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Basic science

Stratified genetic analysis reveals sex differences in MPO-ANCA-associated vasculitis

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Abstract

Objective: To identify and genetically characterize subgroups of patients with ANCA-associated vasculitides (AAV) based on sex and ANCA subtype.

Methods: A previously established SNP dataset derived from DNA sequencing of 1853 genes and genotyping of 1088 Scandinavian cases with AAV and 1589 controls was stratified for sex and ANCA subtype and analysed for association with five top AAV SNPs. rs9274619, a lead variant at the *HLA-DQB1/HLA-DQA2* locus previously associated with AAV positive for myeloperoxidase (MPO)-ANCA, was analysed for association with the cumulative disease involvement of ten different organ systems.

Results: rs9274619 showed a significantly stronger association to MPO-ANCA-positive females than males [$P = 2.0 \times 10^{-4}$, OR = 2.3 (95% CI 1.5, 3.5)], whereas proteinase 3 (PR3)-ANCA-associated variants rs1042335, rs9277341 (*HLA-DPB1/A1*) and rs28929474 (*SERPINA1*) were equally associated with females and males with PR3-ANCA. In MPO-ANCA-positive cases, carriers of the rs9274619 risk allele were more prone

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to disease engagement of eyes [$P = 0.021$, OR = 11 (95% CI 2.2, 205)] but less prone to pulmonary involvement [$P = 0.026$, OR = 0.52 (95% CI 0.30, 0.92)]. Moreover, AAV with both MPO-ANCA and PR3-ANCA was associated with the PR3-ANCA lead SNP rs1042335 [$P = 0.0015$, OR = 0.091 (95% CI 0.0022, 0.55)] but not with rs9274619.

Conclusions: Females and males with MPO-ANCA-positive AAV differ in genetic predisposition to disease, suggesting at least partially distinct disease mechanisms between the sexes. Double ANCA-positive AAV cases are genetically similar to PR3-ANCA-positive cases, providing clues to the clinical follow-up and treatment of these patients.

Keywords: ANCA-associated vasculitis, ANCA, sex differences

Rheumatology key messages

- Sex differences in genetic predisposition to MPO-ANCA+ AAV.
- HLA-DQ variant associated with clinical manifestations in MPO-ANCA+ AAV.
- PR3- and MPO-ANCA double-positive cases share AAV risk variant with PR3-ANCA+ cases.

Introduction

Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV) comprise the three rare disorders: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic GPA. AAV are clinically heterogeneous and may manifest with a spectrum of symptoms, ranging from mild signs to life-threatening organ failure. The aetiology of AAV remains poorly understood; large-scale genetic analyses have, however, identified disease susceptibility loci associated with the presence of either of two autoantibodies: proteinase 3 (PR3)-ANCA (HLA-DP, *SERPINA1*, *PRTN3*) and myeloperoxidase (MPO)-ANCA (HLA-DQ, *BACH2*) in GPA and MPA [1–3]. In contrast, further subgroup analysis based on clinical organ involvement has not revealed any specific genetic associations [2], and potential sex differences in genetic susceptibility have not been investigated to date.

Improved understanding of the pathogenesis of AAV and deconvolution of the heterogeneity among patients are important steps in order to advance clinical diagnosis, decision-making and, ultimately, disease outcome. Moving forward, identifying subgroups within the patient population defined by shared clinical manifestations, molecular mechanisms or genetic background, for example, is being prioritized, which may enable enhanced estimation of risk of relapse, organ engagement and treatment response.

In this study we aimed to genetically define and characterize subgroups of patients with GPA and MPA, based on sex and ANCA subtype. We analysed key SNPs in a DNA sequencing and genotyping dataset of 1088 patients with AAV and intersected central findings with clinical data of disease manifestations.

Material and methods

Subjects

Patients included in the study were recruited at units of rheumatology or nephrology in hospitals in ten cities in Sweden, Norway and Denmark (Supplementary Data S1, available at *Rheumatology* online). Patients clinically diagnosed with GPA or MPA were included at diagnosis or at follow-up visits to the outpatient clinics, after informed and written consent. All patients met the corresponding classification criteria of the European Medicines Agency algorithm [4]. Healthy controls were blood donors or population controls from Sweden and Norway. The study complies with the Declaration of Helsinki. The locally appointed ethics committees approved the research protocol.

Genetic analyses

Genetic analyses are described in detail in Supplementary Data S1, available at *Rheumatology* online and previously [3]. Briefly, DNA samples of cases in the discovery cohort and controls of both the discovery and replication cohorts were subject to liquid capture and sequencing of exons and conserved regions of 1853 immune-related genes [5] using a SeqCap EZ Choice XL library (Roche NimbleGen, Basel, Switzerland) and Illumina HiSeq 2500 (San Diego, CA, USA). Sequence data were processed according to GATK best practices [6] and quality assessed concerning, for example, read depth, allelic imbalance, Hardy–Weinberg equilibrium, population stratification (LASER [7, 8]) and cryptic relatedness (KING [9]). DNA samples of cases of the replication cohort were analysed for 37 candidate AAV SNPs, selected based on the discovery analysis [3], using iPLEX MassARRAY (Agena Bioscience, San Diego, CA, USA).

After quality control, 588 ANCA-positive cases and 999 controls remained for analysis in the discovery cohort and 500 cases and 590 controls remained in the replication cohort (Supplementary Table S1, available at *Rheumatology* online). To determine sex-related differences in genetic associations, five independent SNPs previously identified as significantly associated with PR3-ANCA positive (+) AAV (rs1042335, rs9277341, rs28929474) and MPO-ANCA+ AAV (rs9274619, rs78275221), respectively [3], were selected for analysis. The five SNPs were analysed using logistic regression in the discovery and replication datasets, separately. The analyses were stratified for sex (cases *vs* controls) and with female *vs* male cases, within each ANCA subgroup (PR3/MPO), using an additive model with Plink v.1.9 [10] (4 PCs as covariates). For the five SNPs, the threshold for significance in the analyses of females *vs* males was set to $P < 0.05$. Next, the summary statistics of the sex-stratified discovery and replication analyses were combined in one meta-analysis per sex per ANCA subgroup, as well as for the female *vs* male analyses. Meta-analyses were performed using GWAMA v.2.2.2 [11]. Sex-SNP interactions at rs9274619 were analysed in MPO-ANCA+ female/male mixed cohorts, *vs* controls. The discovery and replication cohorts were analysed separately, using a logistic regression framework as implemented in Plink v.1.9, and a joint analysis of all MPO-ANCA+ cases *vs* all controls was performed using logistic regression in SPSS v.28.0.1.1.

In addition, the five SNPs were analysed separately in AAV cases positive for both PR3- and MPO-ANCA, *vs* controls. Since the 'double-positive' sample size was small (discovery cohort: $n = 13$; replication cohort: $n = 2$), the cases of the discovery and replication cohorts were combined and analysed

against controls of both cohorts ($n = 1589$), using Fisher's exact test (RStudio 2022.07.01 build 554) with $P < 0.05$ as threshold for significance.

Clinical disease manifestations

Clinical data concerning presence of ANCA (measured using enzyme linked immunosorbent assay) and cumulative AAV involvement of any of ten different organ systems were collected from medical records of all patients. The organ systems were ear-nose-throat, lungs, eyes, kidneys, joints/muscles, skin, central nervous system, peripheral nervous system, gastrointestinal tract and heart. Any kind of involvement assessed as a manifestation of AAV by the patient's attending physician according to the medical record, and that had occurred at any time before data collection [mean (SD) years of disease duration at data collection: 9.1 (±7.1)] was recorded as 'present' and otherwise 'absent'; e.g. pulmonary haemorrhage and pulmonary granulomas would both be recorded as 'pulmonary involvement = present'.

Associations between the SNP rs9274619 [homo- or heterozygosity of risk allele (A) *vs* homozygosity of wild type allele (G)] and clinical disease manifestations were analysed using logistic regression (RStudio 2022.07.01 build 554) with a Bonferroni corrected threshold at $P < 0.005$.

Results

Differential genetic predisposition to disease between females and males with MPO-ANCA+ AAV

In a large-scale targeted resequencing study with replication in an independent cohort, we have previously confirmed genetic associations to the *HLA-DPB1/DPA1* and *SERPINA1* loci and to the *HLA-DQB1/DQA2* locus in PR3-ANCA+ and MPO-ANCA+ AAV, respectively. We identified a novel association with the *BACH2* locus in MPO-ANCA+ AAV [3]. Now, we set out to investigate whether these AAV loci are strong predisposing factors to AAV in both males and females by analysing the lead SNP(s) for each locus (Table 1) with cohorts stratified by sex, as well as for females *vs* males. We will here, for brevity, focus on the results of the meta-analyses (results of the discovery-, replication- and meta-analyses are detailed in Table 1).

The SNP with the previously strongest association with MPO-ANCA+ AAV, rs9274619 ($P < 10^{-24}$), showed an almost equally strong association with MPO-ANCA+ AAV in females alone [$P = 3.4 \times 10^{-24}$, odds ratio (OR) = 4.9, (95% CI 3.6, 6.7)], but a weaker association in males [$P = 0.0030$, OR = 1.9 (95% CI 1.3, 3.0); Table 1]. There was a statistically significant difference between females and males [$P = 2.0 \times 10^{-4}$, OR = 2.3 (95% CI 1.5, 3.5)], with minor allele frequencies (MAFs) 0.41 and 0.23, respectively. SNP-sex interaction analyses of the discovery and replication datasets, respectively, showed support of an interaction (Table 1), as did a joint analysis of all MPO-ANCA+ cases *vs* controls [$P = 0.002$, OR = 2.5, (95% CI 1.4, 4.6)]. To investigate whether MPO-ANCA+ males instead harbour the PR3-ANCA lead risk variant at *HLA-DPB1*, the MAF of rs1042335 was compared between the sexes but did not reveal any significant difference [$P = 0.13$, OR = 0.71 (95% CI 0.45, 1.1)]. Furthermore, there was a stronger association in females than in males for the *BACH2* variant rs78275221 ($P = 1.4 \times 10^{-6}$ *vs* $P = 0.17$), but no significant difference when comparing females to males (Table 1).

Next, the two independently PR3-ANCA-associated lead SNPs at the *HLA-DPB1/DPA1* locus, rs1042335 and rs9277341, were analysed in PR3-ANCA+ females and males, respectively. The SNPs were strongly associated with disease in both females and males ($P < 10^{-20}$), with no significant differences between the sexes (Table 1). Neither was there a difference for rs28929474 (*SERPINA1*), although the association was stronger in females than males compared with controls ($P = 1.1 \times 10^{-8}$ *vs* $P = 0.0025$; Table 1).

The HLA-DQ locus is associated with clinical manifestations in MPO-ANCA+ AAV

Previous reports have suggested that there may be sex-dependent differences in clinical manifestations in AAV [12, 13]. The difference in genetic contribution of the *HLA-DQB1/HLA-DQA2* locus to MPO-ANCA+ AAV in females and males prompted us to investigate if there is an association between rs9274619 and clinical presentation of disease. Clinical data comprising AAV involvement of ten different organ systems were available for 258 MPO-ANCA+ cases with known rs9274619 genotype, from the combined discovery and replication cohort. There were no significant associations with clinical manifestations after correction for multiple testing, but there was a suggestive association with eye involvement in carriers of the rs9274619 risk allele [10% *vs* 0%; $P = 0.021$, OR = 11 (95% CI 2.2, 205)] and with pulmonary involvement in non-carriers [46% *vs* 34%; $P = 0.026$, OR = 0.52 (95% CI 0.30, 0.92); Table 2]. The distribution of eye and pulmonary involvement in regard to rs9274619 was similar in females and males (Supplementary Table S2, available at *Rheumatology* online).

Double ANCA positive AAV is associated with PR3-ANCA risk variant

Among the 1088 cases in the combined cohort comprising the discovery and replication datasets, 15 were positive for both PR3- and MPO-ANCA. Fourteen of these individuals were classified as GPA and one as MPA. To investigate whether these double-positive individuals share genetic predisposition to AAV with 'single-positive' PR3-ANCA+ or MPO-ANCA+ cases, they were analysed for association with the five SNPs. There was a significant association with the PR3-ANCA SNP rs1042335 [$P = 0.0015$, OR = 0.091 (95% CI 0.0022, 0.55)], where the double-positive cases shared the low MAF with PR3-ANCA+ AAV (0.033 and 0.043, respectively). There was no association with the remaining SNPs (Supplementary Table S3, available at *Rheumatology* online).

Discussion

Our findings confirmed a strong association to the *HLA-DPB1/DPA1* locus in both females and males with PR3-ANCA+ AAV, but revealed a significant difference in association to the lead *HLA-DQB1/HLA-DQA2* locus in MPO-ANCA+ AAV, with a strong association in females but only a modest association in males. Although previous studies have demonstrated a skewed male-to-female ratio in the incidence of GPA (1.35±0.1) and MPA (0.56±0.3) in Europe [14], few studies have investigated putative sex differences in the pathogenesis of AAV. Recently, however, an analysis of

Table 1. Genetic association analysis of discovery, replication and combined cohorts, with individuals stratified according to sex and presence of ANCA

						Discovery analysis						Replication analysis						Meta-analysis			
	Position (hg19)	SNP	Gene(s)	Minor allele	Groups analyzed (1 *vs* 2)	*n* group 1 *vs* 2	MAF group 1	MAF group 2	*P*	OR	95% CI	*n* group 1 *vs* 2	MAF group 1	MAF group 2	*P*	OR	95% CI	*n* group 1 *vs* 2	*P*	OR	95% CI
PR3-ANCA + AAV																					
	Chr6: 33052958	rs1042335	*HLA-DPB1*	T	Females *vs* Controls	185 *vs* 707	0.027	0.27	1.5×10^{-15}	0.07	0.036, 0.13	179 *vs* 458	0.056	0.27	1.6×10^{-15}	0.13	0.082, 0.22	364 *vs* 1165	6.8×10^{-29}	0.11	0.071, 0.16
					Males *vs* Controls	241 *vs* 292	0.046	0.29	2.0×10^{-18}	0.11	0.068, 0.18	222 *vs* 132	0.041	0.29	1.4×10^{-16}	0.09	0.047, 0.15	463 *vs* 424	3.1×10^{-33}	0.10	0.068, 0.14
					Females *vs* Males	185 *vs* 241	0.027	0.046	0.17	0.59	0.29, 1.2	179 *vs* 222	0.056	0.041	0.34	1.4	0.73, 2.5	364 *vs* 463	0.87	0.96	0.60, 1.5
	Chr6: 33039625	rs9277341	*HLA-DPA1*	C	Females *vs* controls	185 *vs* 707	0.060	0.30	2.1×10^{-16}	0.15	0.093, 0.23	179 *vs* 458	0.092	0.27	1.6×10^{-10}	0.28	0.19, 0.41	364 *vs* 1165	2.1×10^{-24}	0.21	0.16, 0.29
					Males *vs* controls	241 *vs* 292	0.081	0.28	2.4×10^{-13}	0.23	0.16, 0.34	222 *vs* 132	0.070	0.25	3.2×10^{-9}	0.24	0.15, 0.38	463 *vs* 424	4.8×10^{-21}	0.23	0.17, 0.32
					Females *vs* Males	185 *vs* 241	0.06	0.081	0.26	0.74	0.43, 1.3	179 *vs* 222	0.092	0.070	0.25	1.3	0.81, 2.2	364 *vs* 463	0.95	1.0	0.70, 1.5
	Chr14: 94844947	rs28929474	*SERPINA1*	T	Females *vs* controls	185 *vs* 707	0.064	0.023	0.00017	3.1	1.7, 5.5	179 *vs* 458	0.087	0.025	1.5×10^{-5}	3.5	2.0, 6.2	364 *vs* 1165	1.1×10^{-8}	3.3	2.2, 4.9
					Males *vs* controls	241 *vs* 292	0.049	0.024	0.032	2.1	1.1, 4.3	222 *vs* 132	0.065	0.024	0.030	2.6	1.1, 6.3	463 *vs* 424	0.0025	2.3	1.3, 4.0
					Females *vs* Males	185 *vs* 241	0.064	0.049	0.33	1.4	0.74, 2.5	179 *vs* 222	0.087	0.065	0.28	1.32	0.80, 2.2	364 *vs* 463	0.15	1.3	0.91, 2.0
MPO-ANCA + AAV																					
	Chr6: 32635954	rs9274619	*HLA-DQB1*, *HLA-DQA2*	A	Females *vs* controls	119 *vs* 707	0.38	0.14	7.6×10^{-11}	4.2	2.7, 6.5	62 *vs* 458	0.48	0.13	3.9×10^{-15}	5.8	3.7, 9.0	181 *vs* 1165	3.4×10^{-24}	4.9	3.6, 6.7
					Males *vs* controls	56 *vs* 292	0.25	0.15	0.048	1.9	1.0, 3.7	39 *vs* 132	0.26	0.14	0.027	1.9	1.1, 3.5	95 *vs* 424	0.0030	1.9	1.3, 3.0
					Females *vs* Males	119 *vs* 56	0.38	0.25	0.011	2.4	1.2, 4.9	62 *vs* 39	0.48	0.26	0.0068	2.2	1.2, 2.8	181 *vs* 95	2.0×10^{-4}	2.3	1.5, 3.5
					SNP*sex interaction[a]				0.051	0.52	0.27, 1.0				0.0032	0.33	0.16, 0.69				
	Chr6: 90900544	rs78275221	*BACH2*	A	Females *vs* controls	119 *vs* 707	0.084	0.018	5.6×10^{-6}	4.4	2.3, 8.2	62 *vs* 458	0.065	0.029	0.044	2.4	1.0, 5.5	181 *vs* 1165	1.4×10^{-6}	3.5	2.1, 5.8
					Males *vs* controls	56 *vs* 292	0.054	0.027	0.17	2.0	0.74, 5.5	39 *vs* 132	0.026	0.019	0.71	1.4	0.26, 7.4	95 *vs* 424	0.17	1.9	0.78, 4.3
					Females *vs* Males	119 *vs* 56	0.084	0.054	0.37	1.5	0.60, 3.9	62 *vs* 39	0.065	0.026	0.22	2.7	0.55, 14	181 *vs* 95	0.17	1.8	0.79, 4.0

[a] SNP*sex interaction analysis of mixed females and males positive for MPO-ANCA, *vs* controls.

AAV: ANCA-associated vaculitis; MAF: minor allele frequency; MPO: myeloperoxidase; OR: odds ratio; PR3: proteinase 3; PR3-ANCA+/MPO-ANCA+: AAV patients positive for indicated ANCA; SNP: single nucleotide polymorphism.

Table 2. Comparison of organ involvement between carriers and non-carriers of the rs9274619 risk allele in MPO-ANCA+ AAV cases

	rs9274619		G/G vs (A/G + A/A)		
	G/G	A/G, A/A	Unadjusted *P*	OR	95% CI
Total, *n*	107	151			
ENT; *n* (%)	30 (29)	38 (26)	0.70	0.87	0.44, 1.7
Pulmonary; *n* (%)	48 (46)	51 (34)	0.026	0.52	0.30, 0.92
Eye[a]; *n* (%)	0 (0)	14 (10)	0.021	11	2.2, 205
Renal; *n* (%)	91 (85)	127 (86)	0.50	0.75	0.32, 1.7
Joint/muscle; *n* (%)	36 (34)	56 (38)	0.78	1.1	0.60, 2.0
Skin; *n* (%)	13 (12)	18 (12)	0.51	0.75	0.32, 1.7
CNS; *n* (%)	3 (3)	2 (1)	0.46	0.50	0.061, 3.3
PNS; *n* (%)	20 (19)	16 (11)	0.19	0.58	0.25, 1.3
GI; *n* (%)	1 (1)	5 (3)	0.24	3.7	0.56, 72
Heart[a]; *n* (%)	4 (4)	0 (0)	0.12	0.18	0.0089, 1.2

[a] Logistic regression analysis was performed with one individual positive.
G/G, A/G, A/A represent genotypes of rs9274619.
A: AAV risk allele; AAV: ANCA-associated vasculitis; GI: gastrointestinal tract; MPO: myeloperoxidase; OR: odds ratio; PNS: peripheral nervous system.

AAV with biopsy-verified glomerulonephritis identified significant histopathological differences between females and males [13], suggesting that sex-specific immunological mechanisms may exist in AAV. Our findings suggest a differential impact of genetic variation on disease risk in males and females with MPO-ANCA+ AAV. These results motivate a focus on potential sex differences in the clinical outcome in AAV.

Moreover, we identified a suggestive association between rs9274619 at the *HLA-DQB1/DQA2* locus and clinical manifestations of AAV. Future replications are warranted, but the results raise the hypothesis that an association of rs9274619 with both sex and organ involvement may manifest in differential clinical presentation of disease in males and females.

Whether the rs9274619 variant exerts a disease-modifying effect in AAV remains to be elucidated. *In silico* functional analysis of the large set of SNPs in LD with this variant suggests, however, that several of the SNPs may affect coding and non-coding functional sequences in the HLA-DQ region [3].

AAV cases positive for both PR3- and MPO-ANCA at some point during the disease course are well known, but rare and scarcely studied. In our cohort, 1.4% were at some point double-positive, in contrast to a Korean cohort, where 9.4% were positive for both ANCAs [15]. In accordance with the high rate of GPA diagnosis among the double-positive cases (93%), we found a strong association with the top genetic locus for PR3-ANCA+ AAV. Although caution should be taken with regard to the small double-positive sample size, these findings indicate that in the clinical care, double-positive cases of European descent should be regarded as PR3-ANCA+ patients for the assessment of organ involvement and prognosis.

A limitation of this study was small sample sizes, in particular for MPO-ANCA+ males, leading to inconclusive results for the rare *BACH2* and *SERPINA1* variants. Although the replication of the results for the common variants in an independent cohort supports the robustness of the results, future analyses in larger cohorts of different ancestry would be desirable.

In conclusion, in this study, we identified and characterized subgroups of patients with AAV, with implications for clinical assessment and follow-up of vasculitis patients. Specifically, our results suggest sex-dependent genetically distinct subgroups of patients within MPO-ANCA+ AAV. In addition, patients positive for both PR3- and MPO-ANCA are more likely to share characteristics with PR3-ANCA+ patients.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

The datasets generated and/or analysed in the present study are not publicly available due to them containing information that could compromise research participant privacy and consent. However, they are available from the corresponding author Johanna Dahlqvist upon reasonable request and on a collaborative basis.

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