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Genetically Distinct Subsets within ANCA-Associated Vasculitis

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Abstract

BACKGROUND—Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis is a severe condition encompassing two major syndromes: granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis) and microscopic polyangiitis. Its cause is unknown, and there is debate about whether it is a single disease entity and what role ANCA plays in its pathogenesis. We investigated its genetic basis.

METHODS—A genomewide association study was performed in a discovery cohort of 1233 U.K. patients with ANCA-associated vasculitis and 5884 controls and was replicated in 1454 Northern European case patients and 1666 controls. Quality control, population stratification, and statistical analyses were performed according to standard criteria.

RESULTS—We found both major-histocompatibility-complex (MHC) and non-MHC associations with ANCA-associated vasculitis and also that granulomatosis with polyangiitis and microscopic polyangiitis were genetically distinct. The strongest genetic associations were with the antigenic specificity of ANCA, not with the clinical syndrome. Anti–proteinase 3 ANCA was associated with *HLA-DP* and the genes encoding α_1-antitrypsin (*SERPINA1*) and proteinase 3 (*PRTN3*) ($P = 6.2\times10^{-89}$, $P = 5.6\times10^{-12}$, and $P = 2.6\times10^{-7}$, respectively). Anti–myeloperoxidase ANCA was associated with *HLA-DQ* ($P = 2.1\times10^{-8}$).

CONCLUSIONS—This study confirms that the pathogenesis of ANCA-associated vasculitis has a genetic component, shows genetic distinctions between granulomatosis with polyangiitis and microscopic polyangiitis that are associated with ANCA specificity, and suggests that the response against the autoantigen proteinase 3 is a central pathogenic feature of proteinase 3 ANCA–associated vasculitis. These data provide preliminary support for the concept that proteinase 3 ANCA–associated vasculitis and myeloperoxidase ANCA–associated vasculitis are distinct autoimmune syndromes. (Funded by the British Heart Foundation and others.)

Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis is a systemic small-vessel vasculitis comprising three clinical syndromes: granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis),[1] microscopic polyangiitis, and the Churg–Strauss syndrome.[2] ANCA-associated vasculitis commonly causes life-threatening kidney failure or pulmonary hemorrhage, has a fatality rate of 28% at 5 years, and causes substantial long-term morbidity in survivors.[3] Granulomatosis with polyangiitis and microscopic polyangiitis are the major clinical syndromes, both often featuring a pauci-immune necrotizing glomerulonephritis. Granulomatosis with polyangiitis is characterized by granulomatous inflammation of the respiratory tract and by autoantibodies against the neutrophil granule serine protease proteinase 3 in 66% of patients (considered to have proteinase 3 ANCA–associated vasculitis) or against another neutrophil granule component, myeloperoxidase, in 24% of patients (considered to have myeloperoxidase ANCA–associated vasculitis).[4] Microscopic polyangiitis is associated with myeloperoxidase ANCA in 58% of cases and with proteinase 3 ANCA in 26% of cases.[4] Some patients are ANCA-negative,[4] as are more than 50% of patients with the uncommon Churg–Strauss syndrome.[5]

Evidence of an important genetic contribution to ANCA-associated vasculitis has been growing, including evidence of a familial association.[6] The most convincing association has

been with the major histocompatibility complex (MHC),[7-9] especially the locus *HLA DPB1*0401*.[10] An association has been suggested between ANCA-associated vasculitis and the rare Z (or null) allele of the serpin A1 gene (*SERPINA1*), which encodes α_1-antitrypsin, a serine proteinase inhibitor for which proteinase 3 is one of several substrates. Other genetic associations require confirmation.[7-9]

There is debate as to whether the clinical syndromes of granulomatosis with polyangiitis and microscopic polyangiitis represent distinct diseases or are part of a single disease spectrum.[11-13] The concept of a single disease spectrum has resulted in similar treatment strategies being used in trials involving patients with ANCA-associated vasculitis, irrespective of whether they have granulomatosis with polyangiitis or microscopic polyangiitis (e.g., Jayne et al.,[14] Jones et al.,[15] and Stone et al.[16]), and in suggestions that genetic studies should consider the two clinical syndromes together.[7] Clear evidence that these clinical syndromes are etiologically distinct might provide a rationale for devising syndrome-specific therapeutic strategies.

Another controversy is the role of autoantigen reactivity in the pathogenesis of ANCA-associated vasculitis.[17] ANCA is an important diagnostic tool and is loosely associated with disease activity.[18] In vitro evidence suggests that ANCA can be proinflammatory, and transfer models of myeloperoxidase ANCA and proteinase 3 ANCA in animals mimic some aspects of disease.[19,20] Thus, proteinase 3 ANCA and myeloperoxidase ANCA are potentially important in pathogenesis but nonetheless might represent epiphenomena rather than drivers of disease.

We performed a genomewide association study of ANCA-associated vasculitis to identify genetic risk factors and to evaluate whether there is a common or distinct genetic background for granulomatosis with polyangiitis and microscopic polyangiitis.

METHODS

STUDY PARTICIPANTS

The discovery cohort was from the United Kingdom, with control data from the Wellcome Trust Case Control Consortium (WTCCC).[21] The replication cohort and controls were from Northern Europe. Case patients had a clinical diagnosis of either granulomatosis with polyangiitis or microscopic polyangiitis according to the European Medicines Agency algorithm (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org), supported by either a positive ANCA assay or a diagnostic biopsy.[22] The important issue of diagnostic classification is discussed in the Supplementary Appendix, including in Figures S1 and S2 and Tables S1 through S5. All patients provided written informed consent.

GENOTYPING

Genotyping was performed with the use of the Affymetrix SNP 6.0 Platform in the discovery cohort and the Sequenom MassARRAY platform in the replication cohort (see the Methods section in the Supplementary Appendix).

STATISTICAL ANALYSIS

Single-nucleotide polymorphism (SNP) association was determined by applying a standard 1-degree-of-freedom Cochran–Armitage test for additive association. The association analysis was stratified by U.K. geographic region for the discovery cohort and country of origin for the replication cohort (see the Methods section in the Supplementary Appendix).

RESULTS

ASSOCIATIONS OF MHC AND NON-MHC LOCI WITH ANCA-ASSOCIATED VASCULITIS

We studied a total of 2687 patients with ANCA-associated vasculitis who were of European ancestry and 7650 matched controls. In the discovery cohort, 1233 U.K. case patients underwent genotyping and comparison with 5884 WTCCC controls; data from 914 and 5259 of these participants, respectively, were of sufficient quality for inclusion in analyses (see Fig. S3A, Table S4, and the Methods section in the Supplementary Appendix). The resulting quantile–quantile plot shows deviation from the null distribution only at the extreme end, consistent with several loci showing association with disease (Fig. 1A). Removal of SNPs mapping to the MHC locus showed that non–MHC-linked loci also contribute to susceptibility.

In the replication cohort, 156 nonredundant SNPs were genotyped across 1454 case patients and 1666 controls. The SNPs included those from the discovery cohort (Fig. 1B), imputation, and previous association (see the Methods section, Fig. S3B, and Table S4 in the Supplementary Appendix). Three additional SNPs not represented on the Affymetrix SNP 6.0 Platform were included: the interleukin-2–receptor alpha gene (*IL2RA*) and the protein tyrosine phosphatase, nonreceptor type 22 gene (*PTPN22*) because of prior associations and the proteinase 3 gene (*PRTN3*) because it is a major ANCA autoantigen. (The other major ANCA autoantigen, myeloperoxidase, was already represented on the array.) Significant associations with ANCA-associated vasculitis were found (Fig. 1A, and Table S6 in the Supplementary Appendix).

Combined analysis of the discovery and replication cohorts revealed that four SNPs exceeded the significance threshold for genomewide association. Three were in the MHC region, the most significant of which was within the gene encoding HLA-DPB1 (Table 1). Stepwise logistic-regression analysis seeking effects that were independent of rs3117242 failed to find evidence for additional distinct susceptibility loci within the MHC (Table S7 in Supplementary Appendix). The fourth association was in the *SERPINA1* locus at 14q32 (Table 1). Also shown are the four previously associated SNPs closest to reaching genomewide significance, and the three SNPs added to the replication analysis on the basis of a priori hypotheses. Of these, a SNP in *PRTN3* was significantly associated with disease after Bonferroni correction ($P = 7.1\times10^{-5}$) and confirmed in subgroup analyses (reported below). Together, these data provide evidence of a genetic basis of ANCA-associated vasculitis that extends beyond the MHC.

DISTINCT GENETIC ASSOCIATIONS BETWEEN DISEASE SUBTYPES

To determine whether granulomatosis with polyangiitis and microscopic polyangiitis represent parts of a single disease spectrum or distinct clinical entities,[11,12] the discovery cohort was reanalyzed after it was subdivided into two on the basis of clinical diagnosis. The seven most significant SNP associations with ANCA-associated vasculitis were compared between the two subtypes. All three MHC-associated SNPs differed between granulomatosis with polyangiitis and microscopic polyangiitis, and essentially all of the association was found in the cohort with granulomatosis with polyangiitis (Table 2). This was also true of *SERPINA1* — while it did not show a significant difference when granulomatosis with polyangiitis and microscopic polyangiitis were compared directly, perhaps owing to small numbers of patients and thus reduced power, it was associated with granulomatosis with polyangiitis, but not microscopic polyangiitis, when each subtype was compared with controls (Table 2). *PRTN3* (rs62132295) was genotyped by means of a TaqMan assay in the discovery cohort owing to its suggested association with ANCA-associated vasculitis in the replication cohort (Table 1). It did not reach genomewide significance in the overall analysis

(odds ratio for developing the disease, 0.83; $P = 6.6\times10^{-4}$), but there was a suggestion of an association with granulomatosis with polyangiitis rather than microscopic polyangiitis (Table 2). Some less significant SNPs also appeared to be associated with granulomatosis with polyangiitis (e.g., the gene encoding Rho GTPase-activating protein 18 [*ARHGAP18*] and the gene encoding motile sperm domain–containing protein 2 [*MOSPD2*]), but the reduced power of the subgroup analyses makes it impossible to draw firm conclusions without larger cohorts (Table S2 and Fig. S4 in the Supplementary Appendix). Overall, there was evidence that granulomatosis with polyangiitis had genetic associations distinct from microscopic polyangiitis at both the MHC and *SERPIN* loci.

GENETIC SUSCEPTIBILITY, ANCA SPECIFICITY, AND CLINICAL SYNDROME

Further subgroup analysis was performed according to proteinase 3– and myeloperoxidase-ANCA specificity. Subgroups defined by the non–antigen-specific immunofluorescence ANCA assays were also analyzed (Table S8 in the Supplementary Appendix). The relationship between clinical status and ANCA specificity is shown in Figure 2A (and both Fig. S2 and Table S2 in the Supplementary Appendix).

Direct comparisons between the subgroup of patients with proteinase 3 ANCA and the subgroup with myeloperoxidase ANCA showed significant differences at the MHC, *SERPINA1*, and *PRTN3* loci, all consisting of there being a genetic association with proteinase 3 ANCA but not myeloper-oxidase ANCA (Table 2 and Fig. 2B through 2E, and Fig. S5 in the Supplementary Appendix). In all cases, both the odds ratios and P values show a stronger association with proteinase 3 ANCA than with granulomatosis with polyangiitis. This was particularly clear for *PRTN3*, which was conclusively associated with proteinase 3 ANCA status whereas it had not been with total ANCA-associated vasculitis or granulomatosis with polyangiitis (Table 2).

We repeated the whole genomewide association analysis in patient cohorts defined by ANCA specificity; this revealed no new associations with the proteinase 3 ANCA subgroup but one — a SNP in *HLA-DQ* — with the myeloperoxidase ANCA subgroup (Table 2 and Fig. 2D). This SNP was not convincingly associated with proteinase 3 ANCA, granulomatosis with polyangiitis, or microscopic polyangiitis in this cohort. However, after genotyping by means of a TaqMan assay in the replication cohort, it reached “genomewide” significance with the myeloperoxidase ANCA subgroup (odds ratio, 0.65; $P = 2.1\times10^{-8}$ in the combined cohort) (Table 2). (This significant association was replicated in an independent cohort of Italian patients and controls [Table S9 in the Supplementary Appendix].)

The primary association of all these genetic variants thus appeared to be with ANCA specificity rather than the clinically defined syndromes. To test this hypothesis, we examined the SNP associations in the subgroups of granulomatosis with polyangiitis and microscopic polyangiitis that were positive for the two ANCA specificities. Although analysis of these smaller subgroups was characterized by a loss of power, the trends were clear. Within the granulomatosis with polyangiitis subgroup, the associations of *HLA-DP* and the related SNPs *SERPINA1* and *PRTN3* were seen in the patients with proteinase 3 ANCA, not in those with myeloperoxidase ANCA (Table 3). In the microscopic polyangiitis group, the sub-groups were much smaller, but the findings were the same: an association of proteinase 3 ANCA and the *HLA-DP* SNP and *SERPINA1*. The converse was true for the *HLA-DQ* SNP, which was associated with myeloperoxidase ANCA rather than proteinase 3 ANCA among patients with microscopic polyangiitis (Table 3).

VARIANTS WITHIN THE *SERPINA1–SERPINA11* LOCUS AND SUSCEPTIBILITY

The most prominent non-MHC association with both the proteinase 3 ANCA and granulomatosis with polyangiitis subgroups was found at SNP rs7151526, located in the *SERPINA1–SERPINA11* locus at 14q32. Correlation with ANCA-associated vasculitis was found for the Z allele (odds ratio, 0.3; $P = 1.25\times10^{-5}$) but not the S allele (odds ratio, 1.09; P=0.65). A haplotype analysis of the two SNPs examined at the *SERPINA1–SERPINA11* locus (Table S10 in the Supplementary Appendix) indicated that the haplotype combining the minor Z allele with the risk allele of rs7151526 was associated with disease more strongly than was rs7151526 alone. The rs7151526 allele did not appear to be significantly associated with disease independently of the Z allele, indicating that the causal variant at the *SERPINA1–SERPINA11* locus is, or is linked to, the Z allele of *SERPINA1* rather than rs7151526.

DISCUSSION

This genomewide association study of patients with ANCA-associated vasculitis shows that there is a significant genetic contribution to pathogenesis of the disease. Considering ANCA-associated vasculitis as a single entity, we found associations of “genomewide” significance with the MHC and a SNP in the *SERPINA1* locus. Other loci fell short of this significance level, though for these an association might be detected in larger studies or meta-analyses, as has been observed for other genomewide association studies.[23] The present study did not confirm some loci for which associations were found previously, either because the initial associations were spurious or because our study was underpowered to replicate them.

The MHC SNP with the strongest association with ANCA-associated vasculitis is in *HLA-DP*, and there is evidence for only a single genetic association with the region. This indicates that the SNPs in the collagen, type XI, alpha 2 gene (*COL11A2*) are most likely to be associated by virtue of linkage disequilibrium but does not yet directly implicate *HLA-DP.* A more detailed analysis taking advantage of the comprehensive coverage of the MHC locus on the Immunochip[24] could be used to define this association and that between myeloperoxidase ANCA and *HLA-DQ.*

Because granulomatosis with polyangiitis and microscopic polyangiitis share many clinical and histopathologic features, have been thought to represent each end of a single disease spectrum, and cannot always be reliably distinguished on clinical grounds,[25] patients with either disease are typically treated similarly.[14-16] However, the extreme cases of granulomatosis with polyangiitis are easily distinguished from cases of microscopic polyangiitis, and granulomatosis with polyangiitis and microscopic polyangiitis have statistically different clinical outcomes[26] and ANCA specificities.[4] In the current study, we found striking differences in genetic association between granulomatosis with polyangiitis and microscopic polyangiitis that were not driven by the clinically defined syndromes but by the underlying autoantibody specificity. Specifically, both the *HLA* and *SERPINA1* associations with granulomatosis with polyangiitis are seen in patients with granulomatosis with polyangiitis who are positive for proteinase 3 ANCA, not for myeloperoxidase ANCA, and can also be seen in the proteinase 3 ANCA subgroup of patients with microscopic polyangiitis. This clear association of genetic background with autoantibody specificity suggests that it might contribute to the clinical classifications of granulomatosis with polyangiitis and microscopic polyangiitis. The genetic difference between proteinase 3–ANCA and myeloperoxidase-ANCA polyangiitis could have immunopathogenic and therapeutic implications (see below as well as Suppiah et al.[27] and Stegeman[28]), and will require future genetic studies powered to detect associations with these conditions rather than with ANCA-associated vasculitis as a whole. Of course, these results throw no light on

the genetic basis of ANCA-negative granulomatosis with polyangiitis or microscopic polyangiitis.

Although increasing evidence suggested that proteinase 3 ANCA is important in the pathogenesis of ANCA-associated vasculitis, the possibility that it was an epiphenomenon remained. There is strong in vitro evidence that ANCA can bind to neutrophils and drive endothelial inflammation,[29] and proteinase 3 ANCA has induced vasculitis, but not granulomata, in transfer studies in mice.[20] Proteinase 3 expression is increased on neutrophils in patients with granulomatosis with polyangiitis,[30] and pathogenic mechanisms for its involvement have been suggested.[31] One small study has also suggested a genetic association, though this was not replicated.[32] Our study shows that variants at the MHC, *SERPINA1*, and *PRTN3* loci confer a significant risk of proteinase 3–ANCA polyangiitis. The actual causal variants at all three loci remain to be determined by means of detailed fine-mapping and functional studies. Nonetheless, this firm association between key components of the autoimmune response — MHC, the proteinase 3 autoantigen itself, and perhaps α_1-antitrypsin — puts anti–proteinase 3 autoreactivity at the center of the cause of disease, potentially resolving a long-standing controversy.

Proteinase 3–ANCA polyangiitis, which is the antibody subtype of two thirds of patients with granulomatosis with polyangiitis and one third of those with microscopic polyangiitis, is a condition in which there is genetic variation in an autoantigen itself, together with HLA, implying that antigen-specific autoimmunity plays a role in pathogenesis. Other examples in which similar associations directly implicate antigen-specific autoimmunity include diabetes with autoimmunity to insulin[33] and membranous nephropathy with autoimmunity to PLA2R1.[34]

SERPINA1 encodes α_1-antitrypsin, a neutral serine protease inhibitor, enzymatic targets of which include proteinase 3. Associations with the rare Z (null) allele of *SERPINA1* have been described but have been statistically weak.[9,35] The Z allele was not, however, shown to be the causative variant.[36] The SNP we found to be associated, with genomewide significance, is in linkage disequilibrium with the Z allele. Haplotype analysis suggests that the causal variant at the *SERPINA1–SERPINA11* locus is either the Z allele of *SERPINA1* or is in close linkage disequilibrium with it, rather than with the *SERPINA1* SNP rs7151526.

We found myeloperoxidase-ANCA polyangiitis to be associated with a SNP in *HLA-DQ*. This subgroup analysis had reduced power (Fig. S4 in the Supplementary Appendix) and it is possible that larger studies will uncover more variants associated with myeloperoxidase ANCA. These data raise the possibility that, like proteinase 3–ANCA polyangiitis and consistent with in vitro and animal-model evidence,[19] myeloperoxidase-ANCA polyangiitis might be driven by antimy-eloperoxidase autoimmunity.

Despite having distinct genetic risk factors, proteinase 3–ANCA and myeloperoxidase-ANCA polyangiitis have overlapping clinical phenotypes. This could be because, once generated, either type of ANCA drives a similar pathologic process that is largely independent of precise antigen specificity but is due to antigen similarity — thus, because both autoantigens are found in neutrophil granules, on apoptotic neutrophils, and in neutrophil extracellular traps (NETs, which have been implicated in autoimmune processes),[37] for example, antibodies against them might generate inflammation through similar mechanisms. This would be consistent with the similar proinflammatory effects of proteinase 3 ANCA and myeloperoxidase ANCA in vitro[38,39] and upon transfer in animal models.[19,20] This clinical similarity between proteinase 3 ANCA and myeloperoxidase ANCA disease is likely to be augmented by shared genetic associations, which are

commonly seen between distinct autoimmune diseases.[40] Differences between the syndromes could be caused by additional independent genetic associations that this study has not been powered to detect. Environmental influences will also play a role.

Our genomewide association study of ANCA-associated vasculitis provides clear evidence of a genetic contribution to disease susceptibility, which differs between granulomatosis with polyangiitis and microscopic polyangiitis. Associations with *HLA*, *SERPINA1*, and *PRTN3* are primarily aligned with ANCA specificity rather than with the clinically defined syndromes granulomatosis with polyangiitis and microscopic polyangiitis, making it logical to consider including ANCA specificity in the diagnostic criteria for ANCA-associated vasculitis. Moreover, the fact that proteinase 3–ANCA and myeloperoxidase-ANCA polyangiitis have distinct genetic causes suggests that clinical trials that have considered ANCA-associated vasculitis as a single entity must be interpreted carefully, since subsets defined by ANCA specificity may respond differently to therapeutic intervention. Future genetic and clinical studies of ANCA-associated vasculitis should be sufficiently powered to allow for independent analysis of proteinase 3–ANCA and myeloperoxidase-ANCA polyangiitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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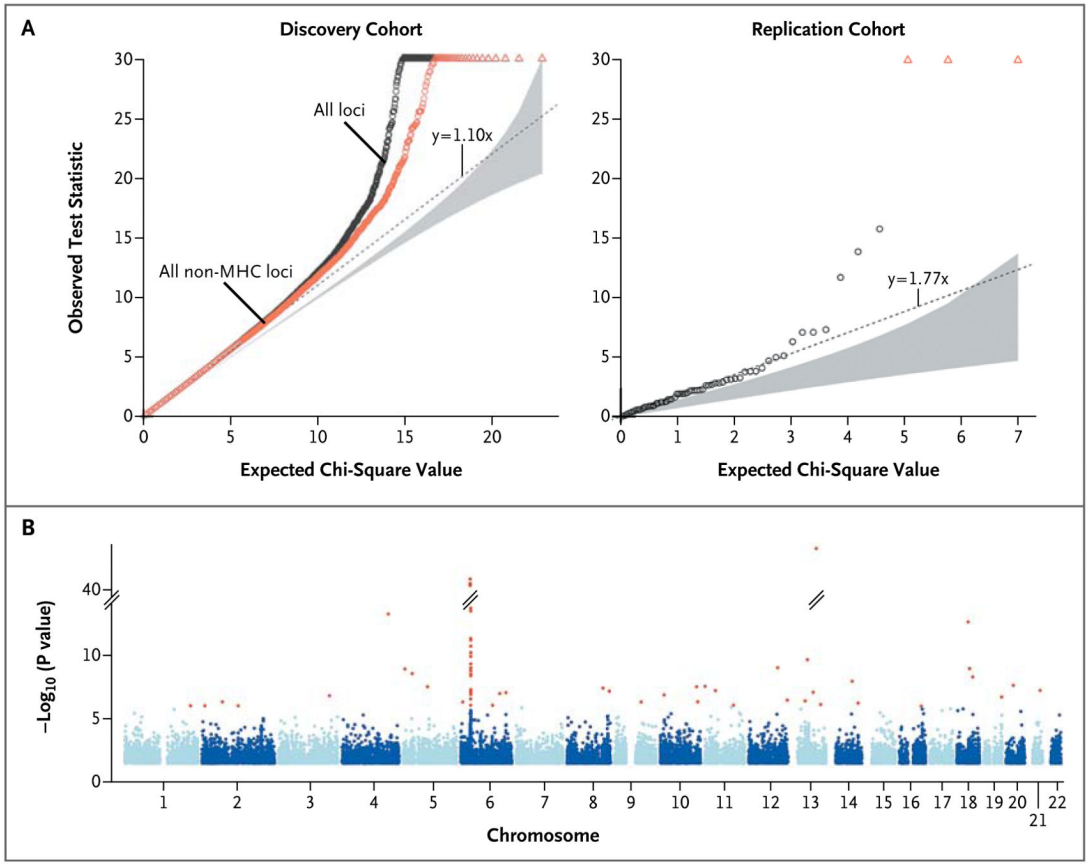

Figure 1. Associations of MHC and Non-MHC Loci with Antineutrophil Cytoplasmic Antibody (ANCA)–Associated Vasculitis

Panel A shows quantile–quantile plots of the results of the association test for all single-nucleotide polymorphisms (SNPs) with data of sufficient quality from the discovery cohort (left) and replication cohort (right). Triangles at the upper right of each graph indicate SNPs with $-\log_{10}$ P values exceeding 30. The red symbols indicate SNPs other than those mapping to the major histocompatibility complex (MHC) for the discovery cohort and the three MHC SNPs specifically chosen for investigation in the replication cohort. The interrupted line indicates the estimated dispersion factor, lambda, and is estimated according to the ratio of the observed trimmed mean (calculated from the leftmost, linear half of the graph) to its expected value under the chi-square assumption. The shaded area indicates the concentration band for the plot and is defined by 95% probability bounds for each order statistic. Panel B shows the $-\log_{10}$ P values for each SNP plotted against its chromosomal location in the discovery cohort. The red points indicate in the discovery cohort SNPs with a P value of less than 1×10^{-5} and a minor allele frequency of greater than 5%.

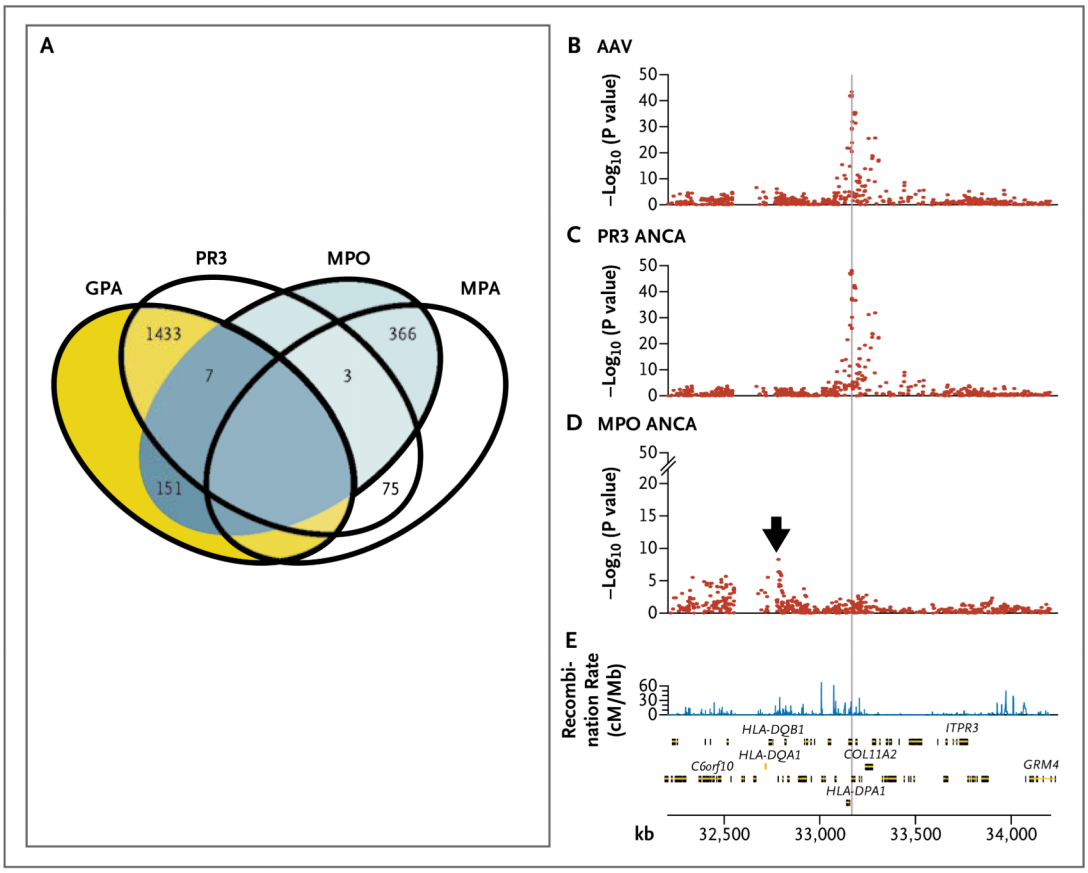

Figure 2. Relationships between Clinical Subtype and ANCA Specificity in ANCA-Associated Vasculitis and Associations of the MHC Locus with Proteinase 3 ANCA and Myeloperoxidase ANCA

A Venn diagram shows the overlap between clinical diagnosis (granulomatosis with polyangiitis [GPA] or microscopic polyangiitis [MPA]) and ANCA specificity (proteinase 3 [PR3] or myeloperoxidase [MPO]) in the combined cohort (Panel A). Also shown are the $-\log_{10}$ P values for the association of SNPs at the MHC locus in all case patients with ANCA-associated vasculitis (AAV) (Panel B), case patients with PR3 ANCA only (Panel C), and case patients with MPO ANCA only (Panel D). The gray vertical line (Panels B, C, and D) indicates the genomic location of the most associated SNP. The arrow (Panel D) indicates the position of rs5000634 (*HLA-DQ*). The genomic architecture of the MHC locus (Panel E) shows the recombination rates along the sequence, as well.

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Table 1

Associations of Single-Nucleotide Polymorphisms (SNPs) and Antineutrophil Cytoplasmic Antibody (ANCA)–Associated Vasculitis, According to Cohort.*

Chromosome	Locus	SNP (Minor Allele)	Discovery Cohort (N = 914 Case Patients, 5259 Controls)			Replication Cohort (N = 1353 Case Patients, 1599 Controls)			Combined Cohort (N = 2267 Case Patients, 6858 Controls)	
			MAF	Odds Ratio	P Value	MAF	Odds Ratio	P Value	Odds Ratio	P Value
SNPs with genomewide significance in combined cohort										
6	*HLA–DP*	rs3117242 (G)	0.25	2.73	4.1×10^{-36}	0.17	4.28	2.1×10^{-38}	3.67	1.5×10^{-71}
6	*COL11A2*	rs3130233 (G)	0.47	1.33	2.3×10^{-6}	0.44	1.51	9.0×10^{-11}	1.51	7.8×10^{-15}
6	*COL11A2*	rs3117016 (A)	0.38	1.48	3.6×10^{-9}	0.32	2.00	3.1×10^{-17}	1.83	6.4×10^{-24}
14	*SERPINA1*	rs7151526 (A)	0.05	0.56	5.2×10^{-7}	0.06	0.58	1.9×10^{-4}	0.59	2.4×10^{-9}
SNPs reported to be associated with ANCA-associated vasculitis†										
1	*IL10*	rs1554286 (A)	0.17	0.89	2.2×10^{-1}	0.18	1.04	7.5×10^{-1}	0.92	4.7×10^{-1}
2	*CTLA4*	rs16840252 (T)	0.17	0.77	1.1×10^{-5}	0.17	0.88	7.7×10^{-2}	0.87	1.3×10^{-5}
18	*CD226*	rs1790947 (A)	0.48	0.91	1.1×10^{-1}	0.48	0.83	7.7×10^{-3}	0.81	7.0×10^{-3}
21	*ITGB2*	rs2070947 (G)	0.31	0.92	0.08	ND	ND	NA	NA	NA
1	*PTPN22*	rs6679677 (A)	ND	ND	ND	0.12	0.80	2.6×10^{-1}	NA	NA
10	*IL2RA*	rs41295061 (A)	ND	ND	ND	0.08	0.91	2.8×10^{-1}	NA	NA
19	*PRTN3*	rs62132295 (A)	ND	ND	ND	0.33	0.78	7.1×10^{-5}	NA	NA

*P values were calculated with the use of the Cochran–Armitage trend test. Genomewide significance was defined as $P<5\times10^{-8}$. MAF denotes minor allele frequency, and NA not applicable.

†Some previously reported SNPs were not directly genotyped in the discovery cohort (indicated as “not done” [ND]). One rs2070947 (*ITGB2*) was not genotyped in the replication cohort because it could not be included in the genotyping assay used for that cohort.

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Table 2

Associations of SNPs and ANCA–Associated Vasculitis, According to Clinical and ANCA Subgroups.*

Chromosome	Locus	SNP	Overall Analysis of Combined Cohort (N = 2267 Case Patients, 6858 Controls)		Clinical Syndrome						ANCA Specificity					
					GPA vs. MPA (N = 1683 vs. 489)		GPA vs. Control (N = 1683 vs. 6858)		MPA vs. Control (N = 489 vs. 6858)		Proteinase 3 vs. Myeloperoxidase (N = 1521 vs. 556)		Proteinase 3 vs. Control (N = 1521 vs. 6858)		Myeloperoxidase vs. Control (N = 556 vs. 6858)	
			odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value
6	*HLA–DP*	rs3117242	3.67	1.5×10^{-71}	3.49	1.9×10^{-27}	5.39	3.1×10^{-85}	1.60	1.3×10^{-3}	5.10	2.5×10^{-46}	7.03	6.2×10^{-89}	1.55	3.2×10^{-2}
6	*HLA–DQ*	rs5000634	0.80	2.9×10^{-9}	1.17	9.0×10^{-2}	0.83	2.2×10^{-6}	0.67	1.4×10^{-5}	1.28	8.2×10^{-3}	0.86	3.3×10^{-5}	0.65	2.1×10^{-8}
6	*ARHGAP18*	rs1705767	0.80	6.2×10^{-7}	0.92	9.2×10^{-1}	0.78	3.3×10^{-7}	0.84	1.8×10^{-2}	0.90	7.2×10^{-1}	0.73	5.2×10^{-8}	0.87	1.0×10^{-2}
14	*SERPINA1*	rs7151526	0.59	2.4×10^{-9}	0.74	1.7×10^{-1}	0.54	4.4×10^{-10}	0.76	1.7×10^{-1}	0.64	1.1×10^{-2}	0.53	5.6×10^{-12}	0.84	2.8×10^{-1}
19	*PRTN3*	rs62132295	0.83	6.6×10^{-4}	0.81	3.9×10^{-2}	0.78	2.6×10^{-5}	0.99	9.3×10^{-1}	0.67	6.9×10^{-6}	0.73	2.6×10^{-7}	1.10	2.2×10^{-1}
X	*MOSPD2*	rs6628825	0.79	9.7×10^{-6}	0.91	5.1×10^{-1}	0.80	2.6×10^{-6}	0.79	2.2×10^{-1}	0.79	8.3×10^{-2}	0.77	6.1×10^{-7}	0.86	6.3×10^{-1}

*P values were calculated with the use of the Cochran–Armitage trend test. GPA denotes granulomatosis with polyangiitis, and MPA microscopic polyangiitis.

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Table 3

Associations of SNPs and ANCA-Associated Vasculitis, According to Clinical Syndromes Stratified on the Basis of ANCA Specificity.

Chromosome	Locus	SNP	Granulomatosis with Polyangiitis						Microscopic Polyangiitis					
			Proteinase 3 vs. Myeloperoxidase (N = 1433 vs. 151)		Proteinase 3 vs. Control (N = 1433 vs. 6858)		Myeloperoxidase vs. Control (N = 151 vs. 6858)		Proteinase 3 vs. Myeloperoxidase (N = 75 vs. 366)		Proteinase 3 vs. Control (N = 75 vs. 6858)		Myeloperoxidase vs. Control (N = 366 vs. 6858)	
			odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value	odds ratio	P value
6	*HLA–DP*	rs3117242	5.24	4.9×10^{-24}	7.51	3.7×10^{-86}	1.60	9.2×10^{-2}	2.76	6.9×10^{-4}	2.49	9.8×10^{-5}	1.50	1.4×10^{-1}
6	*HLA–DQ*	rs5000634	1.46	3.1×10^{-2}	0.86	7.8×10^{-5}	0.62	2.1×10^{-5}	1.34	6.9×10^{-1}	0.79	4.7×10^{-1}	0.68	1.4×10^{-5}
6	*ARHGAP18*	rs1705767	0.77	3.7×10^{-1}	0.72	2.2×10^{-7}	NA	1.0×10^{-1}	1.13	6.8×10^{-1}	1.13	2.6×10^{-1}	0.76	6.8×10^{-3}
14	*SERPINA1*	rs7151526	0.63	1.9×10^{-1}	0.52	1.2×10^{-10}	0.72	4.3×10^{-1}	0.37	2.8×10^{-3}	0.31	1.5×10^{-5}	0.78	9.8×10^{-1}
19	*PRTN3*	rs62132295	0.61	2.3×10^{-3}	0.73	3.9×10^{-7}	1.22	2.2×10^{-1}	0.60	1.1×10^{-1}	0.65	1.3×10^{-1}	1.03	7.7×10^{-1}
X	*MOSPD2*	rs6628825	0.77	2.1×10^{-1}	0.79	1.4×10^{-6}	NA	9.8×10^{-1}	0.62	7.2×10^{-2}	0.31	5.4×10^{-3}	0.87	5.4×10^{-1}