On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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Chapter II

Genetic variants in ANCA-associated vasculitis: a meta-analysis

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Abstract

Background

Genetic factors may influence the pathogenic pathways leading to antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). We performed a meta-analysis to determine the genetic variants most likely associated with AAV and investigated whether diagnostic and serological subtypes within AAV have distinct genetic backgrounds.

Methods

Studies investigating the association between genetic variants and AAV in humans were searched in PubMed, EMBASE and Web of Science. All variants investigated in at least two studies were selected. Subsequently, all studies assessing these variants were included in this meta-analysis. Additionally, data on these variants from the largest genome-wide association studies in AAV were included to increase the validity of this meta-analysis.

Results

The literature search yielded 5180 articles. 62 articles investigating 140 genetic variants were included, 33 of which were associated with AAV in a meta-analysis. These genetic variants were in or near the following genes: *CD226*, *CTLA-4*, *FCGR2A, HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, *HSD17B8*, *IRF5*, *PTPN22*, *RING1/RXRB*, *RXRB*, *STAT4, SERPINA1* and *TLR9*. Moreover, we identified genetic distinctions between granulomatosis with polyangiitis and microscopic polyangiitis and between proteinase 3 ANCA vasculitis and myeloperoxidase ANCA vasculitis. In 76% of the genetic variants, subdivision based on ANCA serotype resulted in higher ORs than subdivision based on clinical diagnosis.

Conclusions

This meta-analysis identified 33 genetic variants associated with AAV, supporting a role for alpha-1-antitrypsin, the major histocompatibility complex system, and several distinct inflammatory processes in AAV pathogenesis. Our results indicate that subdivision of AAV based on ANCA serotype has a stronger genetic basis than subdivision based on clinical diagnosis.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disease in which patients often have circulating proteinase 3 (PR3)-ANCA or myeloperoxidase (MPO)-ANCA.[1,2] The clinical syndromes within the spectrum of AAV are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis.

The subtypes within AAV show significant differences in clinical and epidemiological characteristics,[3,4] leading to debate regarding whether these subtypes are part of a single disease spectrum or represent distinct diseases.[5–7] To date, the prevailing concept of a single disease spectrum has resulted in similar treatment strategies in clinical trials[8–10] and to suggestions that genetic studies should consider the subtypes together.[11] Evidence that these subtypes are pathogenically distinct may lead to the development of syndrome-specific therapeutic strategies.

Both environmental and genetic factors are thought to be involved in the pathogenesis of AAV.[12] Evidence for the role of genetic factors comes from differences in the prevalence of AAV between ethnic groups,[13] familial association studies[14] and genetic associations studies including the two genome-wide association studies (GWAS) performed in AAV.[15,16]

Attempts to replicate findings of genetic association studies performed in AAV have yielded inconsistent outcomes. Small sample sizes and false-positive results arising from the low prior probabilities of genetic associations may be responsible for these inconsistencies.[17] These factors are especially relevant in complex diseases such as AAV.[18]

The aim of this study was to determine the genetic variants associated with AAV. Therefore, we performed a meta-analysis to assess the pooled effect of all genetic variants that have been investigated in AAV in at least two studies. To increase the validity of this meta-analysis, we also included previously unpublished data from the largest GWAS performed in AAV.[15] Moreover, we conducted stratified analyses based on clinical diagnosis and ANCA serotype to investigate whether these different AAV subtypes have distinct genetic backgrounds.

Methods

Literature search and eligibility

A comprehensive search string was carried out in collaboration with a librarian. PubMed, EMBASE and Web of Science were searched until April 2014 for studies investigating genetic variants in patients with AAV. The search strategy consisted of multiple queries combining 'Anti-Neutrophil Cytoplasmic Antibody-Associated

Vasculitis', 'ANCA', 'vasculitis', 'Granulomatosis with Polyangiitis', 'Wegener's Granulomatosis', 'Microscopic polyangiitis', 'Eosinophilic granulomatosis with polyangiitis', 'Churg Strauss Syndrome', 'PR3', 'MPO', 'Polymorphisms' or 'Genes'. To minimise the chance of omitting references, a second broader search was performed for genetic variants in vasculitis in general rather than AAV alone (supplementary table S1). The specific genes and polymorphisms that resulted from the previous searches were added in a next search to minimise the chance of omitting references. To ensure maximum sensitivity, no limits, filters or language restrictions were placed on the searches.

Two observers (CR and DvH) independently reviewed the titles and abstracts of the citations retrieved by the search and read potentially relevant studies independently. Studies that compared genetic variants between patients with AAV and controls without AAV derived from the general population were eligible. Cases had to have an AAV by fulfilling either the Chapel Hill Consensus Conference criteria,[19] American College of Rheumatology criteria,[20] European Medicines Agency algorithm,[21] 1998 Japanese criteria proposed by the Research Committee on Intractable Vasculitides, the Ministry of Health, Labour and Welfare of Japan,[22] or clinical, histological and serological criteria. Definitions of cases and controls in each included study are depicted in supplementary table S2. All genetic variants investigated in at least two studies were included. Genetic variants investigated in multiple cohorts in one publication were also included. This was the case for genetic variants in *GHSR*,[23] *LEPR*[23] and *TLR9*.[24] For the included genetic variants, all genetic studies were identified to estimate the pooled effect of the genetic variant in a meta-analysis, irrespective of their p values. To increase the validity of this meta-analysis, we also included unpublished data on these genetic variants from the Lyons *et al*[15] GWAS. This was possible for the genetic variants in this meta-analysis that were not human leucocyte antigen (HLA) serotypes or tandem repeats and that were genotyped in the Lyons *et al* GWAS. Moreover, we included data from the stage 1 analysis including all single-nucleotide polymorphisms (SNPs) with a p value $<10^{-4}$ from the Xie *et al*[16] GWAS.

Data extraction

Minor allele frequencies of the included genetic variants were extracted from included studies. Studies investigating the same genetic variant published by the same author(s) were checked for overlapping patient groups, in which case only the study with the largest patient group was included. Studies that reported insufficient data to calculate an OR were excluded.

Statistical analysis

ORs and 95% CIs were calculated at the allele level. To account for potential heterogeneity, random-effects model was performed in all analyses that included

at least five studies.[25] Because the HLA serotypes are not completely independent from the HLA alleles, the following HLA variants were collapsed in the analyses: *HLA-DR1*, *HLA-DRB1*01* and *HLA-DRB1*0101*; *HLA-DR3* and *HLA- DRB1*03*; *HLA-DR7* and *HLA-DRB1*07*; *HLA-DR8* and *HLA-DRB1*08*; and *HLA-DR9*, *HLA-DRB1*09* and *HLA-DRB1*0901*. Collapsing other HLA variants was not possible because some studies investigated multiple, closely related, HLA variants in the same patients. If these HLA variants had been collapsed, the same patients would have been included in the same analysis multiple times, increasing their weight in the analysis and introducing a systematic error. To determine whether the disease subtypes within AAV represent parts of a single disease spectrum or distinct clinical entities, we performed pre-specified subgroup analysis stratifying patients according to clinical diagnosis (GPA/MPA) and ANCA serotype (PR3-ANCA/MPO-ANCA and cytoplasmic (c)-ANCA/perinuclear (p)-ANCA), if enough data were available. Moreover, we performed stratified analyses based on ethnicity for all genetic variants investigated in both Caucasian and Asian patients. We assumed difference in effect estimates likely to be present in case subgroups showed significant effects in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup. Heterogeneity within studies was displayed by I^2, which reflects the percentage of total variation across studies beyond chance.[26] Egger and Harbord tests were used to assess publication bias.[27] All of the p values presented are nominal p values and are not corrected for multiple testing. Correction for multiple testing was not performed as the strong linkage disequilibrium between variants tested in the major histocompatibility complex (MHC) region makes calculating an appropriate multiple correction factor impractical. All analyses were performed using STATA V.12 (StataCorp. 2011; Stata Statistical Software: Release 12, College Station, Texas, USA).

Results

Initial search and results

Our literature search yielded 5180 articles, from which we identified 140 genetic variants published in 62 articles. All included studies were case–control studies, two of which were GWAS. These articles were published from 1978 through 2014, and the number of patients with AAV included ranged from 12 to 1445. The characteristics of all included studies are provided in supplementary table S2. Additionally, previously unpublished data from the Lyons *et al*[15] GWAS were available and included in the meta-analysis for 18 genetic variants: *CD226* rs763361, *CTLA-4* rs231775, *CTLA-4* rs3087243, *FCAR* rs16986050, *FCGR2A*rs1801274, *GHSR* rs509035, *HLA-DPB2* rs3130215, *IL1*β rs1143634, *IL6* rs1800795, *IL10* rs1800896, *IRF5* rs10954213, *PTPN22* rs2476601, *RING1/RXRB*

rs213213, *RXRB* rs6531, *RXRB* rs9277935, *STAT4* rs7574865, *SERPINA1* Z allele and *TNF*αrs1800629.

Thirty-three genetic variants were significantly associated with AAV after meta-analysis (table 1 and supplementary figure S1), and 107 genetic variants were not associated with AAV after meta-analysis (supplementary table S3). The ORs for the significant associations after meta-analysis ranged from 0.35 to 0.81 for protective genetic variants and from 1.13 to 2.94 for the genetic variants associated with an increased risk of AAV.

AAV is associated with the Z and S alleles of SERPINA1

Both the S allele and Z allele of *SERPINA1* were significantly associated with AAV, with pooled ORs of 1.30 (95% CI 1.03 to 1.63) and 2.94 (95% CI 2.22 to 3.88), respectively. Subgroup analysis showed that the association with the *SERPINA1* Z allele was present in both PR3-ANCA (pooled OR 2.58 (95% CI 1.57 to 4.25)) and MPO-ANCA (pooled OR 2.01 (95% CI 1.04 to 3.87)) positive patients and in both c-ANCA (pooled OR 3.53 (95% CI 2.28 to 5.49)) and p-ANCA (pooled OR 3.13 (95% CI 1.21 to 8.13)) positive patients (supplementary table S4 and figure S2).

AAV is associated with genetic variants in the MHC region

Seventeen genetic variants in *HLA-B*, *HLA-DP*, *HLA-DQ* and *HLA-DR* remained significantly associated with AAV after meta-analysis (table 1). *HLA-DPA1* rs9277341 had the strongest protective effect (pooled OR 0.35 (95% CI 0.30 to 0.40)), and *HLA-DPB1*0401*was the strongest contributor to an increased risk of AAV (pooled OR 1.99 (95% CI 1.44 to 2.74)). *RING1/RXRB* rs213213, *RXRB* rs6531 and *RXRB* rs9277935 were also significantly associated with AAV with pooled ORs of 1.71 (95% CI 1.57 to 1.86), 1.63 (95% CI 1.50 to 1.77) and 0.44 (95% CI 0.37 to 0.50), respectively.

AAV is associated with genetic variants involved in inflammatory processes

CTLA-4 rs231775 was associated with an increased risk of AAV (pooled OR of 1.16 (95% CI 1.06 to 1.28)) while *CTLA-4* rs3087243 and *CTLA-4* $(AT)_{86}$ had a protective effect (pooled ORs of 0.81 (95% CI 0.75 to 0.87) and 0.54 (95% CI 0.43 to 0.67), respectively). *PTPN22* rs2476601, *CD226* rs763361 and *IRF5* rs10954213 were also significantly associated with AAV, with pooled ORs of 1.39 (95% CI 1.24 to 1.56), 1.14 (95% CI 1.07 to 1.21) and 0.77 (95% CI 0.70 to 0.83), respectively. Moreover, *TLR9* rs352162 and rs352140 were significantly associated with AAV with pooled ORs of 1.58 (95% CI 1.43 to 1.75) and 1.13 (95% CI 1.02 to 1.25), respectively.

Table 1. Genetic variants significantly associated with AAV after meta-analysis

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry[a]
CD226 rs763361 (T)	3	2422/17898	1.14 (1.07 – 1.21)	<0.001	0	0.444	0.792
CTLA-4 $(AT)_{86}$	4	303/543	0.54 (0.43 – 0.67)	<0.001	89	<0.001	0.946
CTLA-4 rs231775 (G)	3	1002/6179	1.16 (1.06 – 1.28)	0.002	60	0.080	0.080
CTLA-4 rs3087243 (A)	3	2015/7855	0.81 (0.75 – 0.87)	<0.001	25	0.262	0.122
FCGR2A rs1801274 (C)	6	1239/6209	0.90 (0.82 – 0.99)	0.028	0	0.834	0.788
HLA-B5	2	335/6573	0.59 (0.38 – 0.92)	0.019	0	0.432	N/A
HLA-B8	6	475/7855	1.48 (1.04 – 2.11)	0.028	47	0.096	0.063
HLA-DPA1 rs9277341 (C)	2	1032/2200	0.35 (0.30 – 0.40)	<0.001	54	0.116	0.215
*HLA-DPB1*0301*	5	1154/1337	0.38 (0.21 – 0.69)	0.002	78	<0.001	0.938
*HLA-DPB1*0401*	5	1154/1337	1.99 (1.44 – 2.74)	<0.001	84	<0.001	0.738
HLA-DPB2 rs3130215 (A)	3	1417/7249	1.40 (1.29 – 1.52)	<0.001	99	<0.001	0.446
*HLA-DQB1*0303*	3	176/218	1.82 (1.09 – 3.03)	0.021	17	0.301	0.916
HLA-DR6	5	487/6222	0.50 (0.27 – 0.95)	0.033	55	0.062	0.997
*HLA-DRB1*1101*	2	268/465	1.89 (1.15 – 3.08)	0.011	0	0.487	N/A
*HLA-DRB1*1201*	2	216/465	0.37 (0.15 – 0.91)	0.031	0	0.491	N/A
*HLA-DRB1*13*	4	233/833	0.47 (0.32 – 0.70)	<0.001	0	0.504	0.884
*HLA-DRB1*14*	4	322/862	1.91 (1.07 – 3.42)	0.029	0	0.728	0.700
*HLA-DRB1*15*	3	236/633	1.86 (1.39 – 2.50)	<0.001	69	0.021	0.347
*HLA-DRB1*1501*	2	216/465	1.68 (1.20 – 2.34)	0.002	0	0.925	N/A
HLA-DRB3	4	260/1845	0.62 (0.49 – 0.79)	<0.001	68	0.024	0.689
HLA-DRB4	4	260/1845	1.69 (1.36 – 2.10)	<0.001	61	0.055	0.533

Table 1. Genetic variants significantly associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry[a]
HSD17B8 rs421446 (C)	2	738/1872	0.40 (0.34 – 0.48)	<0.001	0	0.620	N/A
IRF5 rs10954213 (G)	3	1535/6977	0.77 (0.70 – 0.83)	<0.001	99	<0.001	0.948
PTPN22 rs2476601 (A)	4	2099/8678	1.39 (1.24 – 1.56)	<0.001	0	0.693	0.500
RING1/RXRB rs213213 (A)	3	1414/7238	1.71 (1.57 – 1.86)	<0.001	73	0.026	0.187
RXRB rs6531 (C)	3	1557/6955	1.63 (1.50 – 1.77)	<0.001	96	<0.001	0.292
RXRB rs9277935 (T)	3	1417/7233	0.44 (0.37 – 0.50)	<0.001	73	0.025	0.393
SERPINA1 S allele	5	1474/5762	1.30 (1.03 – 1.63)	0.025	0	0.464	0.547
SERPINA1 Z allele	8	3662/8581	2.94 (2.22 – 3.88)	<0.001	41	0.092	0.078
STAT4 rs7574865 (T)	3	1520/6956	1.11 (1.01 – 1.22)	0.029	3	0.357	0.590
TLR9 rs352162 (T)	1	1289/1898	1.58 (1.43 – 1.75)	<0.001	96	<0.001	N/A
TLR9 rs352140 (T)	1	1289/1898	1.13 (1.02 – 1.25)	0.018	0	0.432	N/A
TLR9 rs352139 (T)	1	1289/1898	1.11 (1.00 – 1.23)	0.041	0	0.756	N/A

[a] Harbord test for funnel plot asymmetry was performed for all genetic variants, except for *CD226* rs763361, *CTLA-4* rs3087243, and *PTPN22* rs2476601. In these cases the Harbold test was not applicable and the Egger test was performed.

Genetic associations differ for the different diagnostic and serological subtypes of AAV

A significant association with GPA and /or MPA was present for 25 genetic variants, and a significant association with both GPA and MPA was present for ten genetic variants (supplementary table S4 and figure S2). In six of these ten genetic variants (60%), the associations were in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup (figure 1). A significant association with PR3-ANCA and/or MPO-ANCA was present for 25 genetic variants, and a genetic association with both PR3-ANCA and MPO-ANCA was present for seven genetic variants. In four of these seven genetic variants (57%), the associations were in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup (figure 1). Moreover, ORs were higher for ANCA serotype than for clinical diagnosis in 76% (16/21) of the genetic variants that were significantly associated with both clinical diagnosis and ANCA serotype.

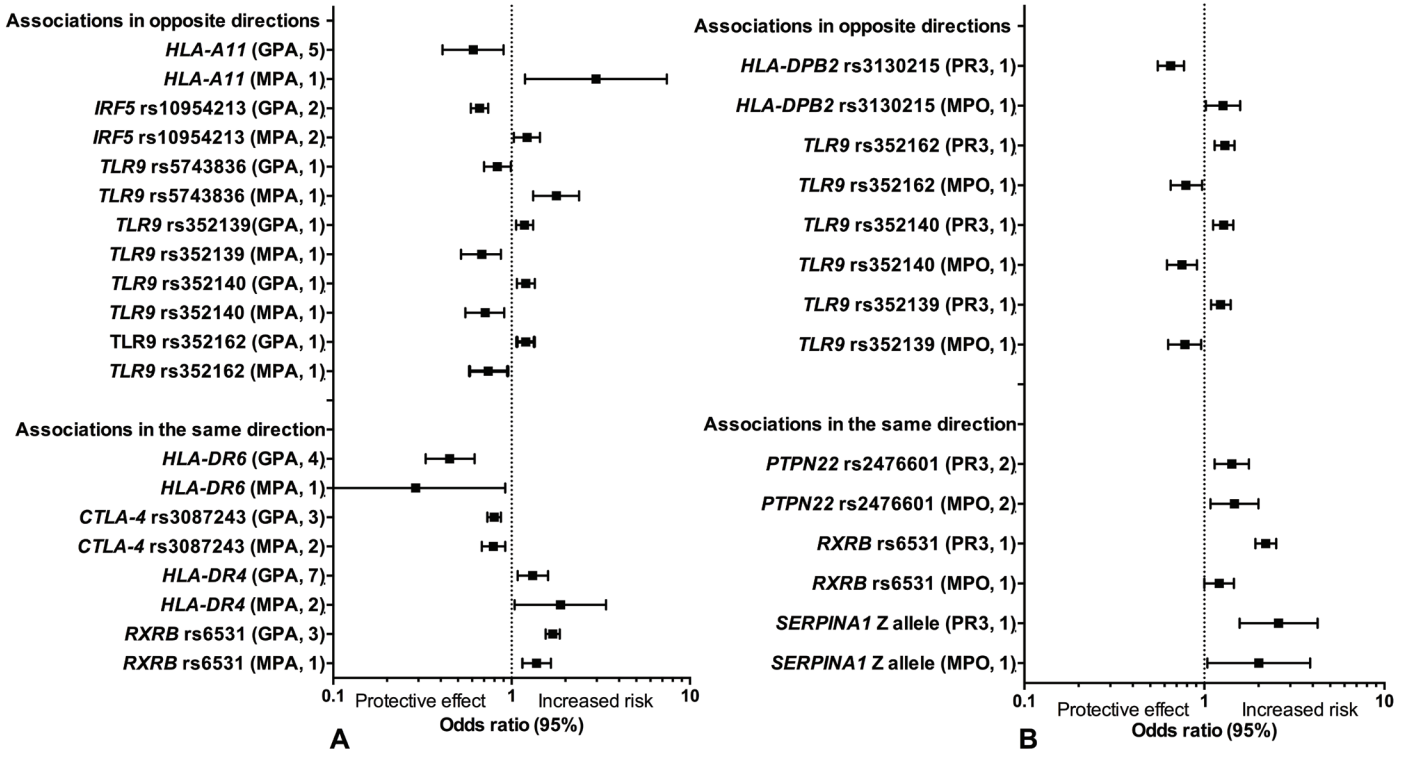
Genetic associations differ for AAV patients of Caucasian and Asian origin

The results of the stratified analyses performed for 42 variants investigated in both Caucasian and Asian patients are depicted in supplementary table S5. CTLA-4 $(AT)_{86}$, CTLA-4 $(AT)_{106}$ and *HLA-DR6* were significantly associated with AAV in the Caucasian patients but not in the Asian patients. Conversely, *HLA-B55* was significantly associated with AAV in the Asian patients but not in the caucasian patients. Of interest, *IRF5* rs10954213 (G) was significantly associated with AAV in both the Caucasian and Asian patients; however, it had a protective effect in the Caucasian patients while it increased the risk of AAV in the Asian patients. The results of these analyses should be interpreted with caution, because each analysis included only one study involving Asian patients.

Genetic variants identified by GWAS

To date, two GWAS have been performed in AAV. The first GWAS included patients with GPA and MPA and found *HLA-DP* rs3117242, *COL11A2* rs3130233, *COL11A2* rs3117016 and *SERPINA1* rs7151526 to be associated with AAV.[15] Moreover, *HLA-DP* rs3117242, *ARHGAP18* rs1705767 and *SERPINA1* rs7151526 were associated with PR3-ANCA vasculitis, and HLA-*DQ* rs5000634 was associated with MPO-ANCA vasculitis. The second GWAS included only patients with GPA and found *HLA-DPB1* rs9277554 and *HL-DPA1* rs9277341 to be associated with GPA.[16] *SEMA6A* rs26595 was associated with GPA at a genome-wide significance level when the results of the two cohorts that were included in this GWAS were combined. However, although *SEMA6A* rs26595 was not genotyped in the first GWAS,[15] data for a large number of proxy SNPs

Figure 1. Subgroup analysis based on clinical diagnosis (A) and antineutrophil cytoplasmic antibody (ANCA) serotype (B), with the clinical diagnosis (A) or ANCA serotype (B) and number of included publications depicted between the parentheses.



(A) In 6 of the 10 genetic variants (60%) in which there was an association with both granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), the associations were in opposite directions. (B) In four of the seven genetic variants (57%) in which there was an association with both proteinase 3 (PR3)-ANCA vasculitis and myeloperoxidase (MPO)-ANCA vasculitis, the associations were in opposite directions.

across this locus were available, and these did not reach statistical significance (data not shown).

Discussion

This meta-analysis identified 33 genetic variants, in or near 15 genes, associated with AAV. Twenty of these 33 genetic variants were present in the MHC region. This study provides the first complete and comprehensive overview including all genetic variants investigated in AAV in at least two studies. Genetic variants in or near *CD226*, *CTLA-4*, *FCGR2A*, *HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, *HSD17B8*, *IRF5*, *PTPN22*, *RING1/RXRB*, *RXRB*, *STAT4*, *SERPINA1* and *TLR9* were associated with AAV in this meta-analysis. Of interest, *FCGR2A* and *STAT4* were not associated with AAV in the individual studies, but were significantly associated with AAV after meta-analysis. Moreover, we showed genetic distinctions between the clinical diagnoses GPA and MPA and between the ANCA serotypes PR3-ANCA and MPO-ANCA. Additionally, our results confirm that subdivision of AAV based on ANCA serotype has a stronger genetic basis than subdivision based on clinical diagnosis.

A number of the genetic variants associated with AAV in this meta-analysis have also been associated with other autoimmune diseases, such as *CTLA-4* rs3087243 in rheumatoid arthritis[28,29] and type 1 diabetes[30,31] and *PTPN22* rs2476601 in Crohn's disease,[32–34] Behçet's disease,[35] systemic lupus erythematosus[36–38] and giant cell arteritis.[39,40] These findings are in line with the fact that first-degree relatives of patients with AAV have an increased risk of other autoimmune diseases.[41, 42] Overlapping genetic variants may form the basis of a disturbed immune system, and together with environmental factors and other, more distinct genetic factors, form a 'bad hand of cards' that leads to the development of AAV.

The results of this meta-analysis support a role for the intricate relationship among alpha-1-antitrypsin and ANCA, the MHC system and other inflammatory processes in the pathogenesis of AAV. The association between *SERPINA1* and AAV supports the concept that ANCA are important in AAV pathogenesis. Alpha-1-antitrypsin is coded by *SERPINA1* and is a major inhibitor of PR3. It has been hypothesised that lower levels of alpha-1-antitrypsin, resulting from the presence of the Z and S alleles of *SERPINA1*, lead to increased levels of circulating PR3 and possibly trigger the synthesis of anti-PR3-ANCA.[43] This hypothesis implies that the association of AAV with alpha-1-antitrypsin deficiency is restricted to PR3-ANCA positive patients; however, in this meta-analysis, the association between the Z allele of *SERPINA1* was present in both PR3-ANCA-positive and MPO-ANCA-positive patients and in both c-ANCA-positive and p-ANCA-positive patients. Another hypothesis is that patients with AAV and alpha-1-antitrypsin

deficiency have a reduced ability to bind PR3 released by previously activated neutrophils, thus promoting PR3-mediated proteolytic vessel damage.

As noted, 20 genetic variants in the MHC region were associated with AAV in this meta-analysis. We, therefore, confirm an important role for the MHC region, but because of linkage disequilibrium, were unable to determine the nature of this association, that is, whether it represents single or multiple independent associations. Both GWAS showed that the SNP association signal in the MHC region was fully accounted for by *HLA-DPB1*, dramatically diminishing the associations of other SNPs in this region.[15, 16] The results of our meta-analysis also support a role for other inflammatory processes in the pathogenesis of AAV, with the associations with *CTLA-4* and *PTPN22* suggesting a role for a threshold of activation or suppression of T cells.

The different subtypes generally grouped under the umbrella term AAV have profound differences in ANCA specificities[3] and clinical outcomes.[4] The results of this meta-analysis indicate that these different AAV subtypes also have distinct genetic backgrounds, as previously shown in a GWAS.[15] Moreover, we found significant associations in opposite directions for the different AAV subgroups. Significant associations of the same SNP in opposite directions for different types of autoimmune diseases have been described before and could be indicative of different mechanisms of disease.[44] Larger studies are required to investigate this issue further in AAV.

The subdivision of AAV based on ANCA serotype had the stronger genetic basis in our meta-analysis; in 76% of the genetic variants, subdivision based on ANCA serotype resulted in higher ORs than subdivision based on clinical diagnosis. The results of these analyses should, however, be interpreted with caution because of the limited number of studies included in some of the analyses and need to be validated in other studies. Although until now the concept of a single disease spectrum has resulted in similar treatment strategies in patients with AAV, our limited results suggest that syndrome-specific therapeutics based on ANCA serotype strategies may be considered.

Our study has some limitations. First, in some of our analyses, the number of subjects or studies was limited; this limitation was especially the case in the subgroup analyses. Second, publication bias is an issue of concern in all meta-analyses. Authors might omit non-significant genetic associations and report only those associations that reach statistical significance. However, none of the tests performed to assess funnel plot asymmetry in this meta-analysis were significant. Furthermore, the studies included in this meta-analysis show heterogeneity with respect to clinical diagnosis, ANCA serotype, disease characteristics, ethnicity and study design. The clinical heterogeneity was accompanied by statistical heterogeneity for 16 of the 140 included genetic variants. However, there is no fully accepted statistical measure that precisely determines clinical heterogeneity.[27] To account for heterogeneity, random-effects models were

performed where possible.[25] Nevertheless, estimates reported in this study should be interpreted with caution, especially when statistical heterogeneity was present or when a small number of studies and/or relatively small groups of participants were included. Finally, it should be kept in mind that the genetic associations identified do not imply causality. While they provide insight into pathogenicity and suggest the involvement of certain pathways, these may not represent therapeutic targets.

In summary, this meta-analysis identified 33 genetic variants, in or near 15 genes, associated with AAV. Moreover, we showed genetic distinctions among the different AAV subtypes, supporting the concept that these subtypes may represent distinct autoimmune syndromes. These subtypes are most likely driven by ANCA serotype and not by clinical diagnosis.

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