

# A Genome-wide Association Study Identifies Risk Alleles in Plasminogen and *P4HA2* Associated with Giant Cell Arteritis

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Giant cell arteritis (GCA) is the most common form of vasculitis in individuals older than 50 years in Western countries. To shed light onto the genetic background influencing susceptibility for GCA, we performed a genome-wide association screening in a well-powered study cohort. After imputation, 1,844,133 genetic variants were analyzed in 2,134 case subjects and 9,125 unaffected individuals from ten independent populations of European ancestry. Our data confirmed HLA class II as the strongest associated region (independent signals: rs9268905,  $p = 1.94 \times 10^{-54}$ , per-allele OR = 1.79; and rs9275592,  $p = 1.14 \times 10^{-40}$ , OR = 2.08). Additionally, *PLG* and *P4HA2* were identified as GCA risk genes at the genome-wide level of significance (rs4252134,  $p = 1.23 \times 10^{-10}$ , OR = 1.28; and rs128738,  $p = 4.60 \times 10^{-9}$ , OR = 1.32, respectively). Interestingly, we observed that the association peaks overlapped with different regulatory elements related to cell types and tissues involved in the pathophysiology of GCA. *PLG* and *P4HA2* are involved in vascular remodelling and angiogenesis, suggesting a high relevance of these processes for the pathogenic mechanisms underlying this type of vasculitis.

## Introduction

During the last decade, genome-wide association studies (GWASs), in which common genetic variation across the whole genome is interrogated in a hypothesis-free fashion, were a breakthrough in biomedical research methodology and have led to the identification of thousands of robust genetic associations within a wide spectrum of complex human diseases.<sup>1</sup> However, some diseases of low preva-

lence have received less attention due to the difficulty in recruiting well-powered study cohorts, even though rare diseases result in a significant disease burden. An example is giant cell arteritis (GCA [MIM: 187360]), the most common form of vasculitis in Western countries in people over 50 years old. GCA is characterized by chronic inflammation of large arteries, such as the aorta and the carotid arteries and its extracranial branches, which may lead to severe clinical sequelae if not treated promptly, including

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vision loss, scalp and tongue necrosis, aortic dissection/rupture, and cerebral infarction.<sup>2–4</sup> Although the genetic component of GCA has previously been investigated following a candidate gene approach, most of the described genetic associations were based on underpowered analyses and usually failed to be replicated in independent populations.<sup>5</sup>

Recently, an international collaborative effort involving European and North American research consortia has made possible more powerful studies, including an Immunochip study, that have identified firm risk signals for GCA predisposition, such as HLA molecules and key genes of the immune response like protein tyrosine phosphatase, non-receptor type 22 (*PTPN22* [MIM: 600716]), and interleukin 17A (*IL17A* [MIM: 603149]).<sup>6–8</sup> Taking advantage of the large sample collection that this collaboration has enabled, comprising ten independent populations of European ancestry that cover the whole gradient for prevalence of the disease, we performed an agnostic genetic study in GCA at the genome-wide level.

## Subjects and Methods

### Study Population

This study included a total of 2,134 GCA-affected case subjects and 9,125 unaffected control subjects from ten independent populations of European ancestry: Spain (805 case and 1,323 control subjects), UK (352 case and 2,965 control subjects), Italy (271 case and 960 control subjects), North America (176 case and 1,181 control subjects from USA and Canada), Germany (160 case and 667 control subjects), France (114 case and 488 control subjects), Norway (104 case and 121 control subjects), the Netherlands (69 case and 638 control subjects), Switzerland (46 case and 500 control subjects), and Ireland (37 case and 282 control subjects). The diagnosis of GCA was established according to the 1990 American College of Rheumatology classification criteria for this disease.<sup>9</sup> In addition, the diagnosis was

subsequently confirmed by either a biopsy of the temporal artery (89.83%) or arterial imaging (10.17%) consistent with GCA. A detailed description of the main clinical characteristics of the different case cohorts is provided in Table S1. All participants signed an informed consent form before being enrolled in the study. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) of all participant centers.

### Genotyping and Quality Controls

Genomic DNA from peripheral blood samples of all GCA-affected case subjects as well as the Spanish and Irish control subjects were screened using the GWAS platform “Infinium HumanCore Beadchip” in an iScan System and the Genotyping Module (v.1.9) of the GenomeStudio software (Illumina). The genotyping was conducted in the Genomics and Genotyping Unit of the Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO, Granada, Spain), according to the manufacturer’s protocol. SNPs with a cluster separation < 0.4 were removed after the calling.

Genotyping data from the remaining control population were obtained from nine cohorts of geographically matched European ancestries included in previous studies<sup>10–17</sup> (Table S2).

All studies were subjected to stringent quality-control measures separately based on the same analytical protocol using PLINK v.1.07.<sup>18</sup> Specifically, we filtered out SNPs with call rates < 0.98 and minor allele frequencies (MAF) < 0.01 and those that deviated from Hardy-Weinberg equilibrium (HWE;  $p < 0.001$  in both case and control subjects). Similarly, samples with less than 95% of successfully called SNPs, and one subject per pair of first-degree relatives (identity by descent > 0.4) were removed. Sex chromosomes were also excluded from the analysis.

Finally, to check the consistency of the results, we re-genotyped the Spanish samples for the associated signals using predesigned TaqMan 5’ SNP genotyping assays (assay IDs: C\_16222465\_10, C\_25614474\_20, and C\_2397211\_10) in a 7900HT Fast Real-Time PCR System (Applied Biosystems) and compared the TaqMan types with the corresponding imputed data.

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## Imputation Methods

After applying the QC filters, whole-genome SNP genotype imputation was carried out with IMPUTE v.2<sup>19</sup> using the combined 1000 Genome Project Phase III (1KGPh3) data as reference panel, which includes 2,504 individuals.<sup>20</sup> In brief, for each individual dataset, the strand orientation, chromosome position, and SNP identification were updated in PLINK to match build 37 (hg19) of the 1KGPh3. Next, PLINK-formatted files were converted to the appropriate format with GTOOL and the genotyping data were split into chunks of 50,000 Mb covering whole-genome regions. Imputation was done separately for each study. To ensure quality of imputed SNPs, the most likely call was used for merging genotypes, but only if the most likely call exceeded a probability threshold of 0.9 (the genotype was set to missing otherwise). As the individual imputation of each case/control set could lead to stratification, imputed data were also subjected to rigorous quality filters in PLINK, including removal of SNPs with call rates < 0.98, MAFs < 0.01, and those that were not in HWE ( $p < 0.001$ ). Moreover, after merging case/control sets, singleton SNPs and those showing strong evidence of discordance in the genotype distribution between cases and controls due to possible miscalling were removed using an in-house Perl script. Finally, principal component (PC) analyses were conducted to control for possible population stratification using PLINK and the GCTA64 and R-base software under GNU Public license v.2. For that, we calculated and plotted the ten first PCs of each individual and those samples located at >4 standard deviations from the cluster centroids of each cohort were considered outliers and, consequently, excluded from the analyses.

The final numbers of individuals and SNPs that remained in the filtered datasets are shown in [Table S2](#).

## Statistical Analyses

[Table S3](#) shows the estimation of the overall statistical power of this study accordingly with the CaTS Power Calculator for Genetic Studies software, which implements the methods described in Skol et al. assuming an additive genetic model.<sup>21</sup>

PLINK was used to conduct all the case/control analyses. First, the genotype frequencies of all markers were compared between case and control subjects of every individual dataset by logistic regression on the best-guess genotypes (>0.9 probability) assuming an additive model and using the ten first study-specific PCs and the gender as covariates. Next, a combined analysis of all studies was performed using the inverse variance weighted meta-analysis under a fixed effects model. In order to maximize the total number of SNPs analyzed without compromising the consistency of the results, we tested all SNPs present in the largest GCA sample set (Spain) and in one or more additional studies (i.e., ranging from 2 to 10 studies but including always the Spanish set). A total of 1,844,133 were evaluated in the meta-analysis. To identify independent effects across associated regions, dependency analyses at the cohort level genome-wide scans were conducted in PLINK by stepwise logistic regression with adjustment for the most associated signals in the common set of SNPs, followed by inverse variance weighted meta-analysis under a fixed effects model. The heterogeneity of the odds ratios (OR) across the different studies was estimated using both  $I^2$  and Cochran's Q tests. The Manhattan plots were obtained with an in-house R script, and the zooms of the associated regions were obtained with LocusZoom v.1.1.<sup>22</sup>

## Functional Annotation of Associated Variants

We evaluated the putative functional implications of the GCA risk loci by implementing our data with publicly available functional annotation data.

Despite the high efficiency of the imputation process, it was not possible to obtain imputed types for every known polymorphism. Therefore, in a first step, we identified all the SNP taggers ( $r^2 > 0.8$ ) of the associated signals of our GWAS using the 1KGPh3 data for the European populations and PLINK. All taggers were considered equally as candidates for prioritizing casualty or hypothesizing possible molecular causes of the observed associations in the subsequent bioinformatic approaches. Then, we explored whether the taggers of each GWAS signal had possible functional implications. The tools PolyPhen-2<sup>23</sup> and Combined Annotation Dependent Depletion (CADD)<sup>24</sup> were used to evaluate possible damaging effects on the protein sequence of coding non-synonymous SNPs. Regarding the intronic and intergenic variants, we explored whether they lay within known or predicted regulatory DNA elements (including regions of DNAase hypersensitivity, binding sites of transcription factors, promoter regions, chromatin marks, etc.) and whether they had predictive effects on clinical phenotypes using the online tools for exploring annotations of the noncoding genome RegulomeDB<sup>25</sup> and HaploReg v.4.1.<sup>26</sup> Sources of these databases include public datasets from the projects Gene Expression Omnibus (GEO), the Roadmap Epigenomics, the Encyclopedia of DNA Elements (ENCODE), and 1KGPh3, as well as published literature.

In addition, to provide an illustrative picture of the current knowledge on the GCA genetics, we conducted a molecular pathway enrichment analysis, considering both previously suggested GCA genes and those showing an association with disease susceptibility in this study, using the tool for that purpose of the Gene Ontology (GO) reference genome project,<sup>27,28</sup> powered by the Protein Analysis Through Evolutionary Relationships (PANTHER) Classification System.<sup>29</sup> Biological pathways showing  $p$  values lower than 0.05 after Bonferroni correction were considered associated with the disease. Finally, predictive protein-protein relationships among these same genes were also tested using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.<sup>30</sup> Only candidate genes that showed statistically significant signals after correction for multiple testing (which was performed with a previously validated method to control the genome-wide type 1 error rate at 0.05<sup>31</sup>) were included in the above analyses.

## Results

### Testing for Association with Disease Susceptibility

Three genomic regions contained association signals at the genome-wide level of significance in the overall meta-analysis: human leukocyte antigen (HLA) class II, plasminogen (*PLG* [MIM: 173350]), and prolyl 4-hydroxylase subunit alpha 2 (*P4HA2* [MIM: 600608]) ([Tables 1](#) and [S4](#); [Figure 1](#)).

Within the HLA region, the top hit was rs9268905 ( $p = 1.94 \times 10^{-54}$ , OR = 1.79, 95% CI 1.67–1.93), located between *HLA-DRA* (MIM: 142860) and *HLA-DRB1* (MIM: 142857). Dependence analyses suggested a possible independent effect on disease susceptibility within the *HLA-DQA1* (MIM: 146880)/*HLA-DQA2* (MIM: 613503) genomic region after controlling for the rs9268905 signal (lead

**Table 1. Independent Association Signals with Giant Cell Arteritis Susceptibility at the Genome-wide Level of Significance**

SNP	Location (GRCh37)	Locus	Change p Value	OR (CI 95%)	P(Q) i <sup>2</sup>	Minor Allele Frequency (GCA/CTRL) <sup>a</sup>											
						Meta-analysis	Spain	UK	Italy	North America	Germany	France	Norway	Netherlands	Switzerland	Ireland	
rs9268905	6:32432077	HLA-DRA/ HLA-DRB1	C<G	1.94 × 10 <sup>-54</sup>	1.79 (1.67–1.93)	0.75	0	0.46/0.32	0.52/0.37	0.33/0.23	0.48/0.32	0.39/0.28	0.48/0.29	0.47/0.33	0.37/0.28	0.42/0.34	0.45/0.34
rs9275592	6:32680620	HLA-DQA1/ HLA-DQA2	T<G	1.14 × 10 <sup>-40</sup>	2.08 (1.87–2.32)	0.98	0	0.15/0.08	0.18/0.11	0.09/0.05	0.20/0.09	0.18/0.10	0.18/0.10	0.28/0.16	0.18/0.10	0.19/0.11	0.15/0.10
rs4252134	6:161153527	PLG	C<T	1.23 × 10 <sup>-10</sup>	1.28 (1.19–1.39)	0.04	49	0.33/0.29	0.36/0.28	0.36/0.33	0.32/0.30	0.32/0.28	0.34/0.33	0.38/0.23	0.38/0.28	0.47/0.28	0.41/0.30
rs128738	5:131540875	P4HA2	T<G	4.60 × 10 <sup>-9</sup>	1.32 (1.20–1.45)	0.49	0	0.19/0.14	0.22/0.17	0.16/0.14	0.17/0.16	0.20/0.17	0.18/0.16	0.22/0.18	0.23/0.15	0.18/0.16	0.16/0.18

Abbreviations are as follows: GCA, giant cell arteritis; CTRL, controls; GRCh37, genome reference consortium human genome build 37; OR, per-allele odds ratio for the minor allele; CI, confidence interval; Q, Cochran's Q test. <sup>a</sup>N (GCA/CTRL): Spain = 805/1,323, UK = 352/2,965, Italy = 271/960, North America = 176/1,181, Germany = 160/667, France = 114/488, Norway = 104/121, Netherlands = 69/638, Switzerland = 46/500, Ireland = 37/282, combined = 2,134/9,125.

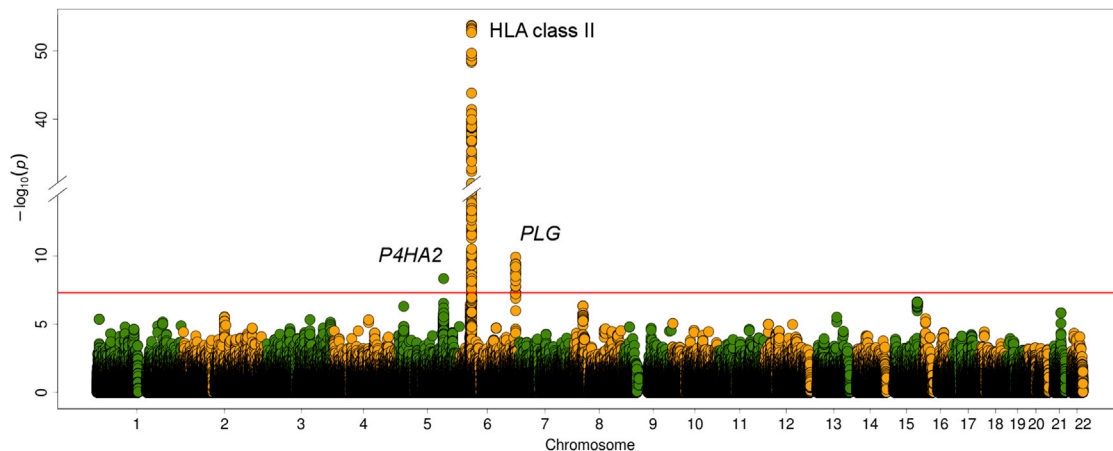
SNP = rs9275592, adjusted  $p = 1.78 \times 10^{-10}$ , OR = 1.48, 95% CI 1.31–1.66). No association at the genome-wide level of significance remained within the HLA after conditioning on both rs9268905 and rs9275592 (Table S5 and Figure S1).

Outside the HLA region, rs4252134, located in an intron of *PLG* at chromosome 6, represented the most strongly associated variant ( $p = 1.23 \times 10^{-10}$ , OR = 1.28, 95% CI 1.19–1.39, Figure 2). Although some heterogeneity was observed between studies ( $I^2 = 48.8$ ), consistent OR directions of the minor allele (toward risk) were observed in all sets (Tables 1 and S4; Figure S2). Other SNPs within the gene (both intronic and exonic) also showed significant  $p$  values, but their statistical significance was lost when conditioned on rs4252134 (Table S6 and Figure S3). Finally, another intronic SNP of *P4HA2* at chromosome 5, rs128738, surpassed the statistical threshold ( $p = 4.60 \times 10^{-9}$ , OR = 1.32, 95% CI 1.20–1.45). This genomic region also showed additional suggestive signals, but none of them was significant at the genome-wide level (Figure 2).

To confirm the above results, we obtained direct genotypes of the whole Spanish cohort using TaqMan probes for rs128738 and two perfect proxies ( $r^2 = 1$ ) of rs4252134 and rs9268905 (rs4252125 and rs2395185, respectively). The overall concordance reached after comparing TaqMan types with the corresponding imputed data was 99.94% for rs128738, 99.81% for rs4252134/rs4252125, and 99.94% for rs9268905/rs2395185.

### Functional Annotations of Proxies of the Non-HLA Hits

To prioritize variants that could drive the observed non-HLA associations, we identified all the SNPs in high linkage disequilibrium (LD,  $r^2 > 0.8$ ) with the lead signals in the European populations of 1KGPh3 (Tables S7 and S8) and used public online annotation tools to evaluate their possible functional implications. One *PLG* polymorphism (rs4252125) was annotated as missense in the dbSNP database. However, this variant was predicted to be benign according to PolyPhen-2 and showed no evidence of being potentially pathogenic after scoring the deleteriousness with CADD (raw score =  $-0.63$ , PHRED-like scaled C-score = 0.104). Then, using RegulomeDB, we identified those SNPs overlapping with known and predicted DNA elements with a higher probability of regulatory effects (score  $\leq 3$ ) (Tables S7 and S8) and performed a detailed functional annotation of these tagger variants and the lead SNPs using HaploReg (Tables 2 and S9–S11). Most of them overlapped with DNase hypersensitivity sites and histone marks enriched at promoters and enhancers (Table 2). Interestingly, some of these annotations were related to cell types and tissues involved in GCA pathophysiology. For example, *PLG* rs4252135 co-localized with DNase peaks in different immune cell lines and had a predicted enhancer chromatin state in lymphoblastoid cells (Tables S9 and S10). A higher enrichment of promoter and enhancer



**Figure 1. Manhattan Plot Representation of the GWAS Results**

The  $-\log_{10}$  of the inverse variance-weighted meta-analysis  $p$  values are plotted against their physical chromosomal position. The red line represents the genome-wide level of significance ( $p < 5 \times 10^{-8}$ ). The most relevant associations are highlighted.

epigenetic marks in these tissues was observed for the prioritized *P4HA2* variants (Table S9). Specifically, the lead signal rs128738 overlapped with enhancer histone marks in immune cells and with the imputed Transcription 3' Enhancer mark in the aorta (Table S10). Additionally, *P4HA2* rs156023 showed evidence of influencing enhancer activity in hematopoietic stem cells and neutrophils as well as promoter activity in mononuclear cells and monocytes (Tables S9 and S10).

In addition, key regulatory proteins (some of them related to the immune response) bound by ChIP-seq experiments as well as relevant motif disruptions were reported in most cases (Table 2).

Consequently, most prioritized SNPs also correlated with eQTL effects in peripheral blood monocytes and lymphoblastoid cells (Tables 2 and S11). Interestingly, rs101194 and rs152054 were reported to affect *P4HA2* expression in arterial tissues in the Genotype-Tissue Expression (GTEx) study,<sup>32</sup> with rs101194 specifically acting as *cis*-eQTL in the aorta (Table S11).

### Candidate Genes and Pathway Analysis

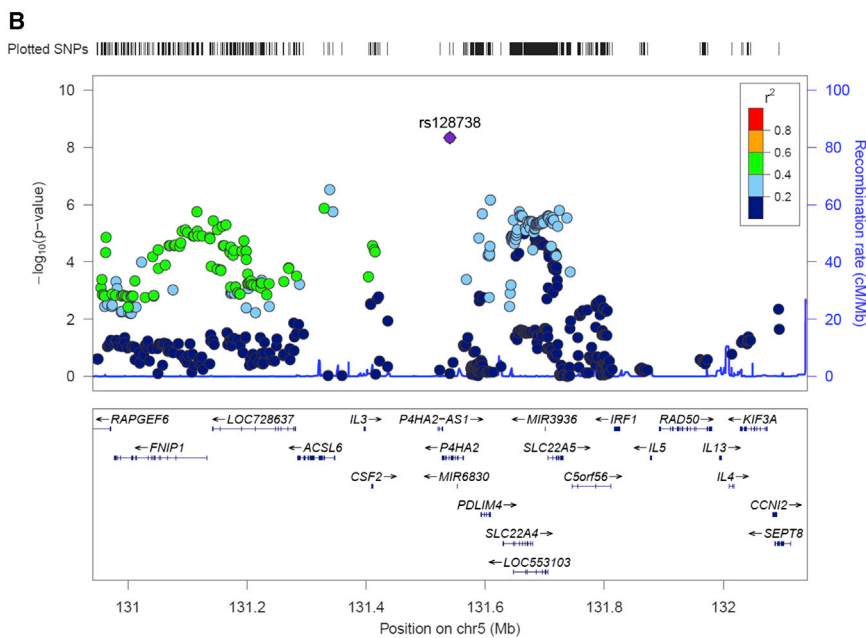
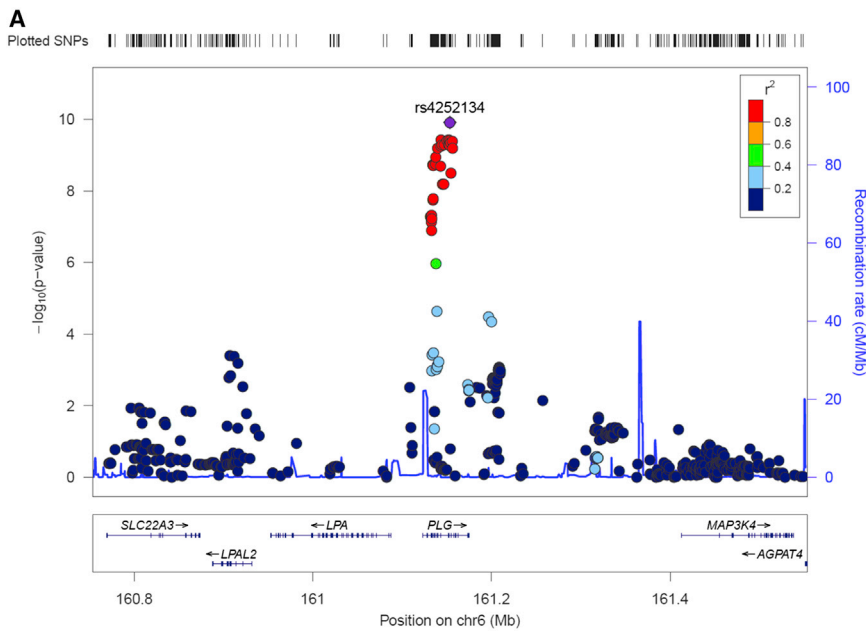
We also checked the statistical significance in our GWAS of previously described GCA-associated genomic regions ( $\pm 100$  kbp 3' and 5' of the reported gene) through candidate gene and Immunochip studies.<sup>5,8</sup> Significant associations after controlling for multiple testing were observed across most of the analyzed regions, with the myeloperoxidase (*MPO* [MIM: 606989]), tumor necrosis factor (*TNF* [MIM: 191160]), interleukin 6 (*IL6* [MIM: 147620]), nitric oxide synthase 2 (*NOS2* [MIM: 163730]), and *PTPN22* regions harboring the strongest non-HLA hits (*MPO* rs10853005,  $p = 7.19 \times 10^{-5}$ , OR = 0.84; *TNF* rs4959077,  $p = 2.08 \times 10^{-4}$ , OR = 1.42; *IL6* rs77741999,  $p = 2.17 \times 10^{-4}$ , OR = 1.20; *NOS2* rs4255826,  $p = 5.75 \times 10^{-4}$ , OR = 0.87; and *PTPN22* rs2476601,  $p = 7.88 \times 10^{-4}$ , OR = 1.24) (Tables

S12 and S13). Subsequently, we accomplished a protein-protein interaction (PPI) and biological pathway enrichment analysis with those candidate hits showing statistically significant signals after correction for multiple testing in our GWAS (Table S13). The molecular network of the selected proteins had significantly more interactions than expected (number of nodes, 13; number of edges, 14; average node degree, 2.15; clustering coefficient, 0.851; expected number of edges, 3; PPI enrichment,  $p = 1.13 \times 10^{-6}$ ; Figure 3). Interestingly, PLG represented a relevant node showing evidence of interaction with different proteins (e.g., NOS3, IL-6, and TNF) (Figure 3). Regarding the functional enrichments of the network, the most significantly associated GO processes were those related to regulation of both cell-cell adhesion and the immune/inflammatory response (Table S14).

### Discussion

This study represents an unbiased screening of genetic variation in GCA at the genome-wide level. GCA was one of the few types of vasculitis in which GWAS data were not available. Therefore, the results presented here may help to better understand the pathogenic mechanisms underlying this condition and its genetic similarities with other vasculitides. In this sense, our data reinforce the idea of GCA as an archetypal HLA class II disease mediated by an antigen-driven immune response,<sup>33</sup> which is in contrast not only to Takayasu's arteritis (TAK [MIM: 207600]), another large-vessel vasculitis, but also to other forms of vasculitis associated with class I molecules like Behçet disease (MIM: 109650).<sup>34</sup>

Two independent association signals with GCA predisposition were observed within the HLA region, one located between *HLA-DRA* and *HLA-DRB1* and another one between *HLA-DQA1* and *HLA-DQA2*. This is consistent with



**Figure 2. Regional Plots of the Associated Loci with GCA outside the HLA Region in the Overall Meta-analysis**  
 (A) Plasminogen (*PLG*) region.  
 (B) Prolyl 4-hydroxylase subunit alpha 2 (*P4HA2*) region.  
 Lead variants are highlighted in violet.

signal according to the dependence analysis. This gene encodes a secreted blood zymogen that can be converted through a complex conformational modification into two different active proteins, plasmin and angiostatin.<sup>35</sup> The plasminogen system has an important role in a wide spectrum of physiological processes, including wound healing, fibrinolysis, angiogenesis, and lymphocyte recruitment and inflammation via production of cytokines and reactive oxygen species,<sup>36,37</sup> all of them relevant processes in GCA.<sup>38</sup> Considering the opposite roles of plasmin and angiostatin in the induction of pro-angiogenic processes,<sup>36,37</sup> we hypothesize that the *PLG* risk alleles could unbalance the metabolism of its encoded protein leading to the characteristic pro-inflammatory phenotypes of GCA, although there is a lack of experimental support for this assumption. Interestingly, anti-plasminogen antibodies have been correlated with systemic disease activity in ANCA-associated vasculitis (MIM: 608710),<sup>39</sup> a type of vasculitis involving small- to medium-sized blood vessels that is also strongly associated with HLA class II molecules.<sup>40</sup> In addition, *PLG* has been shown to be

the amino acid model that we proposed using imputed Immunochip data to explain the HLA class II association with GCA, which comprised the positions 13 and 56 of the DRβ1 and DQα1 molecules, respectively.<sup>8</sup> Indeed, the strongest hit in the Immunochip study (which had partial overlap of the sample collections with this one) was a SNP that tagged the model (rs477515) in high LD with the two independent HLA lead SNPs that we observed in our GWAS (rs9268923:  $r^2 = 0.84$ ,  $D' = 0.95$ ; rs3957146:  $r^2 = 0.23$ ,  $D' = 0.99$ ).

Regarding the non-HLA associations, several variants within *PLG* were firmly associated with risk to develop GCA at the genome-wide level of significance in this study, although they all were in high LD and represented a single

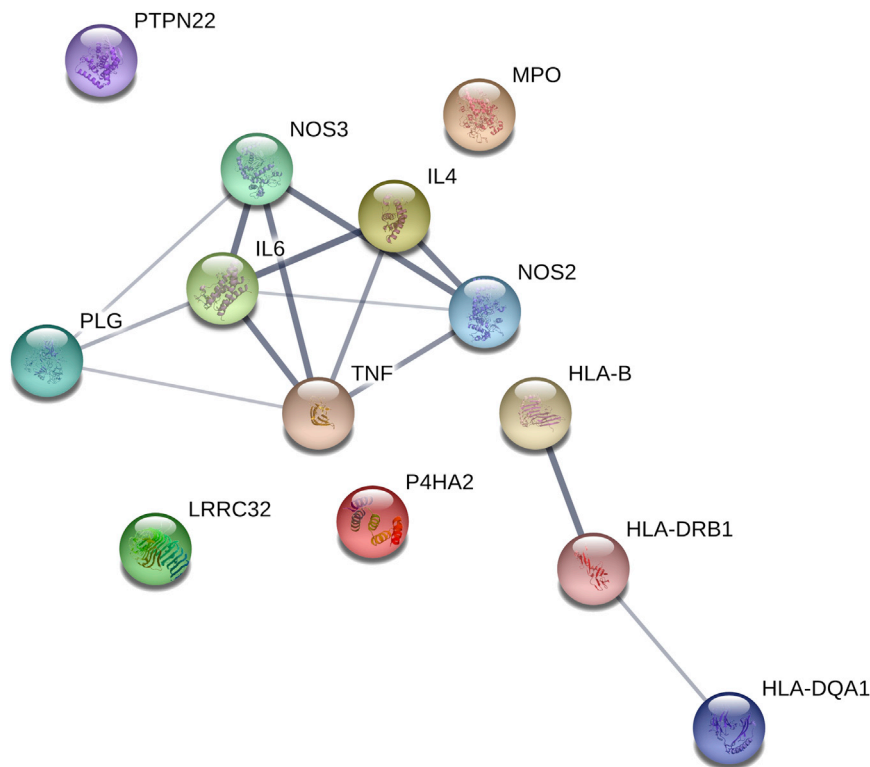
disease (MIM: 608320) and periodontitis (MIM: 260950), characterized by chronic inflammation,<sup>41</sup> and multiple sclerosis (MIM: 126200), which is also an immune-mediated condition.<sup>42</sup> Future studies aimed at improving the understanding of the zymogen activation may shed light into the *PLG* association with GCA.

*P4HA2* represents the second non-HLA hit in our GWAS. This gene encodes an isoform of the alpha subunit of the collagen prolyl 4-hydroxylase, which catalyzes the formation of 4-hydroxyproline from proline residues that is essential for collagen biosynthesis, as it is required for the proper three-dimensional folding of newly synthesized procollagen chains.<sup>43</sup> *P4HA2* is considered an important hypoxia response gene and its

**Table 2. Prioritized Variants of the Giant Cell Arteritis-Associated Non-HLA Regions Accordingly to the Functional Annotations of the Lead Signals and Their Proxies using ENCODE Data**

SNP	Location (GRCh37)	Locus	Distance from Hit (bp)	r <sup>2</sup>	RDB Score	Regulatory Chromatin Marks			Protein Binding and Regulatory Motifs		GRASP QTL and eQTL Annotations			
						Promoter Histone Marks	Enhancer Histone Marks	DNase Hypersensitivity	Proteins Bound (ChIP-Seq)	Regulatory Motifs Altered (PWM)	GRASP QTL Traits	p Value	Ref (PMID)	eQTL Hits
rs4252134	6:161153527	PLG (intronic)	0	NA	6	–	–	–	–	–	gene expression of DDR1 in peripheral blood monocytes	3.31 × 10 <sup>-6</sup>	20502693	–
rs4252126	6:161152294	PLG (intronic)	1,233	0.995	1f	yes	–	yes	CTCF	Pax-6	–	–	–	–
rs4252135	6:161154232	PLG (intronic)	705	1.000	1f	yes	yes*	yes*	CTCF, RAD21, SMC3, ZNF143, NFKB, FOXA1, FOXA2, ZNF263	COMP1	gene expression of DDR1 in peripheral blood monocytes	2.92 × 10 <sup>-6</sup>	20502693	–
rs34126283	6:161130002	PLG (intronic)	23,525	0.920	3a	yes	yes	yes	GATA2	Cdc5, E2F, Evi-1, HDAC2, PLZF, Pou2f2, TATA	–	–	–	–
rs4252125	6:161152240	PLG (missense)	1,287	0.995	3a	yes	–	yes	CTCF, RAD21	AP-3	gene expression of UNQ9391 in liver	4.09 × 10 <sup>-14</sup>	22006096	–
rs128738	5:131540875	P4HA2 (intronic)	0	NA	ND	yes	yes*	–	–	BDP1	–	–	–	24
rs101194	5:131515413	P4HA2 (intergenic)	25,462	0.901	3a	yes	yes*	yes	GATA2, P300	CEBPB	gene expression of P4HA2 in lymphoblastoid cell lines	2.00 × 10 <sup>-7</sup>	17873874	30
											gene expression of SLC22A5 in peripheral blood monocytes	1.16 × 10 <sup>-8</sup>	20502693	–
rs152054	5:131519540	P4HA2 (intergenic)	21,335	0.967	3a	yes*	yes*	yes*	CFOS	BAF155, BCL, Egr-1, Ets, FEV, GATA, NERF1a, Nrf-2, PU.1, Pax-5, SETDB1, SIX5, Tel2, Znf143	–	–	–	25
rs152051	5:131539025	P4HA2 (intronic)	1,850	1.000	3a	yes	yes*	yes*	YY1, NFKB	AIRE, Foxa, GR, VDR	gene expression of P4HA2 in CEU-CHB-JPT-YRI lymphoblastoid cell lines	4.66 × 10 <sup>-9</sup>	17873874	28
rs156023	5:131545168	P4HA2 (intronic)	4,293	0.967	3a	yes*	yes*	–	–	AIRE, LBP-1, LBP-9	–	–	–	22

Chromatin marks in blood tissues are indicated with an asterisk. Abbreviations are as follows: SNP, single nucleotide polymorphism; GRCh37, genome reference consortium human genome build 37; bp, base pair; RDB, Regulome database; PWM, position weight matrix ID; GRASP, genome-wide repository of associations between SNPs and phenotypes; PMID, PubMed identifier; eQTL, expression quantitative trait loci. RDB scores: 1f, eQTL + TF binding / DNase peak; 3a, TF binding + any motif + DNase peak; 6, other; ND, no data; NA, not applicable.



**Figure 3. Interaction Network Formed for GCA Risk Loci**

GWAS genes of this study and those previously identified through Immunochip and consistent candidate gene studies were included in the analysis. STRING database was used to look for both direct and indirect interactions among selected genes. The width of the blue lines indicates the reliability of each interaction.

expression is regulated by hypoxia-inducible factor-1 (HIF-1).<sup>44</sup> Other relevant HIF-1-induced genes include serpin family E member 1 (*SERPINE1* [MIM: 173360], which is the principal inhibitor of the plasmin activation) and genes that have been previously associated with GCA risk through candidate gene studies, such as vascular endothelial growth factor A (*VEGFA* [MIM: 192240], a potent endothelial growth factor), matrix metalloproteinase 9 (*MMP9* [MIM: 120361], involved in the breakdown of extracellular matrix), and *IL6* (a pro-inflammatory cytokine).<sup>5,45,46</sup> Indeed, the hypoxic induction of all these HIF-1 target genes (and, indirectly, the inhibition of plasmin formation) is also related to the typical processes involved in the lesions of GCA-affected individuals, i.e., fibrosis, inflammation, destruction of the internal elastic lamina, and vascular remodeling, with proliferation and migration of medial myofibroblasts and neoangiogenesis.<sup>38,45</sup> The prioritized *P4HA2* SNPs proposed here correlated with *cis*-eQTLs in immune cells and arterial tissues. In particular, expression data indicate that rs101194 may influence *P4HA2* expression in whole blood, lymphoblastoid cells, and the aorta, which is one of the most severely affected vessels in GCA.<sup>32,38,47–49</sup>

Overall, our results are consistent with the currently accepted understanding of the pathophysiology of GCA, in which vascular remodeling and angiogenesis, either under hypoxic conditions or by dysregulation of hypoxia-sensitive genes, are critical to the development of the clinical presentations.<sup>50</sup> Future analysis of the genetic overlap between GCA and other forms of vasculitis

using GWAS data may help to elucidate whether these pathogenic processes are a common feature in vasculitides and to identify other relevant pathways for the development of GCA.

To summarize, through the analysis of common variation across the whole genome, we have identified *PLG* and *P4HA2* as the main non-HLA genetic factors underlying GCA predisposition. Their crucial role in neoangiogenesis highlights the high relevance of this process in

the pathogenic mechanisms leading to this form of vasculitis.

### Supplemental Data

Supplemental Data include 3 figures and 14 tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.11.013>.

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## Web Resources

1000 Genomes, <http://www.internationalgenome.org/>  
BEAGLE, <http://faculty.washington.edu/browning/beagle/beagle.html>  
CADD, <http://cadd.gs.washington.edu/>  
CaTS – Power Calculator, <http://csg.sph.umich.edu/abecasis/CaTS/>  
ENCODE, <https://www.encodeproject.org/>  
GEC: Genetic Type I Error Calculator, <http://statgenpro.psychiatry.hku.hk/gec/>  
Gene Ontology Consortium, <http://geneontology.org/>  
GEO, <http://www.ncbi.nlm.nih.gov/geo/>  
HaploReg, <http://www.broadinstitute.org/mammals/haploreg/haploreg.php>  
IMPUTE2, [http://mathgen.stats.ox.ac.uk/impute/impute\\_v2.html](http://mathgen.stats.ox.ac.uk/impute/impute_v2.html)  
LocusZoom, <http://locuszoom.sph.umich.edu/locuszoom/>  
NCBI, <http://www.ncbi.nlm.nih.gov/>  
OMIM, <http://www.omim.org/>  
PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink/>

PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>  
RegulomeDB, <http://RegulomeDB.org/>  
Roadmap, <http://www.roadmapepigenomics.org/>  
STRING, <http://www.string-db.org/>

## References

1. Manolio, T.A. (2013). Bringing genome-wide association findings into clinical use. *Nat. Rev. Genet.* *14*, 549–558.
2. Jennette, J.C., Falk, R.J., Bacon, P.A., Basu, N., Cid, M.C., Ferrario, F., Flores-Suarez, L.F., Gross, W.L., Guillevin, L., Hagen, E.C., et al. (2013). 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum.* *65*, 1–11.
3. Gonzalez-Gay, M.A., Barros, S., Lopez-Diaz, M.J., Garcia-Porrua, C., Sanchez-Andrade, A., and Llorca, J. (2005). Giant cell arteritis: disease patterns of clinical presentation in a series of 240 patients. *Medicine (Baltimore)* *84*, 269–276.
4. Gonzalez-Gay, M.A., Vazquez-Rodriguez, T.R., Lopez-Diaz, M.J., Miranda-Filloo, J.A., Gonzalez-Juanatey, C., Martin, J., and Llorca, J. (2009). Epidemiology of giant cell arteritis and polymyalgia rheumatica. *Arthritis Rheum.* *61*, 1454–1461.
5. Carmona, F.D., González-Gay, M.A., and Martín, J. (2014). Genetic component of giant cell arteritis. *Rheumatology (Oxford)* *53*, 6–18.
6. Serrano, A., Márquez, A., Mackie, S.L., Carmona, F.D., Solans, R., Miranda-Filloo, J.A., Hernández-Rodríguez, J., Cid, M.C., Castañeda, S., Morado, I.C., et al.; UK GCA Consortium Spanish GCA Consortium (2013). Identification of the PTPN22 functional variant R620W as susceptibility genetic factor for giant cell arteritis. *Ann. Rheum. Dis.* *72*, 1882–1886.
7. Márquez, A., Hernández-Rodríguez, J., Cid, M.C., Solans, R., Castañeda, S., Fernández-Contreras, M.E., Ramentol, M., Morado, I.C., Narváez, J., Gómez-Vaquero, C., et al.; Spanish GCA Consortium (2014). Influence of the IL17A locus in giant cell arteritis susceptibility. *Ann. Rheum. Dis.* *73*, 1742–1745.
8. Carmona, F.D., Mackie, S.L., Martín, J.E., Taylor, J.C., Vaglio, A., Eyre, S., Bossini-Castillo, L., Castañeda, S., Cid, M.C., Hernández-Rodríguez, J., et al.; Spanish GCA Group (2015). A large-scale genetic analysis reveals a strong contribution of the HLA class II region to giant cell arteritis susceptibility. *Am. J. Hum. Genet.* *96*, 565–580.
9. Hunder, G.G., Bloch, D.A., Michel, B.A., Stevens, M.B., Arend, W.P., Calabrese, L.H., Edworthy, S.M., Fauci, A.S., Leavitt, R.Y., Lie, J.T., et al. (1990). The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum.* *33*, 1122–1128.
10. Barrett, J.H., Iles, M.M., Harland, M., Taylor, J.C., Aitken, J.F., Andresen, P.A., Akslén, L.A., Armstrong, B.K., Avril, M.F., Azizi, E., et al.; GenoMEL Consortium (2011). Genome-wide association study identifies three new melanoma susceptibility loci. *Nat. Genet.* *43*, 1108–1113.
11. Bentham, J., Morris, D.L., Cunninghame Graham, D.S., Pinder, C.L., Tomblinson, P., Behrens, T.W., Martín, J., Fairfax, B.P., Knight, J.C., Chen, L., et al. (2015). Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat. Genet.* *47*, 1457–1464.
12. Consortium, W.T.C.C.; and Wellcome Trust Case Control Consortium (2007). Genome-wide association study of

- 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447, 661–678.
13. Dubois, P.C., Trynka, G., Franke, L., Hunt, K.A., Romanos, J., Curtotti, A., Zhernakova, A., Heap, G.A., Adány, R., Aromaa, A., et al. (2010). Multiple common variants for celiac disease influencing immune gene expression. *Nat. Genet.* 42, 295–302.
  14. Gregersen, P.K., Kosoy, R., Lee, A.T., Lamb, J., Sussman, J., McKee, D., Simpfendorfer, K.R., Pirskanen-Matell, R., Piehl, F., Pan-Hammarstrom, Q., et al. (2012). Risk for myasthenia gravis maps to a (151) Pro→Ala change in TNIP1 and to human leukocyte antigen-B\*08. *Ann. Neurol.* 72, 927–935.
  15. Radstake, T.R., Gorlova, O., Rueda, B., Martin, J.E., Alizadeh, B.Z., Palomino-Morales, R., Coenen, M.J., Vonk, M.C., Voskuyl, A.E., Schuerwegh, A.J., et al.; Spanish Scleroderma Group (2010). Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat. Genet.* 42, 426–429.
  16. Rotger, M., Dang, K.K., Fellay, J., Heinzen, E.L., Feng, S., DeScombes, P., Shianna, K.V., Ge, D., Günthard, H.F., Goldstein, D.B., Telenti, A.; Swiss HIV Cohort Study; and Center for HIV/AIDS Vaccine Immunology (2010). Genome-wide mRNA expression correlates of viral control in CD4+ T-cells from HIV-1-infected individuals. *PLoS Pathog.* 6, e1000781.
  17. Stahl, E.A., Raychaudhuri, S., Remmers, E.F., Xie, G., Eyre, S., Thomson, B.P., Li, Y., Kurreeman, F.A., Zhernakova, A., Hinks, A., et al.; BIRAC Consortium; and YEAR Consortium (2010). Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* 42, 508–514.
  18. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575.
  19. Howie, B.N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 5, e1000529.
  20. Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R.; and 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. *Nature* 526, 68–74.
  21. Skol, A.D., Scott, L.J., Abecasis, G.R., and Boehnke, M. (2006). Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat. Genet.* 38, 209–213.
  22. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., and Willer, C.J. (2010). LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 26, 2336–2337.
  23. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. *Nat. Methods* 7, 248–249.
  24. Kircher, M., Witten, D.M., Jain, P., O’Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* 46, 310–315.
  25. Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S., et al. (2012). Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22, 1790–1797.
  26. Ward, L.D., and Kellis, M. (2016). HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res.* 44 (D1), D877–D881.
  27. Gene Ontology Consortium (2015). Gene Ontology Consortium: going forward. *Nucleic Acids Res.* 43, D1049–D1056.
  28. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al.; The Gene Ontology Consortium (2000). Gene ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29.
  29. Thomas, P.D., Campbell, M.J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., Diemer, K., Muruganujan, A., and Narechania, A. (2003). PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* 13, 2129–2141.
  30. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P., et al. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 43, D447–D452.
  31. Li, M.X., Yeung, J.M., Cherny, S.S., and Sham, P.C. (2012). Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Hum. Genet.* 131, 747–756.
  32. GTEx Consortium (2015). Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 348, 648–660.
  33. Carmona, F.D., Martín, J., and González-Gay, M.A. (2016). New insights into the pathogenesis of giant cell arteritis and hopes for the clinic. *Expert Rev. Clin. Immunol.* 12, 57–66.
  34. Carmona, F.D., Martín, J., and González-Gay, M.A. (2015). Genetics of vasculitis. *Curr. Opin. Rheumatol.* 27, 10–17.
  35. Law, R.H., Abu-Ssaydeh, D., and Whisstock, J.C. (2013). New insights into the structure and function of the plasminogen/plasmin system. *Curr. Opin. Struct. Biol.* 23, 836–841.
  36. Miles, L.A., and Parmer, R.J. (2013). Plasminogen receptors: the first quarter century. *Semin. Thromb. Hemost.* 39, 329–337.
  37. Moser, T.L., Stack, M.S., Asplin, I., Enghild, J.J., Højrup, P., Everitt, L., Hubchak, S., Schnaper, H.W., and Pizzo, S.V. (1999). Angiostatin binds ATP synthase on the surface of human endothelial cells. *Proc. Natl. Acad. Sci. USA* 96, 2811–2816.
  38. Ly, K.H., Régent, A., Tamby, M.C., and Mouthon, L. (2010). Pathogenesis of giant cell arteritis: More than just an inflammatory condition? *Autoimmun. Rev.* 9, 635–645.
  39. Hao, J., Wang, C., Gou, S.J., Zhao, M.H., and Chen, M. (2014). The association between anti-plasminogen antibodies and disease activity in ANCA-associated vasculitis. *Rheumatology (Oxford)* 53, 300–306.
  40. Lyons, P.A., Rayner, T.F., Trivedi, S., Holle, J.U., Watts, R.A., Jayne, D.R., Baslund, B., Brenchley, P., Bruchfeld, A., Chaudhry, A.N., et al. (2012). Genetically distinct subsets within ANCA-associated vasculitis. *N. Engl. J. Med.* 367, 214–223.
  41. Schaefer, A.S., Bochenek, G., Jochens, A., Ellinghaus, D., Dom-misch, H., Güzeldemir-Akçakanat, E., Graetz, C., Harks, I., Jockel-Schneider, Y., Weinspach, K., et al. (2015). Genetic evidence for PLASMINOGEN as a shared genetic risk factor of coronary artery disease and periodontitis. *Circ Cardiovasc Genet* 8, 159–167.

42. Sadovnick, A.D., Traboulsee, A.L., Bernales, C.Q., Ross, J.P., Forwell, A.L., Yee, I.M., Guillot-Noel, L., Fontaine, B., Courmu-Rebeix, I., Alcina, A., et al. (2016). Analysis of plasminogen genetic variants in multiple sclerosis patients. *G3 (Bethesda)* *6*, 2073–2079.
43. Myllyharju, J. (2003). Prolyl 4-hydroxylases, the key enzymes of collagen biosynthesis. *Matrix Biol.* *22*, 15–24.
44. Gilkes, D.M., Bajpai, S., Chaturvedi, P., Wirtz, D., and Semenza, G.L. (2013). Hypoxia-inducible factor 1 (HIF-1) promotes extracellular matrix remodeling under hypoxic conditions by inducing P4HA1, P4HA2, and PLOD2 expression in fibroblasts. *J. Biol. Chem.* *288*, 10819–10829.
45. Deng, W., Feng, X., Li, X., Wang, D., and Sun, L. (2016). Hypoxia-inducible factor 1 in autoimmune diseases. *Cell. Immunol.* *303*, 7–15.
46. Dimova, E.Y., Samoylenko, A., and Kietzmann, T. (2004). Oxidative stress and hypoxia: implications for plasminogen activator inhibitor-1 expression. *Antioxid. Redox Signal.* *6*, 777–791.
47. Stranger, B.E., Nica, A.C., Forrest, M.S., Dimas, A., Bird, C.P., Beazley, C., Ingle, C.E., Dunning, M., Flicek, P., Koller, D., et al. (2007). Population genomics of human gene expression. *Nat. Genet.* *39*, 1217–1224.
48. Westra, H.J., Peters, M.J., Esko, T., Yaghootkar, H., Schurmann, C., Kettunen, J., Christiansen, M.W., Fairfax, B.P., Schramm, K., Powell, J.E., et al. (2013). Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* *45*, 1238–1243.
49. Lappalainen, T., Sammeth, M., Friedländer, M.R., 't Hoen, P.A., Monlong, J., Rivas, M.A., González-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G., et al.; Geuvadis Consortium (2013). Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* *501*, 506–511.
50. Kaiser, M., Younge, B., Björnsson, J., Goronzy, J.J., and Weyand, C.M. (1999). Formation of new vasa vasorum in vasculitis. Production of angiogenic cytokines by multinucleated giant cells. *Am. J. Pathol.* *155*, 765–774.