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PTPN22 R620W polymorphism in the ANCA-associated vasculitides

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Abstract

Objectives. PTPN22 is involved in T-cell activation and its R620W single-nucleotide polymorphism (SNP) has been shown to predispose to different autoimmune diseases. The aims of this study were to investigate the role of the *PTPN22* R620W SNP in conferring susceptibility to the ANCA-associated vasculitides (AAVs), and to explore potential associations between the *PTPN22* genotype and the disease manifestations.

Methods. *PTPN22* R620W SNP was genotyped in a cohort of 344 AAV patients [143 with granulomatosis with polyangiitis (Wegener's) (GPA), 102 with microscopic polyangiitis (MPA) and 99 with Churg–Strauss syndrome (CSS)] and in 945 healthy controls.

Results. The frequency of the minor allele (620W) was significantly higher in GPA patients than in controls [$P = 0.005$, $\chi^2 = 7.858$, odds ratio (OR) = 1.91], while no statistically significant association was found with MPA or CSS. Among GPA patients, the 620W allele was particularly enriched in ANCA-positive patients as compared with controls ($P = 0.00012$, $\chi^2 = 14.73$, OR = 2.31); a particularly marked association was also found with ENT involvement ($P = 0.0071$, $\chi^2 = 7.258$, OR = 1.98), lung involvement ($P = 0.0060$, $\chi^2 = 7.541$, OR = 2.07) and skin manifestations of all kinds ($P = 0.000047$, $\chi^2 = 16.567$, OR = 3.73).

Conclusion. The *PTPN22* 620W allele confers susceptibility to the development of GPA (but not of MPA or CSS), and particularly of its ANCA-positive subset.

Key words: vasculitis, ANCA, PTPN22, granuloma, autoimmunity, granulomatosis with polyangiitis

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Introduction

The ANCA-associated vasculitides (AAVs) comprise a spectrum of autoimmune diseases characterized by necrotizing small-vessel vasculitis and the frequent presence of ANCA [1]. The AAVs include granulomatosis with polyangiitis (Wegener's) (GPA), microscopic polyangiitis (MPA) and Churg–Strauss syndrome (CSS). The AAVs range from organ-limited to disseminated, life-threatening disorders; they commonly affect the kidney, the respiratory tract and the peripheral nervous system, but any other organ or system can be involved [2]. The aetiology of the AAVs is only partially elucidated. Infectious agents and environmental factors are likely to play a pathogenetic

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role [3–5], but genetic determinants are also claimed to increase susceptibility to the development of AAVs [6–9]. However, as the AAVs have different clinical phenotypes, it is plausible that their genetics also differs [4, 5, 9]; thus, genetic studies on the AAVs need to include patients with all the three AAV forms to investigate potential similarities or differences in their genetic background.

Genetic risk factors may be disease specific, but some of them may be common determinants of a wide range of conditions, particularly in the setting of autoimmunity [10]. This is the case of the gene encoding protein tyrosine phosphatase type 22 (*PTPN22*), whose R620W single-nucleotide polymorphism (SNP) [rs2476601, NM_015967.5(PTPN22_v001): c.1858C>T] has been described as a common genetic risk factor for many autoimmune and inflammatory diseases [11–14]. *PTPN22* encodes a lymphoid-specific phosphatase (LYP); the R620W SNP, causing substitution of arginine for tryptophan at amino acid residue 620, has been shown to affect protein–protein interactions between LYP and the tyrosine kinase Csk, which is involved in T-cell activation. Furthermore, a role in B-cell signalling has also been suggested [15].

The role of the *PTPN22* R620W polymorphism in the AAVs has been explored in a German cohort of GPA patients [16] and in a UK cohort comprising patients with all AAVs [6]. Both studies demonstrated that it is a susceptibility factor for GPA, and the UK study also found a significant association with MPA. However, the potential association between this SNP and CSS has not been analysed; in addition, the association found with MPA resulted from the analysis of a relatively small cohort of patients (only 74 patients in the UK study had a known diagnosis of MPA), and has never been replicated in other populations. Finally, the association between genetic and clinical findings was only investigated in the German study.

In the present study we genotyped the *PTPN22* R620W polymorphism in a cohort of 344 AAV patients (including GPA, MPA and CSS) and in 945 healthy controls, in order to replicate its association with GPA and MPA and to explore whether it also confers susceptibility to CSS. Additionally, we also investigated potential associations between this SNP and the main clinical manifestations of AAVs or their disease patterns.

Patients and methods

Study subjects

We recruited 344 consecutive patients with AAV [175 women and 169 men, with a median age at diagnosis of 55 (range 13–86) years]. The patients were recruited at the following centres in northern Italy: the Nephrology Departments of the University Hospitals in Parma and Brescia, of the Policlinico and San Carlo Borromeo Hospitals in Milano, and of the general hospitals in Perugia, Legnano and Como; the Rheumatology Department of Reggio Emilia Hospital; the Pulmonary Department of the Milano Bicocca University Hospital; and the Internal Medicine Departments of the Reggio Emilia Hospital and of the University Hospitals in Torino and Ancona.

One hundred and forty-three patients were affected by GPA, 102 by MPA and 99 by CSS. The AAVs were diagnosed based on the presence of clinical manifestations consistent with small-vessel vasculitis, with or without histological confirmation, then the diseases were classified as GPA, MPA or CSS following the 1990 ACR classification criteria for CSS and GPA, and the Chapel Hill Consensus Conference definitions [17–19]. When a diagnostic biopsy was not available and ANCAs were negative or undetermined, the diagnosis had to be supported by a compatible clinical picture, together with surrogate markers for GPA (i.e. persistent lung infiltrates, nodules or cavitations, bronchial stenosis, bloody nasal discharge or crusting, retro-orbital mass, chronic rhino-sinusitis, otitis or mastoiditis, saddle nose deformity, subglottic stenosis) or renal vasculitis (i.e. haematuria with red cell casts or 2+ haematuria and 2+ proteinuria) [20]; in cases of suspected CSS, at least four of the five non-histological ACR criteria had to be fulfilled. As examples, patients with chronic sinusitis and crusts, lung nodules and rapidly progressive renal failure with an active urinary sediment were included as GPA cases, whereas patients with only lung nodules and rapidly progressive renal failure but without active urinary sediment were excluded; equally, patients with asthma, eosinophilia, neuropathy and lung infiltrates were included as CSS cases, whereas patients with only asthma, eosinophilia and lung infiltrates were excluded. Patients with rapidly progressive renal failure but without involvement of other organs were excluded despite the presence of an active urinary sediment because it was not possible to classify them [20]. Importantly, in patients with negative or undetermined ANCA and no diagnostic biopsy, we required a minimum follow-up of 6 months for diagnosis confirmation.

Organ involvement in the AAV patients was assessed by physical examination, routine laboratory tests and a standardized set of imaging studies. ANCA status was determined at the time of diagnosis using IIF and antigen-specific PR3 and MPO ELISAs.

A total of 945 age- and gender-matched healthy subjects with no history of autoimmune or inflammatory diseases served as controls. All the study subjects were of Italian Caucasian origin and, similar to the cases, they all came from northern Italy. Individuals from genetic isolates were not included.

Written informed consent was obtained from all subjects included in the study, and from parents for minors as appropriate. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethical Committees of the University Hospital of Parma, Perugia Hospital, Reggio Emilia Hospital, Ospedali Riuniti in Ancona, University of Torino, Spedali Civili in Brescia, Policlinico and San Carlo Borromeo Hospitals in Milano and Milano Bicocca University.

SNP genotyping

Genomic DNA was extracted from EDTA-treated peripheral blood samples (5 ml of blood per study subject) using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at −20°C until use. The rs2476601 *PTPN22* SNP was genotyped using an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA) with a TaqMan 5′-allele discrimination Assay-By-Design method (Applied Biosystems, Foster City, CA, USA). PCR was performed according to the manufacturer's instructions. In order to confirm TaqMan genotyping, one-third of the samples were sequenced with a Sanger-based method (CEQ 2000XL Sequence Analysis, Beckman Coulter, CA, USA). The primer sequences used to confirm the TaqMan genotypes were as follows: forward, 5′-ATGAGCCACCATGCCCAT-3′; reverse: 5′-ATGTTGCTTCAACGGAATTTAAAT-3′.

Statistical analysis

Association analysis between the R620W polymorphism and each of the phenotypes investigated was performed by allelic test. Furthermore, the association with the three AAV phenotypes (GPA, MPA, CSS) was also investigated using a dominant model for the allele 620W, as suggested by other authors [16].

Multiple testing was treated using the severe Bonferroni's correction as follows. The three AAV phenotypes were investigated using the allelic and dominant models, and correction was applied for the five tests performed for each of the two models (Table 1). The threshold for defining an association significant when five tests are computed is $P_{cor} = 0.01$, in accordance with Bonferroni's correction, and thus an association was defined statistically significant when its nominal *P*-value was lower than $P_{cor} = 0.01$. It is noteworthy that this correction is somewhat too severe since the five tests for each model are not independent.

Association was also investigated for 14 clinical and laboratory characteristics correlated with the AAV phenotypes. A total of 14 tests was computed and an association was defined as significant when its nominal *P*-values were <0.0036. Statistical analyses were performed using the software PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml).

Results

Clinical characteristics and laboratory findings of the patients

The main clinical and laboratory characteristics of the enrolled AAV patients are shown in Table 2. Detailed clinical data were available in 336 (97.7%) of the 344 patients. In the remaining eight patients (two with GPA, four with MPA and two with CSS), the clinical diagnosis was entered by the recruiting clinician into the study database; however, given the lack of a complete clinical phenotyping, all of these cases had to have a positive PR3- or MPO-ANCA test as well as a diagnostic biopsy to be included. The percentages of the clinical manifestations reported

Table 1 Allele and genotype frequencies of the *PTPN22* R620W polymorphism in patients with ANCA-associated vasculitides and healthy controls

	R620W	GPA (*n* = 143)	MPA (*n* = 102)	CSS (*n* = 99)	Controls (C) (*n* = 945)	*P*-value	OR (95% CI)
Allele frequency	620W allele	25/286 (8.74)	15/204 (7.35)	5/198 (2.52)	90/1890 (4.76)	GPA + MPA *vs* C: 0.0031[a]	1.777 (1.208, 2.617)
	R620 allele	261/286 (91.26)	189/204 (92.65)	193/198 (97.48)	1800/1890 (95.23)	GPA *vs* C: 0.0051[a]	1.915 (1.207, 3.041)
						MPA *vs* C: 0.1072	1.587 (0.901, 2.798)
						CSS *vs* C: 0.1508	0.518 (0.208, 1.291)
Dominant model for the allele W (genotype frequencies)	620W/620W + R620/620W	23/143 (16.08)	15/102 (14.71)	4/98 (4.08)	86/945 (9.11)	GPA + MPA *vs* C: 0.0034[a]	1.834 (1.216, 2.765)
	R620/R620	120/143 (83.92)	87/102 (85.29)	95/98 (95.96)	859/945 (90.89)	GPA *vs* C: 0.0095[a]	1.914 (1.16, 3.15)
						MPA *vs* C: 0.0684	1.722 (0.95, 3.11)
						CSS *vs* C: 0.0878	0.420 (0.151, 1.17)

Except where indicated otherwise, values are represented as *n* (%). [a]Association statistically significant also after correction for multiple tests. OR indicates the effect of the minor allele 620W compared with the allele R620 (allelic test) or the effect of the genotypes 620W/620W or R620/620W compared with the most common genotype R620/R620.

Table 2 Demographic and clinical features of the 344 patients with ANCA-associated vasculitides

	GPA ($n = 143$)	MPA ($n = 102$)	CSS ($n = 99$)	All ($n = 344$)
Mean age at disease onset (range), years	51 (13–85)	61 (16–83)	53 (18–86)	55 (13–86)
Male/female, *n*	73/70	46/56	50/49	169/175
Cardiovascular involvement	11/141 (7.8)	11/98 (11.2)	16/97 (16.5)	38/336 (11.3)
Cutaneous involvement, all kinds	35/141 (24.8)	14/98 (14.3)	42/97 (43.3)	91/336 (27.1)
Purpura	19/141 (13.5)	6/98 (6.1)	14/97 (14.4)	39/336 (11.6)
Gastrointestinal involvement	4/141 (2.8)	7/98 (7.1)	13/97 (13.4)	24/336 (7.1)
Peripheral neuropathy, all kinds	32/141 (22.7)	27/98 (27.6)	75/97 (77.3)	134/336 (39.9)
Mononeuritis multiplex	12/141 (8.5)	10/98 (10.2)	36/97 (37.1)	58/336 (17.3)
CNS involvement	10/141 (7.1)	3/98 (3.1)	7/97 (7.2)	20/336 (5.9)
Eye involvement	32/141 (22.7)	5/98 (5.1)	7/97 (7.2)	44/336 (13.1)
ENT involvement	111/141 (78.7)	32/98 (32.7)	84/97 (86.6)	227/336 (67.5)
Lung involvement, all kinds	96/141 (68.1)	52/98 (53.1)	50/97 (51.5)	198/336 (58.9)
Alveolar haemorrhage	26/139 (18.7)	21/98 (21.4)	4/97 (4.1)	51/334 (15.3)
Asthma	6/141 (4.3)	8/98 (8.2)	90/97 (92.8)	104/336 (31.0)
Constitutional symptoms[a]	121/138 (87.7)	80/98 (81.6)	71/97 (73.2)	272/333 (81.7)
Renal involvement, all kinds	94/141 (66.7)	91/99 (91.9)	27/93 (29.0)	212/333 (63.7)
RPGN	60/139 (43.2)	71/99 (71.7)	3/92 (3.3)	134/330 (40.6)
Mean serum creatinine (range), mg/dl	2.9 (0.8–14.6)	4.2 (0.5–17.9)	1.0 (0.5–6.2)	2.8 (0.8–17.9)
Proteinuria >1 g/24 h	41/115 (35.7)	54/87 (62.1)	7/81 (8.7)	102/283 (36.0)
pANCA by IF	29/139 (20.9)	86/99 (86.9)	48/90 (53.3)	163/328 (49.7)
cANCA by IF	111/139 (79.8)	8/99 (8.1)	7/90 (7.8)	126/328 (38.4)
Anti-MPO antibodies	16/113 (14.2)	75/84 (89.3)	22/41 (53.7)	113/238 (47.5)
Anti-PR3 antibodies	93/120 (77.5)	6/81 (7.4)	0/40 (0)	99/241 (41.1)

Except where indicated otherwise, values are the *n* (%) of patients. [a]Fatigue, fever, anorexia and weight loss.

hereafter are calculated out of the number of patients in whom a given manifestation or laboratory test was available.

GPA was the most common diagnosis (42%). Constitutional symptoms (e.g. fatigue, fever, anorexia, weight loss) were found in 121 (87.7%) patients. Seventy-eight per cent of the patients exhibited ENT involvement. Lung involvement was another major clinic manifestation, affecting 96 (68%) patients, 26 of whom had alveolar haemorrhage. Renal involvement was found in 94 (67%) patients and a rapidly progressive glomerulonephritis (RPGN) occurred in 60 (43%) cases. Skin manifestations were found in 35 (25%) patients, 19 of whom had purpura. Peripheral neuropathy occurred in 32 (23%) patients, with 12 exhibiting mononeuritis multiplex.

MPA was diagnosed in 102 (30%) patients. Renal involvement was the most frequent clinical manifestation, affecting 91 (92%) cases, 71 of whom had RPGN. Nineteen (19%) patients had a renal-limited form of MPA, whereas the remaining 71% had a systemic disease with different vasculitic manifestations, especially peripheral neuropathy (28%), ENT and lung involvement (33 and 53%, respectively), and skin manifestations (14%).

Ninety-nine (29%) patients were affected by CSS. Asthma, ENT involvement and peripheral neuropathy were the major clinical manifestations, affecting 90 (93%), 84 (87%) and 75 (77%) patients, respectively. Constitutional symptoms were found in 71 (73%) cases. Lung involvement occurred in 50 (52%) patients, only 4 of whom had alveolar haemorrhage. Forty-three per cent of the patients exhibited skin manifestations, with purpura in 14% of the cases. Renal involvement occurred in 27 (29%) patients; only three patients had RPGN. Gastrointestinal, cardiac and CNS manifestations occurred in 13 (13%), 16 (17%) and 7 (7%) patients with CSS, respectively.

ANCAs were positive by IF in 227 (80.5%) patients with AAV, negative in 51 (14.8%) and undetermined in 16 (4.7%). The cANCA pattern was more frequent in GPA (80% of the patients), whereas pANCA predominated in MPA and CSS patients (87 and 53%, respectively). ELISA showed anti-PR3 antibodies in 78% of the tested GPA cases, whereas 89% of the tested MPA and 54% of the tested CSS cases had anti-MPO antibodies.

A diagnostic biopsy was available in 217 (63%) patients, of whom 105 had GPA, 39 had CSS and 73 had MPA. Thirty-six patients, of whom 28 had CSS and 8 had GPA, had no diagnostic biopsy and negative or undetermined ANCA; however, all of these patients had a typical clinical phenotype and, in those classified as GPA, surrogate markers of GPA or renal vasculitis [20].

Overall, this collection of patients has a clinical phenotype comparable with that of other large published series [21, 22] and probably reflects the true presentation of AAVs, given the multidisciplinary nature of the recruiting centres.

PTPN22 rs2476601 genotyping in AAV

The genotype frequencies of both AAV patients and healthy controls were in Hardy–Weinberg equilibrium.

Table 1 shows the *PTPN22* rs2476601 genotype and allele frequencies in AAV patients and healthy controls. Genotype analysis was not performed because the numbers in most categories were too small. The frequency of the minor allele (620W) was higher in GPA patients than in controls [$P = 0.0051$, $\chi^2 = 7.858$, odds ratio (OR) = 1.91], whereas no statistically significant differences were found in its frequency between CSS patients ($P = 0.1508$, $\chi^2 = 2.065$, OR = 0.51), MPA patients ($P = 0.1072$, $\chi^2 = 2.595$, OR = 1.59) and controls.

When we compared the frequency of the 620W allele between all AAV patients (GPA + MPA + CSS) and healthy controls, the difference was not statistically significant ($P = 0.0729$, $\chi^2 = 3.216$, OR = 1.40). Conversely, when GPA and MPA were grouped together, the difference in allele frequency *vs* healthy controls was even more pronounced ($P = 0.0031$, $\chi^2 = 8.718$, OR = 1.78) than if GPA alone was considered.

We also performed a meta-analysis (Supplementary Table S1, available as supplementary data at *Rheumatology* Online) combining our results on GPA and MPA cases with those of the previous two studies [6, 16]; this reinforced the strength of the association between the rare allele and AAV, although its significance is limited by the fact that the German study only included GPA cases and that the UK study did not provide the allelic frequencies of the single AAV subtypes (GPA and MPA).

We also tested the hypothesis of a dominant model for the allele 620W since other authors suggested a dominant effect of this allele in GPA patients [16]. We confirmed the association between the R620W polymorphism and GPA ($P = 0.0095$, $\chi^2 = 6.72$, OR = 1.91) and we also observed a significant association when GPA and MPA patients were grouped together ($P = 0.0034$, $\chi^2 = 6.72$, 8.563, OR = 1.83). No association was found when CSS patients were tested alone or grouped with GPA and MPA patients.

We next analysed AAV patients with respect to their ANCA status (with ANCA status being determined by IF): a statistically significant difference was found between ANCA-positive (either pANCA, cANCA or P + C) AAV patients and healthy controls ($P = 0.00813$, $\chi^2 = 7.005$, OR = 1.65). When we only considered ANCA-positive GPA patients (Table 3), the association with the SNP was highly pronounced, with a *P*-value ($P = 0.00012$, $\chi^2 = 14.73$, OR = 2.31) more significant than that obtained when all GPA patients were considered. Furthermore, the predisposing allele was more frequent in GPA patients with anti-PR3 antibodies ($P = 0.02319$, $\chi^2 = 5.154$, OR = 1.88). Overall, *PTPN22* 620W was more frequent in all AAV patients with anti-PR3 antibodies than in controls ($P = 0.0088$, $\chi^2 = 6.848$, OR = 2.00), whereas the association with anti-MPO antibodies did not reach statistical significance ($P = 0.131$).

PTPN22 R620W SNP association with organ involvement in GPA patients

Further analyses of this polymorphism focused on its association with organ involvement in GPA patients, as shown in Table 3. The 620W allele was predominantly associated with skin involvement ($P = 0.000047$, $\chi^2 = 16.567$, OR = 3.73), lung involvement ($P = 0.0060$, $\chi^2 = 7.541$, OR = 2.07), ENT manifestations ($P = 0.0071$, $\chi^2 = 7.258$, OR = 1.98) and constitutional symptoms ($P = 0.0098$, $\chi^2 = 6.665$, OR = 1.90). Interestingly, despite the strong enrichment in GPA patients with skin involvement, the 620W allele frequency was not increased in

Table 3 Organ involvement, ANCA status and the *PTPN22* R620W polymorphism in GPA patients

	GPA genotypes WW/WR/RR	Controls genotypes WW/WR/RR	GPA number of alleles, W/R	Controls number of alleles, W/R	*P*-value[a]	OR (95% CI)
Cardiovascular	0/1/10	4/82/859	1/21	90/1800	0.9622	0.952 (0.127, 7.159)
Skin, all kinds	0/11/24	4/82/859	11/59	90/1800	0.000047[b]	3.729 (1.894, 7.343)
Purpura	0/4/15	4/82/859	4/34	90/1800	0.1023	2.353 (0.817, 6.774)
Peripheral neuropathy	0/7/25	4/82/859	7/57	90/1800	0.0253	2.456 (1.089, 5.538)
ENT	2/16/93	4/82/859	20/202	90/1800	0.0071	1.98 (1.194, 3.284)
Lung, all kinds	2/14/80	4/82/859	18/174	90/1800	0.0060	2.069 (1.218, 3.513)
Alveolar haemorrhage	0/6/20	4/82/859	6/46	90/1800	0.0262	2.609 (1.086, 6.268)
Constitutional symptoms	1/19/101	4/82/859	21/221	90/1800	0.0098	1.9 (1.158, 3.118)
Kidney, all kinds	1/15/78	4/82/859	17/171	90/1800	0.0113	1.988 (1.157, 3.417)
ANCA	3/23/114	4/82/859	29/251	90/1800	0.00012[b]	2.311 (1.49, 3.584)
pANCA	1/7/21	4/82/859	9/49	90/1800	0.00024[b]	3.673 (1.75, 7.712)
cANCA	2/16/93	4/82/859	20/202	90/1800	0.0071	1.98 (1.194, 3.284)
Anti-MPO	0/5/11	4/82/859	5/27	90/1800	0.0049	3.704 (1.394, 9.843)
Anti-PR3	1/14/78	4/82/859	16/170	90/1800	0.0231	1.882 (1.081, 3.277)

OR indicates the effect of the minor allele 620W compared with the allele 620R. [a]Significance of association based on the allelic test. [b]Association statistically significant also after correction for multiple tests.

those with purpura as compared with controls ($P = 0.1023$, $\chi^2 = 2.669$, OR = 2.35); purpura is typically due to leucocytoclastic vasculitis. We reviewed the available skin biopsies to ascertain whether non-purpuric lesions actually showed granulomatous inflammation. Thirty-five patients with GPA had skin lesions: of these, 19 had purpura. Skin biopsy was performed in 8 of the 19 patients with purpura (showing in all of them typical leucocytoclastic vasculitis) and in 5 of the 16 patients with non-purpuric lesions; 4 of these 5 cases had histological features consistent with granulomatous inflammation, with or without associated histological signs of vasculitis.

Only weak associations were found between the R620W SNP and other disease manifestations in which organ damage is due to small-vessel vasculitis, such as kidney involvement and peripheral neuropathy (Table 3). Taken together, these findings (especially the marked association with ENT, lung and non-purpuric skin involvement) seem to point towards a particular association between the *PTPN22* R620W SNP and the granulomatous manifestations of GPA. We also examined the potential associations between this SNP and the main disease manifestations or disease subsets of MPA and CSS (e.g. renal-limited or systemic MPA, vasculitic or non-vasculitic CSS) [7, 23, 24], but found no significant differences in allele and genotype frequencies in comparison with controls. Also, there was no association with the ENT-limited or generalized forms of GPA.

Discussion

PTPN22 is located on chromosome 1p13.3–13.1 and encodes an 807-amino acid residue protein also referred to as LYP, which interacts with the tyrosine kinase Csk in the intracellular signalling cascade following T-cell activation [25]. The autoimmunity-predisposing allele of *PTPN22* is a missense C>T variation at position 1858, which changes the amino acid residue 620 from arginine (R) to tryptophan (W) in the encoded LYP protein. The *PTPN22* R620W substitution results in a gain of enzymatic function that increases the threshold for TCR signalling. This SNP also seems to impair B-cell signalling in a way that is similar to that demonstrated for T cells [15]. How an impaired amplification of TCR signalling may predispose to the development of autoimmunity remains unclear. Two theories have been postulated: the first is based on the relevance of thymic selection as a mechanism for establishing predisposition to autoimmune diseases. In the thymus, the increase in TCR signalling threshold caused by the *PTPN22* R620W SNP can lead to positive selection of thymocytes that would otherwise undergo deletion, with resulting appearance of autoreactive T cells in the periphery. The second theory instead involves regulatory T cells, which under physiological conditions are thought to limit the emergence of autoimmunity. An impaired TCR signalling involving particularly the regulatory T-cell compartment may eventually boost autoimmunity [26, 27].

The *PTPN22* 620W allele was previously found to be associated with type 1 diabetes (T1D) [28, 29], RA [30–32], JIA [33–35], SLE [29, 36, 37], Graves' disease [38], myasthenia gravis [39] and generalized vitiligo as well as with AAV [16, 40, 41]. The association with other autoimmune disorders (e.g. SS, SSc) is controversial. Interestingly, some autoimmune conditions (e.g. Crohn's disease) are associated with the alternative allele (R620) [42]. A recent meta-analysis confirmed that T1D, RA, JIA, SLE and Graves' disease were associated with the 620W allele, while multiple sclerosis, IBD, psoriasis and Addison's disease were not [13]. Based on these results, PTPN22 appears to predispose particularly to autoimmune conditions characterized by circulating autoantibodies [26, 27].

The results of our study confirm the previously demonstrated association between the *PTPN22* R620W SNP and GPA [6, 16]; in opposition to the findings obtained by the UK study [6], we failed to demonstrate any association with MPA. Additionally, this SNP did not seem to confer susceptibility to CSS. Our results, however, are difficult to compare with those obtained by the UK and German studies: the UK study was performed on a larger cohort of AAV patients, but only a small part of them had a clinical diagnosis of either GPA or MPA; moreover, this study was not intended to explore associations between genetic and clinical findings [6]. On the other hand, the German study provided detailed clinical and laboratory findings, but only included GPA patients [16].

GPA, MPA and CSS are often grouped under the umbrella term AAVs because of their common association with ANCA, but they have profound differences in clinical and epidemiological characteristics. This makes it likely that they also have distinct genetic backgrounds. Only a few of the gene polymorphisms studied (e.g. CD226) were shown to be common genetic risk factors for the different AAVs [43], whereas most associations with variants of putative autoimmunity genes were restricted to single forms (e.g. *CTLA4* in GPA, *IL10* in ANCA-negative CSS) [44, 45]; the HLA variants associated with the different AAVs also differ (e.g. HLA-DPB1 in GPA and HLADR4 in CSS) [8, 24].

As already pointed out in the German study [16], we also found that the association with the *PTPN22* SNP was stronger for ANCA-positive GPA than for the whole GPA population; this is in line with the hypothesis that PTPN22 R620W SNP mainly predisposes to autoimmune diseases with circulating autoantibodies.

In the present study, a careful clinical characterization of the patients allowed the investigation of genotypic–phenotypic associations. In the German study [16], the 620W allele was particularly associated with kidney, lung, eye and peripheral nervous system involvement. We instead found that this allele was enriched predominantly in GPA patients with ENT and lung involvement, and skin lesions other than purpura, whereas the association with kidney and peripheral nervous system involvement was of borderline significance. While these differences may be accounted for by the small size of the patient subgroups or by differences in clinical phenotyping, our results seem to point to a particular association between the *PTPN22* SNP and a granulomatous disease pattern of GPA. As a

matter of fact, ENT lesions in GPA are typically granulomatous [46], and so are usually the lung ones (with the exception of alveolar haemorrhage, which was not strikingly associated with the 620W allele); skin lesions may be granulomatous or vasculitic, but one of the most typical vasculitic manifestations (i.e. purpura) was again not associated with the 620W allele.

Interestingly, PTPN22 is thought to be involved in granuloma formation in GPA [47]. GPA is believed to arise as an aberrant cell-mediated immune response to microbial pathogens (e.g. *Staphylococcus aureus*) colonizing the upper airway tract; this probably leads to the formation of granulomata and ectopic lymphoid structures, which may promote the breakdown of tolerance towards PR3 and the subsequent formation of PR3-ANCA [3, 47, 48]. PTPN22 is thought to be involved in the exuberant response mediated by T cells, which release pro-inflammatory cytokines (e.g. IFN-γ, TNF-α) able to induce macrophage recruitment and granuloma formation [16, 47, 48]. *In vivo* data also support this view, as mice lacking *Pep* (the murine orthologue of *PTPN22*) display enhanced expansion and function of the effector/memory T-cell pool and spontaneous germinal centre formation [49]. However, further studies are needed to confirm our findings and to clarify whether the *PTPN22* R620W SNP actually predisposes to a particular GPA phenotype. In conclusion, the results of the present study confirm that the *PTPN22* 620W allele is a genetic risk factor for the development of GPA (but not of MPA or CSS) and particularly of its ANCA-positive subset.

Rheumatology key messages

- The *PTPN22* R620W SNP is a susceptibility factor for the development of GPA.
- The *PTPN22* R620W SNP is not associated with susceptibility to develop MPA or CSS.
- The ANCA-positive subset of GPA is strongly associated with the *PTPN22* R620W SNP.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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