Clinical Effect of Alpha-1 Antitrypsin Deficiency in Antineutrophil Cytoplasmic Antibody–associated Vasculitis: Results from a French Retrospective Monocentric Cohort

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ABSTRACT. ***Objective.*** Deficiency in alpha-1 antitrypsin (AAT) is a possible pathogenic cofactor in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). However, the clinical effect of AAT deficiency remains poorly established in this setting. This study aimed to describe the clinical phenotypes and outcomes of AAV according to AAT phenotypes.

Methods. This study was conducted retrospectively at Caen University Hospital and included all consecutive granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) patients with positive proteinase 3-ANCA or myeloperoxidase-ANCA, from January 2000 or September 2011, respectively, to June 2016. AAT dosage (nephelometry) and phenotyping (isoelectric focusing in agarose gel) were performed.

Results. Among the 142 patients with AAV, including 88 GPA and 54 MPA, 102 (72%) had the MM phenotype, 5 (4%) had a nonpolymerogenic M-variant phenotype, 18 (13%) had the deficient allele MZ, 12 (8%) had MS, 2 (1%) had ZZ, 2 (1%) had SZ, and 1 (1%) had SS. M, Z, and S allele frequencies were 84%, 8%, and 6%, respectively. No association was observed between AAT deficiency and ANCA subtype or AAV phenotype, except for intraalveolar hemorrhage (IAH), which was more frequent in patients harboring at least 1 of the deficient Z or S alleles than in those without any deficient alleles ($p < 0.01$). Global, renal, or relapse-free survival rates were similar for all subgroups.

Conclusion. This study shows that AAT deficiency confers, independently of ANCA subtype, a higher risk of IAH. Prospective studies are required to refine these data and to assess the need for replacement therapy in AAT-deficient patients with AAV. (J Rheumatol First Release May 1 2019; doi:10.3899/jrheum.180591)

Key Indexing Terms:
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Antineutrophil cytoplasmic antibodies (ANCA) are associated with pauciimmune systemic vasculitis, which includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). The pathophysiology of these 3 pathologically differentiated ANCA-associated vasculitis (AAV) disorders is not yet fully understood. The phenomenon that in each AAV subtype, ANCA can be directed against proteinase 3 (PR3), myeloperoxidase (MPO), or absent

altogether is also not understood. Moreover, these entities share substantial overlapping but also distinctive epidemiological, clinical, and prognostic features[1].

Alpha-1 antitrypsin (AAT), mainly produced by hepatocytes, belongs to the serine protease inhibitor (serpin) superfamily. AAT provides more than 90% of antiproteinase activity in serum and leads to the inactivation of a wide range of proteases, such as neutrophil elastase and PR3. AAT also exhibits antiinflammatory effects by blocking the effects of human neutrophil peptides and regulating expression of proinflammatory cytokines [such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, IL-1β, IL-8, and IL-32][2,3,4,5]. This protease inhibitor is produced by the *SERPINA1* gene located on chromosome 14, and is a highly polymorphic gene inherited as an autosomal codominant disorder. The most prevalent deficient alleles are the S (caused by the E264V mutation) and Z (secondary to the E342K mutation) alleles, with frequencies in white populations ranging from 5% to 10%, and 1% to 3%, respectively[2]. These pathogenic mutations affect posttranslational folding. Mutated AAT proteins then self-associate through intermolecular β-strand linkage to form nonfunctional AAT polymers[6]. Patients deficient in AAT, mainly homozygous ZZ patients, can have lung (emphysema and bronchiectasis), liver (chronic hepatitis, cirrhosis, and hepatoma), or skin (panniculitis) disorders as well as GPA or MPA. Indeed, the Z allele is carried by 5–27% of patients with GPA[2,7]. Although more recently known, deficient AAT alleles are also associated with perinuclear (p)ANCA or MPO-ANCA patients with AAV[8]. Screening for the AAT deficiency mainly relies on AAT dosage. Serum AAT assay by turbidimetry or nephelometry is one of the simplest screening methods, but it can fail to detect AAT deficiency. Indeed, AAT levels can be within normal range in cases of inflammatory syndrome. AAT phenotype can be determined by isoelectric focusing, which separates proteins according to their isoelectric points, allowing for identification of different AAT isoforms. However, isoelectric focusing cannot identify dysfunctional alleles or the few null alleles, which do not produce circulating proteins but lead to low AAT levels[9].

The American Thoracic Society/European Respiratory Society therefore states that AAT levels should be measured in cases of anti-PR3 vasculitis[10]. Nevertheless, no consistent clinical consequences of the association of AAV with AAT-deficient phenotypes have been established. On the one hand, AAT deficiency is associated with some organ and tissue damage and inflammation. On the other hand, some of these organs are also those that are typically injured in AAV, especially lungs or skin. We therefore formulated the hypothesis that patients with AAV carrying AAT-deficient phenotypes should exhibit more severe organ involvement, including those seen in both diseases, and/or worse global prognosis, in contrast to AAV patients with normal AAT levels. We therefore aimed to describe the overall clinical phenotypes and outcomes of ANCA-positive patients with GPA and MPA according to the AAT phenotype.

MATERIALS AND METHODS

Study design. We conducted a retrospective monocentric study at Caen University Hospital to include patients with AAV who were positive for ANCA, except for patients diagnosed with EGPA. All consecutive patients with GPA or MPA who tested positive for PR3-ANCA from January 2000 to June 2016 and MPO-ANCA from September 2011 to June 2016 were thereby included. The demographic, clinical, and treatment outcome data were collected from the patients' clinical files.

Ethics and consent. Patient data were anonymized in databases before the authors were granted access. This study was conducted in compliance with good clinical practices and the Declaration of Helsinki principles. The serum bank is registered with the French Commission Nationale de l'Informatique et des Libertés (DC-2008-559). In accordance with French public health law (Art. L 1121-1-1, Art. L 1121-1-2), approval was waived, as confirmed by our institutional review board. The manuscript was prepared in accordance with Strengthening The Reporting of OBservational Studies in Epidemiology (STROBE) guidelines.

Patient selection and classification according to AAT phenotype. All patients were diagnosed as having AAV based on the Chapel Hill Consensus Conference criteria[11]. Patients with AAV were classified as either GPA or MPA and as limited or severe vasculitis according to the European Medicines Agency vasculitis algorithm[12] and Wegener's Granulomatosis Etanercept Trial Research Group criteria[13], respectively. AAV activity was determined using the Birmingham Vasculitis Activity Score (BVAS) version 3[14]. Patients with EGPA, which has a different pathophysiology, were excluded. PR3- and MPO-ANCA were determined by ELISA. Using samples from the serum bank, AAT dosage and phenotyping were determined by nephelometry (Immage, Beckman Coulter) and isoelectric focusing in agarose gel (Hydrasys, Sebia), respectively. The other nondeficient and nonpolymerogenic AAT variants, such as X, Y, or ZPratt, were grouped according to the generic name of nonpolymerogenic variant phenotype[15]. AAT levels were determined from the most recent serum sample available, which was selected because it was as far as possible from the AAV diagnosis and relapse, to limit the influence of inflammatory phenomena. Patients were divided into several subgroups based on the presence or the absence of deficient AAT alleles.

Treatment classification and outcomes. Treatment was defined as follows: (1) standard regimen, in cases of new-onset organ-threatening or life-threatening AAV, corresponding to the induction of remission with cyclophosphamide or rituximab associated with corticosteroids, and sometimes plasma exchange mainly because of intraalveolar hemorrhage (IAH) or severe renal impairment, followed by maintenance treatment; and (2) moderate treatment regimen, in cases where patients did not receive a typical induction therapy; treatment was limited to methotrexate, azathioprine, or mycophenolate mofetil, with or without corticosteroids. Musculoskeletal involvement was defined as myalgia, arthralgias, and/or arthritis. Relapse was defined as the reactivation of vasculitis in any organ system that required a change in therapy. Endstage renal disease was defined as the onset of chronic dialysis or renal transplantation.

Statistical methods. Categorical variables were reported as percentages and compared using the chi-square or Fisher's tests, according to expected frequencies. Continuous variables were expressed as medians and interquartile ranges and analyzed using the nonparametric Mann–Whitney U test. Patient data were censored at the time of relapse, death, or last followup visit, whichever occurred first. Associations between overall survival, death-censored renal survival, or relapse-free survival, and AAT phenotype were analyzed using Kaplan-Meier survival curves, and between-group differences were evaluated by the log-rank test. A p value < 0.05 was considered statistically significant. All tests were performed using GraphPad Prism 7 (GraphPad Software Inc.).

RESULTS

Among the 158 patients with AAV identified during the prespecified period, 16 were excluded (8 cases of EGPA and 8 patients with GPA or MPA for whom sera samples were not available). In total, 142 patients with AAV were included, with 88 cases of GPA (62%) and 54 of MPA (38%). Seventy-nine patients (56%) were PR3-ANCA–positive and 63 (44%) were MPO-ANCA–positive. Patient characteristics are summarized in Table 1. The median age at diagnosis was 62 (51–71) years, with 63 women and 79 men (Table 2). AAV clinical presentations mainly included constitutional symptoms (105, 74%), renal (102, 72%), pulmonary (101, 71%), musculoskeletal (77, 54%), and ear, nose, or throat (71, 50%) involvement. The median BVAS was 18 (14–22).

There were 102 patients (72%) with the MM phenotype, 5 (4%) nonpolymerogenic M-variant phenotype, and 35 (25%) patients harbored at least 1 deficient allele: 18 (13%) MZ, 12 (8%) MS, 2 (1%) ZZ, 2 (1%) SZ, and 1 (1%) SS. Ninety-one cases of relapse were observed in 55 patients (39%), and 23 deaths (16%) were recorded after a median followup of 51.5 months (20.25–87.75).

Table 1. Characteristics of the 142 patients with ANCA-positive granulomatous with polyangiitis or microscopic polyangiitis.

Characteristics	ANCA-positive granulomatous with polyangiitis, n = 88	ANCA-positive microscopic polyangiitis, n = 54
Demographics		
Age at diagnosis, yrs	61 (48–70)	64 (54–71)
Women	44 (50)	19 (35)
Vasculitis characteristics		
Constitutional symptoms	67 (76)	38 (70)
Renal involvement	54 (61)	48 (89)
Dialysis patients	17 (19)	22 (41)
Pulmonary involvement	68 (77)	33 (61)
Intraalveolar hemorrhage	23 (26)	14 (26)
Bronchiectasis	15 (17)	6 (11)
Pulmonary emphysema	4 (5)	5 (9)
Musculoskeletal involvement	55 (63)	22 (41)
Ear, nose, and throat involvement	66 (75)	5 (9)
Mucocutaneous involvement	27 (31)	13 (24)
Neurological involvement§	24 (27)	13 (24)
PR3-ANCA	68 (77)	11 (20)
Limited vasculitis*	25 (28)	9 (17)
No typical induction treatment	14 (16)	14 (26)
Initial BVAS	20 (15–24)	16.5 (13–21)
Death	12 (14)	11 (20)
Relapse	46 (52)	9 (17)
Laboratory results		
Alpha-1 antitrypsin level, g/l	1.54 (1.16–1.91)	1.59 (1.33–2.16)
M allele frequency	144 (82)	95 (88)
Z allele frequency	19 (11)	5 (5)
S allele frequency	12 (7)	4 (4)

Unless indicated otherwise, values are displayed as an absolute number (%) or median (interquartile range). * The vasculitis was classified as limited or severe according to the Wegener's Granulomatosis Etanercept Trial Research Group criteria. § Central nervous involvement was noted in 2 cases in each group. ANCA: antineutrophil cytoplasmic antibody; PR3: proteinase 3; BVAS: Birmingham Vasculitis Activity Score.

When comparing patients with at least 1 deficient allele (Z and/or S) to those without any deficient alleles, the first subgroup more frequently exhibited IAH, as seen in Table 2: 15/35 (43%) versus 22/107 (21%), respectively; $p < 0.01$. Other studied variables were not significantly different, including overall, renal, and relapse-free survival rates between both main patient subgroups ($p = 0.08$, $p = 0.36$, and $p = 0.15$, respectively; Supplementary Figure 1, available from the authors on request). In particular, no association was observed between AAT deficiency and ANCA (MPO vs PR3) specificity or AAV (MPA vs GPA) subtype. No difference regarding AAV treatment was observed between these 2 groups ($p = 0.36$), including the use of plasma exchange (12/35 in patients having at least 1 deficient allele vs 21/107, $p = 0.08$; data not shown), which was routinely performed for IAH but also for severe renal involvement.

Specifically, by comparing patients with at least 1 Z allele with patients without any Z alleles, Z carriers more frequently exhibited ear, nose, and throat involvement than non-Z carriers. Even though statistical significance was not reached, a statistical trend for a higher frequency of IAH in Z carriers can be noted ($p = 0.07$; data not shown). Overall, renal and relapse-free survival rates between patients with or without Z alleles were not significantly different ($p = 0.09$, $p = 0.41$, and $p = 0.49$, respectively; Supplementary Figure 2, available from the authors on request). To exclude any influence of the S allele, a comparison between patients with at least 1 Z allele and patients without any deficient alleles (i.e., MM or M-variant phenotypes) yielded the same results, except for IAH, which reached statistical significance in favor of patients with at least 1 Z allele ($p = 0.042$; data not shown). Overall, renal and relapse-free survival rates were similar ($p = 0.07$, $p = 0.39$, and $p = 0.38$, respectively; data not shown).

Similarly, in patients with at least 1 S allele, the same statistical trend ($p = 0.07$; data not shown) was noted for a higher frequency of overall pulmonary involvement, including IAH, than in patients without any S alleles. Overall, renal, and relapse-free survival rates between patients with or without S alleles were not significantly different ($p = 0.33$, $p = 0.56$, and $p = 0.15$, respectively; Supplementary Figure 3, available from the authors on request). To exclude any influence of the Z allele, a comparison between patients with at least 1 S allele and patients without any deficient alleles (i.e., MM or M-variant phenotypes) yielded the same results ($p = 0.07$; data not shown). Overall, renal, and relapse-free survival rates were similar ($p = 0.26$, $p = 0.14$, and $p = 0.50$, respectively; data not shown).

All 5 homozygous ZZ or SS or compound heterozygous–deficient SZ patients had low AAT levels [median AAT level: 0.362 (0.195–0.476) g/l]. In contrast, 10/12 MS patients had normal AAT levels, including 6 with C-reactive protein

Table 2. Comparison between patients with granulomatosis with polyangiitis or microscopic polyangiitis having at least 1 deficient allele (Z or S) with patients without any deficient alleles.

Characteristics	Patients without Any Deficient Alleles, n = 107	Patients with a Z and/or S Allele, n = 35	p
Age at diagnosis, yrs	62 (51–71)	63 (50.5–69.5)	0.95
Women	50 (47)	13 (37)	0.33
Constitutional symptoms	80 (75)	25 (71)	0.70
Renal involvement	80 (75)	22 (63)	0.18
Dialysis patients	32 (30)	7 (20)	0.26
Pulmonary involvement	75 (70)	26 (74)	0.64
Intraalveolar hemorrhage	22 (21)	15 (43)	< 0.01
Bronchiectasis	15 (14)	6 (17)	0.66
Pulmonary emphysema	6 (6)	3 (9)	0.69
Musculoskeletal involvement	58 (54)	19 (54)	1
Ear, nose, and throat involvement	51 (48)	20 (57)	0.34
Mucocutaneous involvement	34 (32)	6 (17)	0.10
Neurological involvement§	28 (26)	9 (26)	0.96
Granulomatosis with polyangiitis	62 (58)	26 (74)	0.09
PR3-ANCA	56 (52)	23 (66)	0.17
Limited vasculitis*	26 (24)	8 (23)	0.87
No typical induction treatment	23 (21)	5 (14)	0.36
Initial BVAS	18 (14–22)	17 (14–21.5)	0.55
Deaths	14 (13)	9 (26)	0.08
Relapse	42 (39)	13 (37)	0.83
Alpha-1 antitrypsin level, g/l	1.6 (1.4–2.08)	1.09 (0.92–1.64)	< 0.001

Unless indicated otherwise, values are displayed as an absolute number (%) or median (interquartile range). * The vasculitis was classified as limited or severe according to the Wegener's Granulomatosis Etanercept Trial Research Group criteria. § Central nervous involvement was noted in 1 patient without any deficient alleles and in 3 MZ patients. PR3-ANCA: proteinase 3–antineutrophil cytoplasmic antibody; BVAS: Birmingham Vasculitis Activity Score.

< 10 mg/l at the time of AAT dosage, and 16/18 MZ patients, including 8 with a C-reactive protein < 10 mg/l at the time of AAT dosage. AAT level was significantly lower in heterozygous patients (MZ or MS) than MM patients [1.31 (1.01–1.68) g/l vs 1.86 (1.39–2.07) g/l, respectively, p < 0.0001].

Considering the specific usual non-AAV-related manifestations of AAT deficiency, no cases of cutaneous panniculitis or hepatic cirrhosis were observed in the cohort of patients with AAV. Nine patients (6%) exhibited pulmonary emphysema, including 3 MZ patients. These patients were former (n = 5) or current (n = 3) smokers (smoking status not available in 1 patient). Three (33%) of these patients exhibited IAH. Twenty-one patients (15%) had bronchiectasis, including 5 MZ patients and 1 ZZ patient. Five (24%) of these patients had IAH.

DISCUSSION

We found that, among consecutive patients with GPA or MPA who exhibited ANCA positivity, one-quarter of the cohort had at least 1 deficient AAT (S and/or Z) allele. Moreover, carrying a deficient AAT allele (S or Z) was associated with a significantly higher frequency of IAH, independent of ANCA specificity (anti-PR3 or anti-MPO). Finally, both AAT deficient phenotype and IAH had no significant influence on overall, renal, or relapse-free survival rates.

We specifically found Z and S allele frequencies at 8% and 6%, respectively[16]. Our frequency of patients with at least 1 Z allele is therefore significantly higher than a previous French estimation of 1.28% in 2006 (p < 0.001), whereas that of S allele at 7.6% appears not significantly different from ours (p = 0.31)[16].

Several heterozygous patients can be missed if the screening method solely relies on the determination of AAT level because (1) AAT level can be within normal range in cases of inflammatory syndrome; and (2) although AAT level was decreased in our homozygous patients, AAT level is frequently within the normal range in cases of heterozygous AAT deficiency[17,18,19,20,21]. We may assume that this is in part secondary to the widespread distribution of deficient AAT alleles and that heterozygous individuals have been inadvertently included to determine the normal AAT range. Mota, *et al* have demonstrated that, besides true AAT deficiency, patients with GPA also have a qualitative AAT deficiency[18].

Cases of ANCA-negative AAV were not included in our study, resulting in a probable underestimation of limited AAV[22]. Additionally, no control group has been included, and France has high prevalence rates of the Z and S alleles, which could result in an overrepresentation of AAT deficiency in AAV.

The pathophysiology supporting the epidemiological

association between AAT deficiency and AAV relies on several findings. Physiologically, AAT has an inhibitory effect on neutrophil chemotaxis and regulates TNF-α–induced neutrophil degranulation by inhibiting the binding of TNF-α to its membrane receptor[23,24,25]. AAT is the main PR3 inhibitor, and thus, AAT deficiency results in excess serum levels of PR3, which can trigger the synthesis of PR3-ANCA. ANCA is responsible for neutrophil degranulation of proteases, which are no longer inhibited[18]. Additionally, PR3-ANCA prevents the binding of PR3 to AAT, interfering with the clearance of PR3, and AAT inhibits the PR3/PR3-ANCA interaction and PR3-ANCA neutrophil activation in a dose-dependent fashion[18,26,27,28]. Patients with a Z or S allele can form AAT polymers, which induce chemotaxis, priming and degranulation of neutrophils, and increase ANCA-stimulated superoxide production[29,30,31,32,33]. The products from neutrophil activation may result in oxidative inactivation of AAT, which is responsible for a further localized AAT deficiency[34,35]. Moreover, neutrophils from ZZ patients cause superactivation of the proinflammatory nuclear factor-ϰB pathway[25]. Another much less likely explanation is the possibility of a linkage disequilibrium with another as yet undiscovered disease susceptibility gene[36,37]. Nevertheless, AAT replacement therapy had proven effective in case reports of GPA patients with AAT deficiency, both on pulmonary and other systemic symptoms[38]. Intrapulmonary cells such as bronchial epithelial cells, type II pneumocytes, neutrophils, and alveolar macrophages secrete AAT, and AAT polymers found in the alveolar and bronchial epithelial cells can act as potent chemoattractants and activate neutrophils. These findings could explain the higher frequency of IAH observed in our patients[6,33].

To our knowledge, since the first study regarding the association of AAV and AAT deficiency in 1993, 15 other distinct studies have been published (Table 3)[7,17–19,21,30,36,37,39–46]. Nevertheless, no clear effect of this association on the clinical expression or prognosis of AAV has been established when specifically investigated. One study found a less severe GPA phenotype in patients with a deficient allele[7]; some studies showed that a deficient allele was associated with either increased mortality, more disseminated disease, or a higher BVAS score[17,20,39]. The largest study to date by Morris, *et al* did not find any association with disease severity, survival, or relapse[30]. Regarding specific

Table 3. Association of alpha-1 antitrypsin deficiency with ANCA-associated vasculitis from literature.

Study	Country	Sample AAV Size	Vasculitis Type	No. MZ Patients	No. MS Patients	No. SS Patients	No. ZZ Patients	No. SZ Patients	Z Allele Frequency, n/N (%)	S Allele Frequency, n/N (%)
O'Donoghue, 1993[41]	Scotland	40	14 PR3 / 26 MPO	5	4	0	1	1	8/80 (10)	5/80 (6)
Lhotta, 1994[36]	Austria	32	29 GPA / 2 MPA / 1 IRPG, 30 cANCA	3	1	0	2	0	7/64 (11)	1/64 (2)
Savige, 1995[19]	Australia	60	31 PR3 / 29 MPO	2	5	1	1	0	4/120 (3)	7/120 (6)
Segelmark, 1995[39]	Sweden	99	73 GPA / 26 MPA, 99 PR3-ANCA	NA	NA	NA	1	NA	19/198 (10)	NA
Baslund, 1996[42]	Denmark	44	44 GPA, 32 cANCA and/or PR3	NA	NA	NA	NA	NA	8/88 (9)	NA
Griffith, 1996[43]	UK	198	51 GPA / 29 MPA / 11 IRPG / 11 other vasculitis / 96 suspected vasculitis, 99 cANCA / 99 pANCA	13	25	0	1	2	17/396 (4)	27/396 (7)
Callea, 1997[21]	Italy	84	33 GPA / 28 MPA / 23 IRPG, 38 cANCA / 46 pANCA	6	5	0	0	0	6/168 (4)	5/168 (3)
Esnault, 1998[46] (including 1993[40])	France	113	37 PR3 / 76 MPO	8	18 (including 1 IS)	1	3	2	16/226 (7)	22/226 (10)
Borgmann, 2001[37]	Germany	79	79 GPA, ANCA status: NA	6	NA	NA	1	NA	8/158 (5)	NA
Mahr, 2010[7]	USA	433	433 GPA, 339 PR3 / 44 MPO	26	44	4	4	2	36/866 (4)	54/866 (6)
Morris, 2011[30]	France, Germany, UK	856	723 GPA / 133 MPA, 605 cANCA / 150 pANCA	59	63	2	7	2	75/1712 (4)	69/1712 (4)
Chorostowska-Wynimko, 2013[44]	Poland	51	51 GPA, 43 cANCA / 2 pANCA	2	1	0	0	1	3/102 (3)	2/102 (2)
Mota, 2014[18]	Iran	27	27 GPA, 27 PR3	3	1	1	0	0	3/54 (6)	3/54 (6)
Pervakova, 2016[17]	Russia	38	38 GPA, 38 PR3	4	0	0	1	0	6/76 (8)	0/76 (0)
Hadzik-Blaszczyk, 2018[45]	Poland	64	64 GPA, 64 PR3	2	1	0	0	1	3/128 (2)	2/128 (2)
Deshayes, 2018 (present study)	France	142	88 GPA / 54 MPA, 79 PR3 / 63 MPO	18	12	1	2	2	24/284 (8)	16/284 (6)
Total		2360	1733 GPA / 272 MPA, 1575 cANCA and/or PR3, 535 pANCA and/or MPO	157	180	10	24	13	236/4720 (5)	213/4276 (5)

ANCA : antineutrophil cytoplasmic antibody ; AAV: ANCA-associated vasculitis; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; PR3: proteinase 3; MPO: myeloperoxidase; IRPG: idiopathic rapidly progressive glomerulonephritis; cANCA: cytoplasmic ANCA; pANCA: perinuclear ANCA; NA: not available.

clinical consequences, Baslund, *et al* found a higher prevalence of eye symptoms in Z patients with GPA[42]. Only O'Donoghue, *et al*, looking at the frequency of IAH according to AAT phenotype, found that patients with Z and/or S alleles had more frequent IAH, as in our study[41]. While the strongest association between the deficient Z allele and GPA or cytoplasmic (cANCA) or PR3-ANCA has been known since 1993 (because AAT is the main PR3 inhibitor), the association of AAV with the S allele or with pANCA or MPO-ANCA is more recent. Indeed, in their metaanalysis to determine the genetic variants most likely associated with AAV, Rahmattulla, *et al* showed that both the S and Z alleles are significantly associated with AAV, with pooled OR of 1.30 and 2.94, respectively, both in PR3-ANCA (pooled OR 2.58) and MPO-ANCA (pooled OR 2.01), and in cANCA (pooled OR 3.53) and pANCA (pooled OR 3.13) patients[8]. While p-ANCA and MPO-ANCA have been associated with AAT deficiency, albeit to a lesser extent than cANCA and PR3-ANCA, to our knowledge, only 6 other studies have included MPA, and ours is the second largest study, with 54 patients with MPA included in this setting (Table 3). These associations diminish the role played by PR3 as the sole pathogenic factor in AAV and reinforce the role of the other mechanisms discussed above.

Finally, our ANCA-positive AAV patients with AAT deficiency did not have other well-known cutaneous or hepatic involvement associated with this metabolic deficiency, except perhaps for some rare cases of pulmonary lesions for which the pathogenic mechanism cannot be specified herein. This could be explained by the scarcity of homozygous patients in our cohort, with very few patients having really low AAT levels. Our study failed to find any prognosis differences between patients with or without deficient AAT alleles, probably because of several confounding factors, including the typical use of additional plasma exchange treatment for IAH or severe renal involvement. That said, a large international randomized controlled trial recently demonstrated that plasma exchange does not reduce the risk of endstage renal disease or death in patients with severe ANCA-associated vasculitis[47]. Moreover, it is also possible that plasma exchange may even be deleterious in the setting of AAT deficiency because this treatment may further reduce AAT levels if performed with albumin rather than fresh plasma replacement[39].

Our study confirms and specifies both the epidemiological and clinical effects of AAT deficiency on both anti-PR3 or anti-MPO AAV, especially with the increased risk of IAH. Even though this association, through the different studies, does not seem to have a clear effect on prognosis, the potential therapeutic consequences of plasma exchange must be addressed. Prospective studies are required to fully specify these data and to assess the real effect of plasma exchange with fresh plasma replacement and of enzyme replacement therapy in patients with both ANCA-positive AAV and AAT deficiency.

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