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A cross-phenotype analysis of Immunochip data identifies \textit{KDM4C} as a relevant locus for the development of systemic vasculitis.

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Word count: 2,997
ABSTRACT

Objective: Systemic vasculitides represent a heterogeneous group of rare complex diseases of the blood vessels with a poorly understood aetiology. To investigate the shared genetic component underlying their predisposition, we performed the first cross-phenotype meta-analysis of genetic data from different clinically distinct patterns of vasculitis.

Methods: Immunochip genotyping data from 2,465 patients diagnosed with giant cell arteritis (GCA), Takayasu’s arteritis (TAK), ANCA-associated vasculitis (AAV), or IgA vasculitis (IgAV), as well as 4,632 unaffected controls were analysed to identify common susceptibility loci for vasculitis development. The possible functional consequences of the associated variants were interrogated using publicly available annotation data.

Results: The strongest association signal corresponded with an intergenic polymorphism located between HLA-DQB1 and HLA-DQA2 (rs6932517, P=4.16E-14, OR=0.74). This SNP is in moderate linkage disequilibrium with the disease-specific HLA class II associations of each type of vasculitis and could mark them. Outside the HLA region, we identified the KDM4C gene as a common risk locus for vasculitides (highest peak rs16925200, P=6.23E-07, OR=1.75). This gene encodes a histone demethylase involved in the epigenetic control of gene expression.

Conclusions: Through a combined analysis of Immunochip data, we have identified KDM4C as a new risk gene shared between systemic vasculitides, consistent with the increasing evidences of the crucial role that the epigenetic mechanisms have in the development of complex immune-mediated conditions.

Keywords: Vasculitides, Immunochip, HLA, KDM4C
INTRODUCTION

The vasculitides constitute a heterogeneous group of diseases of complex aetiology characterised by a chronic inflammation of the blood vessels. They show a wide spectrum of clinical manifestations that depend on the affected vessels in the arterial or venous system. As a consequence, overlapping of pathophysiological mechanisms is frequent amongst them [1]. To better define the different clinical forms of vasculitis, the Chapel Hill Consensus Conference proposed a nomenclature system in which the most common vasculitides were subdivided into three main categories, i.e. large vessel vasculitis (LVV), medium vessel vasculitis, and small vessel vasculitis (SVV), based on the distribution of vessel involvement [2]. In this regard, two diseases account for most cases of LVV, i.e. giant cell arteritis (GCA) and Takayasu’s arteritis (TAK), which mainly involve arteries of large calibre such as the aorta and its major branches. On the other hand, IgA vasculitis (IgAV) and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) are classified as SVV, as inflammation predominantly affects arterioles, small arteries, capillaries, and venules [2, 3].

Although the aetiology of vasculitides remains unclear, cumulating data suggest that they are triggered in genetically predisposed individuals by the concurrence of certain environmental factors [4]. The importance of the genetic component has been consistently supported by evidence of familial aggregation, differential prevalence depending on the ethnicity, and multiple genetic associations with disease susceptibility and progression reported during the last years [5]. In this regard, the strongest association signals in most vasculitides correspond with genetic variants within the human leukocyte antigen (HLA) region, which suggests an important role of the immune system in their pathophysiology. However, each form of vasculitis has distinct HLA association markers that define them, most likely due to disease-specific differences in antigenic drivers [5].
Despite the considerable increase in our understanding of the genetic basis of systemic vasculitis during the last decade, the number of identified risk loci for most types of vasculitis remains significantly lower than other immune-mediated diseases [5, 6]. Taking into account the low prevalence of these pathologies in the general population [1], one of the main limitations is the lack of statistical power to identify susceptibility signals with modest effects, given the large sample size required for that. Therefore, additional strategies are necessary to unravel the genetic background underlying these disorders. In this sense, the combination of the genetic data from different clinical syndromes with vasculitis as a single underpinning phenotype has been successfully applied for identifying shared genetic components [7-10]. One of the most successful genetic platforms to study immune-mediated diseases is the Immunochip, which is a custom high-density array that allows the analysis of genetic variants across multiple known susceptibility loci for autoimmune and autoinflammatory disorders [11]. This platform has been successful in identifying specific and common risk variants for many autoimmune and autoinflammatory diseases [12], including GCA and TAK [9, 13, 14].

Considering the above, and with the aim to improve the knowledge of the genetic component of vasculitides, we performed a cross-phenotype meta-analysis by combining Immunochip data of individuals diagnosed with four different forms of vasculitis.
MATERIAL AND METHODS

Study population

The study population comprised 2,465 cases from four vasculitides (GCA, TAK, AAV, and IgAV) and 4,632 unaffected controls. A detailed description of the study population can be found in the supplementary material.

Genotyping and quality controls

The GCA and TAK raw datasets were obtained from previously published Immunochip studies [13, 14]. For AAV and IgAV, new genotyping data were generated. In this case, genomic DNA was extracted from peripheral blood samples with standard methods. Genotyping was performed using the Illumina Immunochip custom genotyping array (Infinium® ImmunoArray-24 v1.0 and v2.0 BeadChip, respectively) according to the manufacturer’s instructions. To ensure consistency amongst datasets, we obtained the raw data of each of them and applied the same quality control procedures in parallel (including both the previously published studies and the newly genotyped data) using PLINK v1.07 [15]. In this regard, low quality SNPs were filtered out if they had call rates <98%, minor allele frequencies (MAF) <0.01, and deviated from Hardy-Weinberg equilibrium (HWE; P < 0.001). Samples were also removed if they showed a genotyping calling < 95%, had a pair of first-degree relatives (identity by descent > 0.4), or were considered outliers by plotting at > 4 standard deviations from the cluster centroids of each population using the first ten principal components (PC; estimated using the ancestry markers included in the Immunochip). Sex chromosomes were not analysed.

Imputation

SNP genotype imputation of whole-genome data after QC was performed separately for each dataset as implemented in IMPUTE v.2 [16]. The 1000 Genome Project Phase
3 data were used as reference panel [17]. Briefly, we updated the SNP IDs and positions to rs# and build 37 (HG19), respectively, with PLINK. Chunks of 50,000 Mbp were then generated and imputed considering the most likely call for merging genotypes (> 0.9 probability threshold). Imputed data were also subjected to stringent quality filters as follows: call rate < 0.98, MAF < 0.01, HWE P < 0.001.

**Imputation of the HLA region**

We performed a more comprehensive imputation of this genomic region to generate imputed data of classical HLA alleles (with two and four digits), polymorphic amino acid positions, and additional SNPs. In this case, we extracted the non-imputed genotyping data from 20,000,000 to 40,000,000 base-pairs at chromosome six (which comprises the extended HLA region) and used the SNP2HLA v1.0.3 package [18] with a reference panel of 5,225 individuals of European ancestry with available genotyping data for 8,961 genetic variants (common SNPs and INDEL polymorphisms) as well as types for HLA class I and II molecules at four-digit resolution, as described [13, 19].

**Data analysis**

PLINK and the R-base software under GNU Public license v2 were used to analyse the imputed data. First, we used logistic regression on the best-guess genotypes (> 0.9 probability), assuming an additive model and using the ten first PCs and gender as covariates, to analyse each dataset separately. The results were then meta-analysed by means of the inverse variance method under a fixed effects model, except for the HLA region in which a random effects model was considered. Heterogeneity of the odds ratios (OR) across the different datasets was estimated using both Cochran's Q test and $I^2$. The study-wide threshold for both statistical significance and trends of association were set at 1.28E-06 and 2.55E-05, respectively, according to the estimation by the Genetic Type I Error Calculator (CEG) software [20], which implements a Bonferroni-based validated method to control the genome-wide type I
error rate at 0.05. R was used to generate the overall Manhattan plots, and the LocusZoom V.1.1 tool (http://csg.sph.umich.edu/locuszoom/) [21] was used to obtain the regional association plots. Finally, the possible functional implication of the association signals was estimated using the publicly available browser resources RegulomeDB [22], HaploReg v4.1 [23], and Blood NESDA NTR Conditional eQTL Catalog [24].
RESULTS

A total of 2,425 vasculitis cases (1,008 GCA cases, 437 TAK cases, 303 IgAV cases, and 677 AAV cases) and 4,526 controls, as well as 191,948 genetic markers remained after QC filtering and were included in the analyses.

Cross-phenotype meta-analysis

The combined analysis of the Immunochip data from all four pathologies showed evidence for common genetic associations in two loci, i.e. HLA and lysine demethylase 4C (KDM4C), both surpassing the study-wide level of significance (Figure 1).

As the strongest association signals were located within the HLA region, we decided to carry out a more robust analysis of this genomic region in order to fine-map and localise the putative causal variants. Therefore, we conducted another meta-analysis of imputed data of SNPs, classical alleles, and polymorphic amino acid positions. Due to the complex linkage disequilibrium (LD) structure and disease-specific associations of this region, we used a random effect model. As shown in Figure S1 and Table S1, several variants in high LD were significantly associated to vasculitis predisposition, with an intergenic polymorphism located between HLA-DQB1 and HLA-DQA2 representing the peak signal (rs6932517, $P=4.16\times10^{-14}$, OR=0.74). This marker showed both evidence of nominal association with each disease separately and consistent ORs across studies (GCA\textsubscript{Spain}, $P=1.26\times10^{-04}$, OR=0.73; GCA\textsubscript{UK}, $P=1.52\times10^{-03}$, OR=0.78; TAK\textsubscript{Turkey}, $P=3.91\times10^{-02}$, OR=0.78; TAK\textsubscript{USA}=4.03\times10^{-03}, OR=0.60; IgAV, $P=1.51\times10^{-04}$, OR=0.67; AAV, $P=3.80\times10^{-04}$, OR=0.77). The remaining association peaks of the HLA lost their statistical significance after conditioning on rs6932517, thus suggesting that this SNP was sufficient to explain most of the observed HLA signals.

In order to evaluate whether rs6932517 represented a true novel susceptibility marker for the analysed vasculitides or just a marker of the established risk variants for
each type of vasculitis, we decided to calculate the LD between them [13, 14, 25, 26]. As shown in Table S2, evidence of moderate LD was observed in all cases (D' > 0.8).

Outside the HLA region, the most strongly associated polymorphism corresponded with the KDM4C SNP rs16925200 (P=6.23E-07, OR=1.75; Table 1). No heterogeneity amongst the ORs and consistent OR directions were observed across studies (Table S3). This genomic region also harboured additional polymorphisms with significant P-values, all of them in high LD with rs16925200, which suggests that they represent genetic markers of the same signal (Figure S2). In addition, the association of this locus remained significant when the association test was performed under a random effects model (Table S3). Interestingly, one of these polymorphisms (rs12003023) showed a nominal association with Behçet's disease (BD), a variable vessel vasculitis, in a previously published Immunochip study of this form of vasculitis (P=1.11E-02, OR=1.78, 95% CI=1.14-2.79) [27]. Consistent with this, the statistical significance of the overall meta-analysis for the KDM4C rs12003023 variant increased after including the BD data (rs10203023, P=4.08E-08, OR=1.69, P(Q)=7.70E-01, I²=0).

Trend of associations (P < 2.55E-5) were also observed for additional SNPs located in different genomic regions (Table 1), including RGS21/RGS1 (rs6704162, P=4.48E-06, OR=1.25), protein kinase C theta (PRKCO; rs7895774, P=1.40E-05, OR=0.83), LOC10012963/TNFSF15 (rs4574921; P=1.47E-05; OR=0.83), Nedd4 family interacting protein 1 (NDFIP1; rs116760964, P=1.72E-05, OR=1.71), TMEM174/FOXO1 region (rs12517414, P=2.96E-05, OR=1.27), KIAA1841 (rs74995325, P=3.29E-05, OR=1.64), AC008703.1 (rs6869688, P=3.54E-05, OR=0.85), zinc finger, MIZ-type containing 1 (ZMIZ1; rs1250544, P=3.90E-05, OR=0.85), and SERPINE1 mRNA binding protein 1 (SERBP1, rs1890928, P=4.91E-05, OR=0.80).

Variants from known susceptibility loci for each individual form of vasculitis were checked in our cross-phenotype meta-analysis. Although no associations at the
genome-wide level of significance were observed within those genes, three out of the five genes examined harboured at least one SNP with suggestive P-values (P<0.05; Table S4)

**Functional annotation of KDM4C associated polymorphisms**

Given that the associated KDM4C polymorphisms were located in non-coding regions, we decided to further evaluate their possible functional implications using bioinformatics approaches. First, we identified all the SNP taggers (r² > 0.8) of the lead variant (rs16925200) in the European populations of the 1000 genomes project. Fourteen SNPs met this criterion, with eight of them not being included in our merged dataset. Some of these KDM4C variants showed functional annotations of interest, such as overlapping with histone marks and DNAse hypersensitivity peaks enriched at enhancers as well as possible influence to protein binding and regulatory motifs (Table 2). In addition, seven variants have been described as cis-eQTLs regulating KDM4C expression in whole blood. Specifically, rs10975993 had a score 2b in RegulomeDB indicating that this SNP likely affects transcription factor binding, in particular that of small MAF proteins like MAF bZIP transcription factor K (MAFK). These proteins are induced by the TGF-β pathway and seem to influence the metabolism of antioxidant and xenobiotic metabolism, being implicated in the susceptibility to various type of cancer [28]. However, none of these marks were reported in cell types or tissues related to vasculitis pathology.
DISCUSSION

The results of this study suggest the existence of a common genetic component that may influence the susceptibility to develop different forms of vasculitis. Particularly, besides the widely known association with the HLA region, we have identified KDM4C, also known as JHDM3C or JMJD2C, as a new shared locus between GCA, TAK, AAV, and IgAV, which could be also shared with BD. To our knowledge, this is the first time that this gene has been implicated in the genetic predisposition to any immune-mediated disease.

KDM4C is a member of the Jumonji domain 2 (JMJD2) family. It is located on chromosome 9p24.1 and encodes a trimethylation-specific demethylase that controls the methylation state of histone residues, affecting gene expression and chromatin structure [29]. The main function of KDM4C is the activation of genes by removing methyl groups from the repressive histone mark trimethylated lysine 9 of the N-terminal tail of H3 (H3K9me3). Consistent with this, KDM4C has been related to cell growth in different types of cancer, and it has been reported to influence concentrations of both circulating growth factors, such as stem cell growth factor beta (SCGF-β), and cytokines like cutaneous T-cell attracting chemokine (CCL27) [30, 31]. Moreover, a recent GWAS performed in an Ameridian ancestry population evidenced a suggestive association of a KDM4C variant with systemic lupus erythematosus [32].

Interestingly, it has been described that the H3K9me3 mark was significantly enriched in the promoters of both systemic lupus erythematosus (SLE)- and rheumatoid arthritis (RA)-associated genes from GWAS in the Gm12878 EBV-transformed B lymphoblastoid cell line, suggesting that epigenetics may have a relevant role in autoimmunity [33]. Indeed, H3K9me3 and other histone marks (including H3K4Me3, H3K4Me2, and H3R17Me2) seem to be introduced in a transcription-coupled manner during the IFN-γ-induced activation of HLA class II
genes, and it has been proposed that they may be implicated in the transcription elongation and the establishment of transcriptional memory of the \textit{HLA-DRA} gene [34].

On the other hand, it has been described that intronic polymorphisms located near the 3’ end of \textit{KDM4C} affect the expression of this gene and, consequently, the expression levels of its target genes. Hence, genetic variation within \textit{KDM4C} may have an impact on the epigenetic regulation of other genes, which evidences the close link that exists between genetic and epigenetic mechanisms [30].

Genetic-epigenetic relationships like the one described above could explain many genetic associations of non-coding variants with complex diseases for which no functional implication has been established yet. In this sense, the role of epigenetics in the pathogenesis of immune-mediated diseases seems now undeniable [35]. Regarding vasculitides, insights into the high relevance of the epigenetic mechanisms for disease susceptibility have been published during the last years [36]. A recent study, in which chromatin signature patterns within genetic susceptibility regions in four types of systemic vasculitis (including TAK, AAV, Behçet's disease, and Kawasaki disease) were analysed, identified potential specific immune cells involved in vasculitis pathogenesis through epigenetic alterations caused by genetic risk variants. Interestingly, the epigenetic changes mediating disease risk included different methylation states in different immune cells of the histone H3 such as H3K27me3 and H3K4me1 (associated with AAV and TAK, respectively) [37]. Additionally, it has been described that AAV patients show an increased expression of the JMJD3 protein (a histone demethylase similar to KDM4C), which lead to a decreased histone methylation within the AAV-associated genes myeloperoxidase (MPO) and proteinase 3 (PR3), thus resulting in a transcriptionally permissive chromatin structure at these \textit{loci} [38]. Furthermore, a decreased expression of histone demethylases in peripheral blood mononuclear cells, including KDM3A, was observed in IgAV patients [39]. However, depletion of this gene in heterozygous mouse models does not seem to cause
phenotypes that may resemble the clinical features of the vasculitides included in our study (MGI:1924054). Further studies aimed at improving the understanding of the association of \textit{KDM4C} with the predisposition to vasculitis development may definitively shed light into our understanding of the molecular basis underlying these complex pathologies.

On the other hand, our analyses confirm the pivotal role of the HLA region in the genetic susceptibility to systemic vasculitis [5]. However, the fact that an HLA polymorphism represented the strongest association signal in our meta-analysis is intriguing, as specific HLA associations use to define each disease entity [5]. Our results suggest the existence of a shared genetic marker for vasculitides within the HLA in an intergenic region between \textit{HLA-DQB1} and \textit{HLA-DQA2}. The most significant SNP within this region, rs6932517, is in moderate LD (D' > 0.8) with the different peak variants of the individual archetypal HLA class II vasculitides analysed here (GCA, AAV, and IgAV; \textbf{Table S2}) [13, 25, 26]. Although TAK is mainly linked to HLA class I genes, previous studies suggest that some less relevant class II SNPs between \textit{HLA-DQB1} and \textit{HLA-DRB1}, like rs113452171, may be also involved in its predisposition [9, 14]. This SNP has also a considerable LD relationship with rs6932517 (D'=1). Therefore, it is likely that the apparently shared association signal that we observed within the HLA region corresponded to a tag SNP that marks the different disease-specific associations. Despite this, rs6932517 could have a potential value as a possible diagnostic marker of vasculitides. In any case, due to the broad LD structure throughout the HLA region, more comprehensive studies interrogating this question will be necessary to establish the attribution of common susceptibility \textit{locus} for vasculitides to a specific causal variant within the HLA system.

Finally, other interesting genes that have been previously related with immune-mediated diseases were suggested as shared risk factors for vasculitides in our study (\textbf{Table 1}). Amongst them, it is worth mentioning the genes zinc finger, MIZ-type
containing 1 (ZIMZ1), which has been described as a shared risk gene for Crohn’
disease and psoriasis [40], and protein kinase C theta (PRKCQ), a relevant locus for
RA susceptibility [41]. The latter, which encodes a calcium-independent and
phospholipid-dependent protein kinase important for T-cell activation, has been
described as a suggestive genetic marker for GCA development in a previous study
[13, 42]. Future replication studies will be necessary to validate these suggested
associations with vasculitides.

In summary, we have identified KDM4C as a common risk locus for vasculitides,
highlighting the relevance of the epigenetic mechanisms in the development of these
complex diseases. In addition, our data suggest that genetic variation between HLA-
DQB1 and HLA-DQA2 could have a high value in the understanding of the aetiological
mechanisms underlying systemic vasculitides, and support the notion that the HLA
class II region is also relevant in TAK.
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**Contributors:** LOF, FDC and JM were involved in the conception and design of the study as well as to the interpretation of data. LOF performed the statistical analyses and drafted the manuscript. FDC and JM critically revised the manuscript for important intellectual content. RLM, PAL, AWM, AHS, KS, MAGG and the consortium members were involved in the acquisition of data. All authors revised and approved the final version of the manuscript.

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**Competing Interests:** None declared.

**Patient consent:** Obtained.

**Ethics approval:** Comité de Bioética del Consejo Superior de Investigaciones Científicas and the local ethical committees of the different participating centres.
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TABLE LEGENDS

**Table 1.** List of association and suggestive non-HLA shared signals (P<2.55E-5) between GCA, TAK, IgAV, and AAV.

**Table 2.** Functional annotations of the $KDM4C$ polymorphism rs16925200 and its proxies in the European populations of the 1000 genomes project using ENCODE data.

FIGURE LEGENDS

**Figure 1.** Manhattan plot representation of the results of the cross-disease meta-analysis of four types of vasculitis. The -log10 of the inverse variance-weighted meta-analysis p-values are plotted against their physical chromosomal position. The red line represents the study-wide level of significance (P < 1.28E-05).

SUPPLEMENTARY MATERIAL

**Table S1.** List of shared association signals within the HLA region between GCA, TAK, IgAV, and AAV.

**Table S2.** Linkage disequilibrium (LD) in the CEU population of the 1000 genomes project between rs6932517 and the previously reported lead SNPs of the HLA associations with GCA, TAK, IgAV, and AAV.

**Table S3.** List of non HLA suggestive common signals (P<2.55E-5) between GCA, TAK, IgAV, and AAV. Results of the individual analyses are also shown.

**Table S4.** Cross-phenotype association analysis results showing genetic variants outside of the HLA region that have been significantly associated (P< 5E-08) with each vasculitis independently.

**Figure S1.** Manhattan plot representation of the overall cross-disease meta-analysis of the imputed HLA region. The -log10 of the inverse variance-weighted meta-analysis p-
values are plotted against their physical chromosomal position. The diamond size depends on the linkage disequilibrium ($r^2$) with the lead signal (rs6457617).

**Figure S2.** Regional association plot of the $KDM4C$ region. The lead variant (rs16925200) is highlighted in violet.
### Table 1. List of association and suggestive non-HLA shared signals (P<2.55E-5) between GCA, TAK, IgAV and AAV.

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GRCh37, Genome Reference Consortium Human genome build 37; OR; odds ratio for the minor allele, SNP, single-nucleotide polymorphism
Table 2. Functional annotations of the *KDM4C* polymorphism rs16925200 and its proxies in the European populations of the 1000 genomes project using ENCODE data and Blood NESDA NTR Conditional eQTL Catalog.

<table>
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<tr>
<th>SNP</th>
<th>Location (GRCh37)</th>
<th>Change</th>
<th>LD (r²)</th>
<th>RDM score</th>
<th>Promoter histone marks</th>
<th>Enhancers histone marks</th>
<th>DNAse hypersensitivity</th>
<th>Proteins bound (ChiP-seq)</th>
<th>Regulatory motifs altered (PWM)</th>
<th>Cis eQTL</th>
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The most strongly associated SNP in the meta-analysis is marked in bold. eQTL, expression quantitative trait locus; GRCh37, Genome Reference Consortium Human genome build 37; LD, linkage disequilibrium; RDM score, Regulome Data Base Score; SNP, single-nucleotide polymorphism. *Asterisks indicate polymorphisms not included in the combined dataset