 0.3$ were considered as differentially methylated positions (DMPs). The enrichment of DMPs within genomic locations was analyzed using epigenome-wide association study toolkit.[17] Furthermore, we assessed the presence of differentially methylated regions (DMRs) with DMRcate[18] by using default parameters ($\lambda = 1{,}000$) and considering only DMRs containing at least five significant CpGs.

**Pathway enrichment analysis.** To explore the potential pathologic relevance of the gene sets identified in our analyses, we conducted pathway enrichment analysis using the EnrichR tool.[19] The analysis was performed by querying two comprehensive databases: Gene Ontology Biological Process[20] and BioPlanet.[21] Pathways needed to be enriched in at least five genes to be considered, and those presenting an $P_{adj} < 0.05$ were considered significant. As methylation changes can either up-regulate or down-regulate genes depending on their genomic context, we included all annotated genes in the pathway enrichment analysis, regardless of whether they contained hypermethylated or hypomethylated CpGs.

**Comprehensive overview of GCA-affected genes.** To identify genes exhibiting transversal alterations across gene regulation, gene expression, and protein levels, we compared our results on DMPs and DMRs with reported findings from previous bulk omics studies in GCA. Specifically, we evaluated overlaps with (1) DMPs previously reported in temporal arteries,[12] (2) differentially expressed genes identified in temporal arteries,[22] and (3) altered protein levels observed in plasma serum.[23] The characteristics of the experimental design conducted in each of those studies are summarized in Supplementary Table 2. As omics studies reporting a large number of genes were compared, we performed permutation tests to assess whether the observed level of overlap between studies was significantly greater than expected by chance (Supplementary Materials section 7).

**Data availability statement.** Summary results will be made available upon reasonable request. Individual-level data cannot be shared due to privacy and ethical considerations.

**Ethics approval.** The study was approved by the ethical committees of all institutions involved in this study. Participants gave informed consent to participate in the study before taking part.

## RESULTS

**Specific epigenomic signature in GCA-affected arteries.** After quality controls, 770,921 CpGs were analyzed, and all samples were included in the analysis. Notably, 89.2% of CpGs showing significantly altered methylation levels in GCA-affected arteries in a previous study[12] were also significant in our results ($P_{adj} < 0.05$) and showed a strong correlation in their Δβ values between studies (Spearman correlation = 0.75; $P < 2.2 \times 10^{-16}$). In addition, all overlapping significant CpGs displayed methylation changes in the same direction (Supplementary Material section 3). Because of the increased statistical power in our experimental design, a total of 228,152 CpG sites presented significant changes in methylation ($P_{adj} < 0.05$), demonstrating an extensive epigenetic alteration present in the GCA-affected artery. Randomized case and control permutations confirmed that the large differences observed between patients with GCA and controls were unlikely to be due to unaccounted confounding factors in our experimental design (Supplementary Material section 6). Across 100 iterations, a maximum of 10 significant CpGs were identified, whereas the majority of permutations (n = 87) yielded no significant CpGs. Because of the large effect of GCA pathogenesis over arterial tissue, we proceeded to focus on those significant CpGs showing major changes in methylation ($|\Delta\beta| > 0.3$; $P_{adj} < 0.05$), comprising 3,644 DMPs, in which 81.1% of them represent novel DMPs for arteries affected by GCA. Among these, 2,680 were hypermethylated, and 964 were hypomethylated (Figure 1; Supplementary Table 3). These DMPs were predominantly located in OpenSea regions and gene bodies (Supplementary Table 4) and were annotated to 1,517 different genes, including relevant genes involved in GCA pathologic mechanisms related to inflammatory response and vascular remodeling, such as *IL6R*, *IL2RA*, *STAT3*, and *JAG1.* Pathway analyses of these DMPs showed 78 significant pathways (Supplementary Tables 5–6), including fundamental immune pathways related to IL-2 and CTLA-4 signaling as well as mechanisms linked to the angiogenic process, such as integrins affecting angiogenesis and CXCR4 signaling (Figure 2).

**Epigenetic altered regions and affected pathways in GCA.** To determine consistent methylation changes in the epigenome, we conducted a DMR analysis, identifying 12,899 significant regions associated with GCA ($P_{adj} < 0.05$). Among these, 5,393 were hypomethylated and 7,506 hypermethylated, with a mean size of 9.53 CpGs per region. A subset of 918 DMRs showed substantial methylation changes ($|\text{mean } \Delta\beta| > 0.2$) and were annotated to 935 genes (Supplementary Table 7), comprising 282 additional genes from those identified by the annotation of DMPs. This gene set included critical immune and GCA-associated genes, such as *IL17B*, *IL11RA*, *HLA-DPA1*, *TNF*, and *PDGFA*, further supporting a strong alteration of immune activation and tissue remodeling. Pathway enrichment analysis highlighted disruptions in key arterial pathways (155 significant pathways; Supplementary Tables 8–9). Notably, pathways related to arterial remodeling were enriched, including regulation of growth factors VEGF and PDGF. Additionally, signaling pathways relevant for arterial cell migration and immune cell activation were affected, such as leukocyte transendothelial migration, regulation of T cell activation, and natural killer cell–mediated cytotoxicity (Figure 3). These findings emphasize the extensive epigenetic reprogramming that drives the local arterial response in the context of GCA pathogenesis.

**Consistently altered genes in GCA omics studies.** To contextualize our findings, we evaluated the affected genes identified in this study against previously reported omics datasets on GCA. Notably, we identified 37 genes consistently altered in GCA-affected arteries across three major epigenomics and transcriptomics studies of this tissue, including this current work (Figure 4). This list comprises genes annotated either by DMPs or DMRs from our results, DMPs reported by Coit et al,[12] and genes classified as differentially expressed in Ferrigno et al.[22] For all studies considered, permutation tests demonstrated that the number of overlapping genes was significantly greater than expected by chance, including the overlap of the 37 genes consistently identified in GCA-affected arteries ($P < 0.0001$ for all comparisons; Supplemental Material section 7). These 37 genes carried a robust body of omics evidence supporting their involvement in the pathophysiology of GCA and contain genes related to immune signaling (predominantly related to T cell mechanisms), tyrosine kinases, transcription factors, and integrins as well as other elements. Although the list includes several genes known to be affected in GCA pathogenesis, such as *CD28*, *IL2RA*, and *TNF*, it also highlights many genes whose potential role in the pathogenesis of this disease have not been explored, such as *ICOS*, *GZMA*, *IRF5*, *TLR1*, and *FYN*, among others. *BTLA* and *SLAMF1* were particularly outstanding because they also exhibited significantly altered protein levels in plasma serum from patients with GCA.[23] These findings warrant further investigation given their consistent alterations across independent GCA omics studies.

## DISCUSSION

This study represents the largest genome-wide DNA methylation study of GCA-affected arteries conducted to date, providing comprehensive insights into the severe epigenetic dysregulation

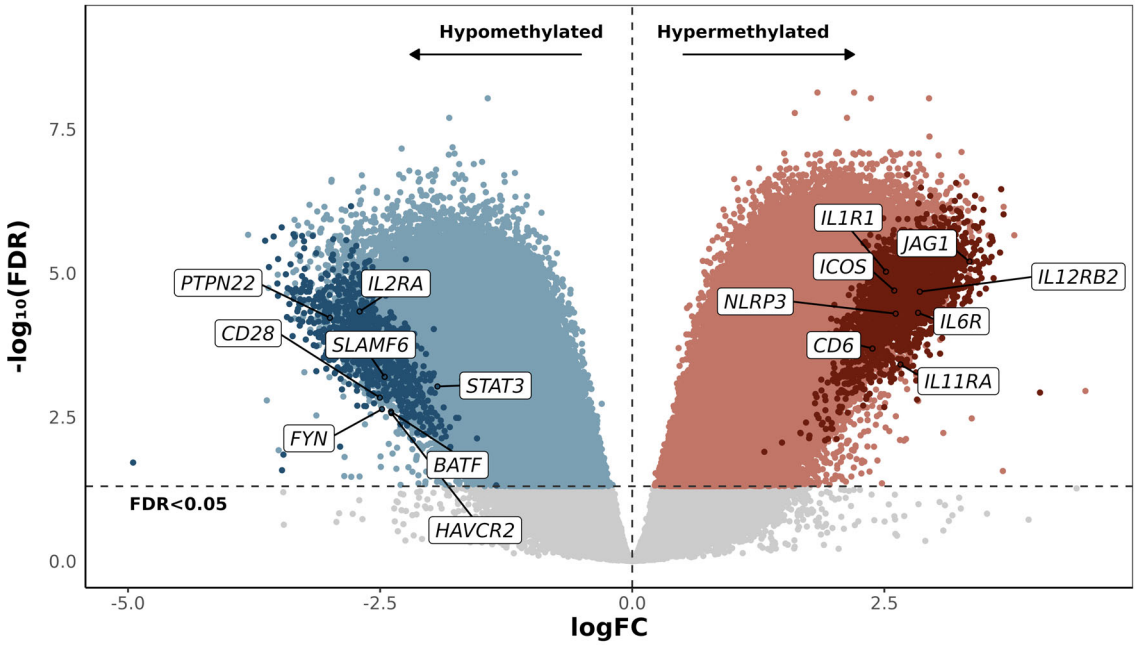


**Figure 1.** Volcano plot of the epigenome-wide comparison between patients with giant cell arteritis and controls. Gray points represent the non-significant ($P_{adj} > 0.05$) CpGs, and blue and red points represent significant hypomethylated and hypermethylated positions, respectively. Darker-colored points represent differentially methylated positions, significant CpGs showing $|\Delta\beta| > 0.3$.

within arterial tissue underlying this pathology. We analyzed the most notable alterations in the GCA-associated signature, revealing major changes in critical immune and vascular pathways, especially those related to immune activation and vascular remodeling. Furthermore, by leveraging findings from epigenomics and transcriptomics studies, we identified key immune-related genes found consistently altered in GCA-affected arteries, comprising 37 genes that may play a fundamental role in driving this condition.

Interpreting omics data in a complex condition such as GCA is challenging due to the sheer number of genes implicated. To address this, we prioritized genes with strong replicability across

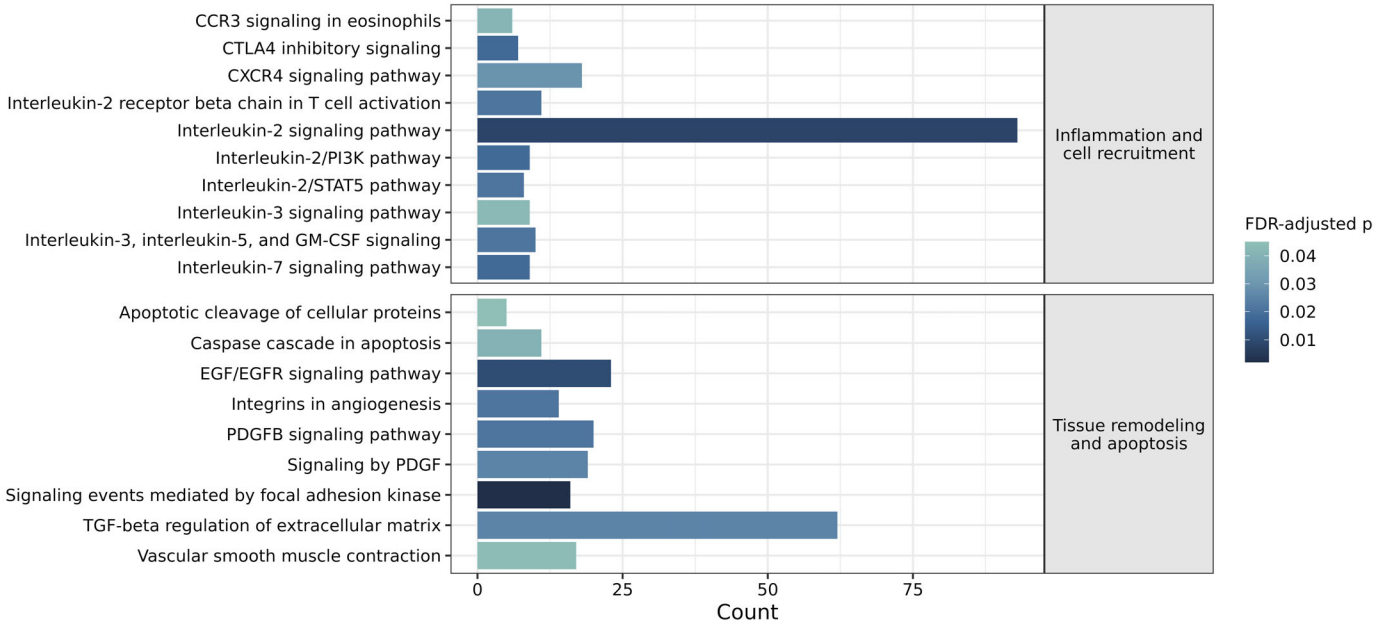


**Figure 2.** Highlighted significant pathways ($P_{adj} < 0.05$) of the 1,517 genes annotated from differentially methylated positions. FDR, false discovery rate. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43358/abstract.

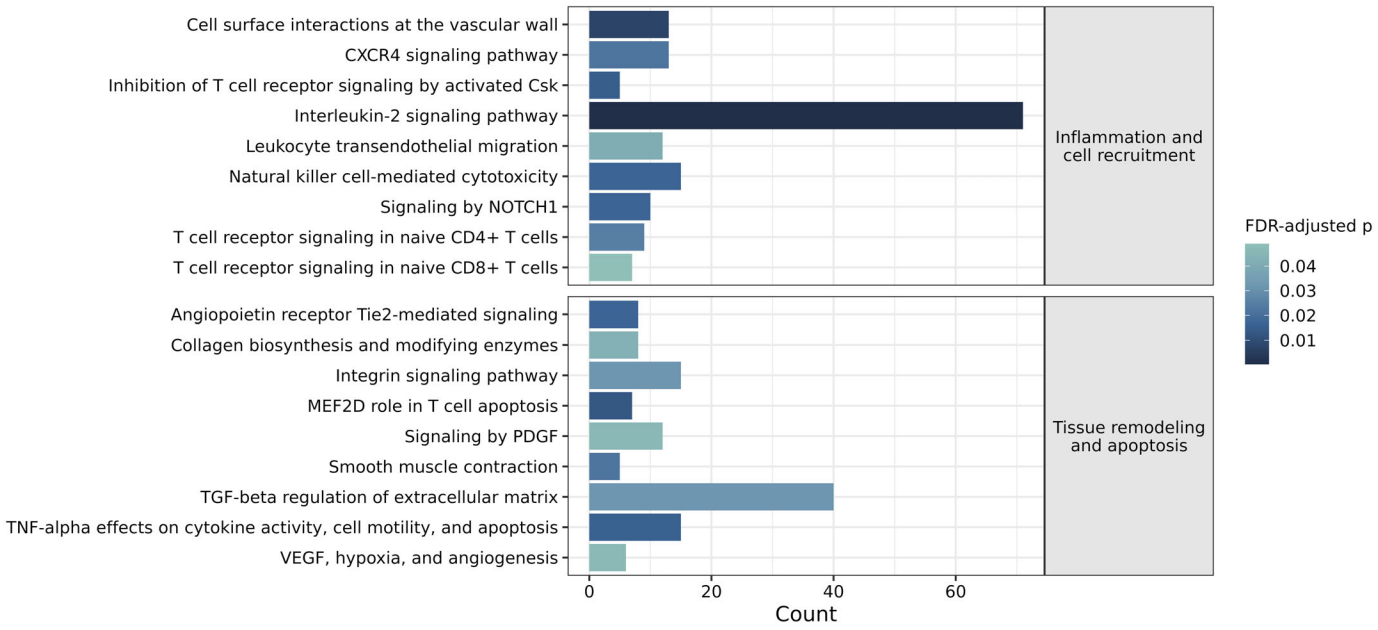


**Figure 3.** Highlighted significant pathways (FDR-adjusted $P < 0.05$) of the 935 genes annotated from differentially methylated regions. FDR, false discovery rate. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43358/abstract.

independent omics studies using TAB samples. Our integrative approach yielded a shortlist of 37 genes exhibiting significant methylation changes (replicated in two independent studies) and altered gene expression levels. This shortlist encompasses a robust set of genes potentially central to GCA, many of them not previously proposed to play a specific role in the pathogenesis of this disease. Among these, we observed a strong representation of costimulatory proteins involved in T cell and antigen-presenting cell interactions, including CD28, CD247, BTLA, ICOS, and SLAM proteins (SLAMF1 and SLAMF6). Additionally, immune-related signaling molecules, such as TNF, FYN, ABL1, PIK3CD, and PIK3CG, as well as transcription factors including RUNX1, RUNX3, and BATF, were prominent. One particularly noteworthy gene in this set is *NLRP3*, which encodes an intracellular pattern recognition receptor that detects pathogens, endogenous damage, and environmental irritants. After activation, it promotes the formation of the NLRP3 inflammasome that leads to proinflammatory signaling and apoptosis. This innate immunity-related pathway plays a central role in cardiovascular diseases[24] and is gaining recognition for its role in autoimmune diseases[25]; therefore, its potential contribution to GCA pathogenesis deserves further study, especially given its established value as a therapeutic target for conditions presenting chronic inflammation.[26]

T cell exhaustion is a process in which T cells, following chronic exposure to antigens and persistent inflammatory signals, progressively lose their effector functions and cytotoxicity while up-regulating inhibitory receptors.[27] Although this phenotype is traditionally linked to chronic infections and cancer, it is now increasingly recognized in autoimmune and inflammatory diseases.[27] Our study, along with other GCA omics studies, identified several markers of exhausted T cells as being altered in GCA-affected arteries at both gene regulation and expression levels. Notable examples include *SLAMF6*, *HAVCR2* (also known as TIM-3), and *CX3CL1* (the ligand for CX3CR1), specific markers of the exhausted T cell phenotype.[28] Additionally, we also observed consistently reported alterations in *BTLA* and *BATF*, regulators of exhausted T cells differentiation.[29] It is also important to note that PD-1 and CTLA4, immune checkpoint molecules implicated in regulating inflammation, are central to the progression of GCA[4,30] and also deeply linked to the development of exhausted T cells.[27] Furthermore, the CXCR4 pathway, significant in our pathway enrichment results, is also related to exhausted T cell differentiation, as it has been reported that CXCR4 can promote T cell exhaustion via JAK2/STAT3.[31] Based on the convergence of GCA-related pathways and T cell differentiation, as well as the replicability of reported alteration of specific markers of exhausted T cells across GCA studies, we hypothesize that the microenvironment of GCA-affected arteries—characterized by persistent inflammation, hypoxia, and extracellular matrix damage—provides favorable conditions for the emergence and maintenance of exhausted T cell phenotypes that would impede the ability of the immune system to regulate the local inflammation occurring in the affected tissue.

Although T cell exhaustion has been primarily described in CD8+ T cells, the role of this T cell subtype in GCA remains poorly understood despite evidence of their presence in inflamed arterial tissue.[32] Emerging evidence suggests that CD4+ T cells can also exhibit an exhausted phenotype,[33] and given the established importance of CD4+ T cells in GCA pathogenesis, it would be particularly relevant to investigate whether exhausted subsets of both

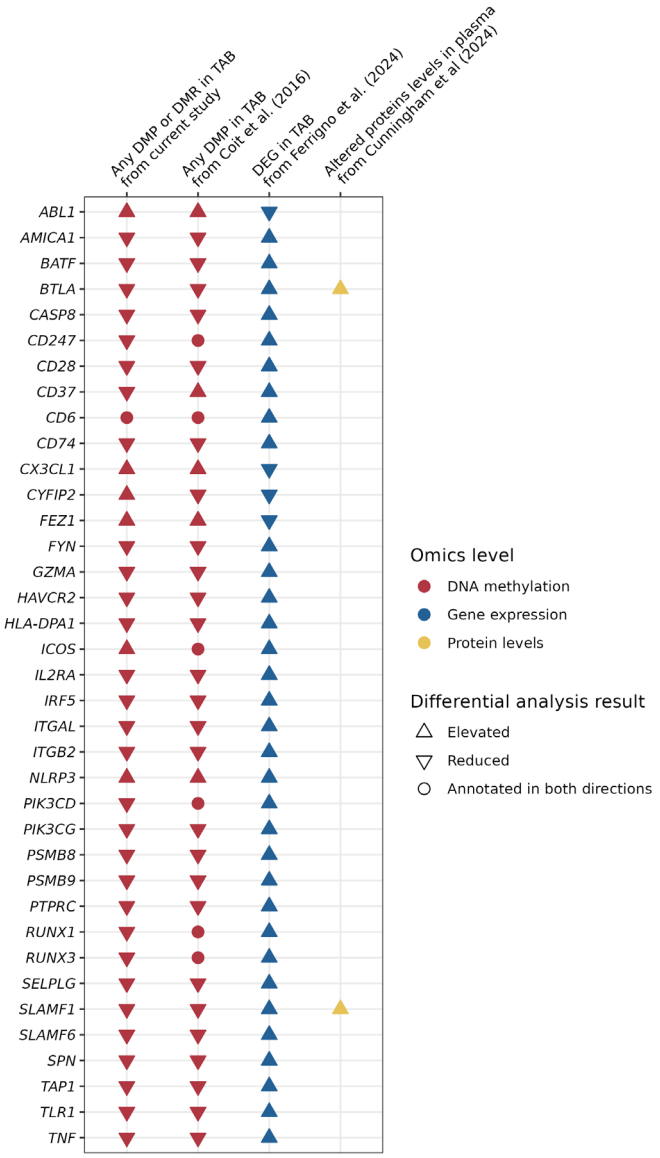


**Figure 4.** Reported alterations of gene regulation, gene expression, and/or protein abundance levels in independent giant cell arteritis omics studies for the 37 consistently reported genes in giant cell arteritis–affected arteries. DMP, differentially methylated position; DMR, differentially methylated region; TAB, temporal artery biopsy. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43358/abstract.

CD8+ and CD4+ T cells contribute to disease progression. Understanding the functional implications of T cell exhaustion in GCA could reveal novel mechanisms of immune dysregulation and provide opportunities for targeted therapeutic interventions aimed at restoring T cell function or mitigating exhaustion pathways, a promising therapeutic strategy currently under active investigation in other conditions.[34,35]

Regarding epigenetic alterations newly identified in this current study, pathway analysis revealed a broad spectrum of disrupted mechanisms in the context of GCA. Among these, the IL-2 signaling pathway emerged prominently, with the highest number of affected genes (n = 93) being annotated to this pathway. This finding emphasizes the contribution of IL-2 signaling in GCA pathophysiology, associated with vascular persistent Th1 cell signature.[36] Notably, low-dose IL-2 therapy has shown significant promise in helping regulate inflammatory responses across a broad range of IMIDs.[37] These results suggest GCA as a compelling candidate for exploring the therapeutic potential of IL-2–based treatments. Similarly, the IL-7 signaling pathway, also related to IL-2, was found to be significantly enriched in our results. Although IL-7 is well documented to play a role in other IMIDs[38] and is known to affect immune cell populations, for example, promoting T cell apoptosis,[39] its involvement in GCA pathogenesis represents a novel finding showing specific therapeutic potential.[40] Furthermore, our study also found a significant change in the regulation of *IL11RA*, which supports the recently proposed contribution of IL-11 in GCA pathogenesis by a multiomics study of blood-derived GCA monocytes.[8] Finally, another notable finding was the novel association of the CXCR4 signaling pathway with this disease, an essential regulator of local inflammation.[41] CXCR4 is instrumental in preserving arterial integrity, regulating vascular inflammation, and promoting angiogenesis and the recruitment of inflammatory cells,[42,43] processes that are central to the pathophysiology of GCA. The implication of CXCR4 in GCA pathogenesis also shows great therapeutic promise, as CXCR4-targeted therapies are under active research.[44] These findings reinforce the relevance of local immune and vascular signaling in GCA and suggest that targeting pathways such as CXCR4 and IL-2 could be pertinent for therapeutic innovation in this disease.

Our study offers valuable insights into the epigenetic changes in GCA-affected arteries, leveraging well-powered genome-wide DNA methylation profiling and multiomics comparison to provide a comprehensive overview of this disease. The use of a notable cohort and robust analytical methods allowed us to identify novel pathways and mechanisms such as T cell exhaustion and CXCR4 signaling. However, several limitations should be acknowledged to fully contextualize these findings. The omics evidence presented here provides statistical evidence but requires further functional studies to clarify how the proposed pathways and genes mechanistically contribute to GCA pathogenesis. It is also important to clarify that because of the lack of reference methods for cell deconvolution in arterial tissue, we were unable to adjust our models for cell composition. Thus, our results represent bulk tissue methylation changes reflecting the combined signal from all cellular components. One important aspect that remains unexplored in our study is how the genetic component of GCA contributes to the extensive epigenomic alterations identified. Achieving this goal will require a comprehensive multiomics approach, including quantitative trait loci analyses. Moreover, the substantial clinical heterogeneity of patients with GCA posed challenges for our analysis. Although we identified a robust epigenetic signature associated with GCA, limitations in the sample size of our cohort prevented the possibility of studying

the effect of particular clinical manifestations in DNA methylation, restricting our ability to detect epigenetic patterns linked to particular phenotypes. It is also noteworthy that, because of the inherent difficulty of obtaining TAB samples from individuals ultimately diagnosed as non-GCA, only 10 control samples were available for analysis, resulting in an imbalanced case–control ratio. These limitations highlight the importance of future research involving larger, clinically well-characterized cohorts and experimental validation to enhance our understanding of the molecular mechanisms driving GCA pathogenesis.

This study represents a methodologically robust analysis into the epigenetic landscape of GCA-affected arteries, leveraging genome-wide DNA methylation profiling and previous omics findings to provide novel insights into the pathogenesis of this disease. Among the key results, the potential involvement of exhausted T cells in GCA pathophysiology represents an intriguing discovery, representing a new potential agent of the immune dysfunction present in GCA-affected arterial tissue. These findings enhance our understanding of GCA pathogenesis and highlight therapeutic targets, paving the way for improved treatment strategies.

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## AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Ortiz-Fernandez confirms that all authors have provided the final approval of the version to be published and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

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