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Alpha$_1$-Antitrypsin Deficiency–Related Alleles Z and S and the Risk of Wegener's Granulomatosis

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Abstract

Objective—Deficiency of α_1-antitrypsin (α_1AT) may be a determinant of susceptibility to Wegener's granulomatosis (WG). Several previous, mainly small, case–control studies have shown that 5–27% of patients with WG carried the α_1AT deficiency Z allele. It is not clear whether the S allele, the other major α_1AT deficiency variant, is associated with WG. This study investigated the relationship of the α_1AT deficiency Z and S alleles with the risk of developing WG in a large cohort.

Methods—We studied the distribution of the α_1AT deficiency alleles Z and S in 433 unrelated Caucasian patients with WG and 421 ethnically matched controls. Genotyping was performed using an allele discrimination assay. Results were compared between cases and controls using exact statistical methods.

Results—Among the patients with WG, the allele carriage frequencies of Z and S were 7.4% and 11.5%, respectively. The frequencies of the 6 possible genotypes differed in a statistically significant manner between cases and controls ($P = 0.01$). The general genetic 2-parameter codominant model provided the best fit to the data. Compared with the normal MM genotype, the odds ratio (OR) for MZ or MS genotypes was 1.47 (95% confidence interval [95% CI] 0.98–2.22), and the OR for ZZ, SS, or SZ genotypes was 14.58 (95% CI 2.33–∞). ORs of similar direction and magnitude were observed within the restricted cohorts that excluded cases and controls carrying ≥1 Z or ≥1 S allele.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Merkel had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conclusion—Both Z and S alleles display associations with risk of WG in a codominant genetic pattern. These findings strengthen the evidence of a causal link between α_1AT deficiency and susceptibility to WG.

Wegener's granulomatosis (WG) is a primary systemic small-vessel vasculitis that is associated in ~80–90% of cases with the production of antineutrophil cytoplasmic antibodies (ANCAs). In WG, ANCAs most commonly display a cytoplasmic immunofluorescence pattern (cytoplasmic ANCAs [cANCA]) and are directed against the neutrophilic enzyme proteinase 3 (anti–PR3 ANCA). Cytoplasmic ANCA/anti–PR3 ANCA has been identified as a highly sensitive and specific biomarker for the diagnosis of WG and may also be involved in its pathogenesis (1). While the etiology of WG remains unclear, current concepts support the involvement of both environmental and genetic factors in the development of this vasculitis (2).

Several studies have shown that the functional genetic polymorphism determining a deficient production of the protease inhibitor α_1-antitrypsin (α_1AT) is significantly overrepresented in patients with WG (or in cANCA/anti–PR3 ANCA–positive vasculitis). Those studies found that the Z polymorphism of the α_1AT gene (also called Serpin A1) was carried by 5–27% of patients with WG as compared with 2–5% of controls (3–11). However, most of these conclusions were based on small case sample sizes (3,5–12) that prevented a thorough investigation of the inheritance pattern of this genetic polymorphism. Data are inconclusive as to whether (6,10) or not (5,8) the S polymorphism, the other major α_1AT deficiency allele, also contributes to the risk of WG. These issues considerably hamper the full acceptance and mechanistic understanding of α_1AT deficiency as a factor predisposing to WG.

Using a large genetic repository for patients with WG, we undertook a case–control association study to reexamine the relationship of the α_1AT-deficiency alleles Z and S to WG.

PATIENTS AND METHODS

Study population

This study was conducted using the WG Genetic Repository. This repository contains DNA samples from 476 patients with WG and 576 controls contributed by 8 academic centers in the US from 2001 to 2005. Patients were eligible if they satisfied the modified American College of Rheumatology classification criteria for WG (13). Controls were unrelated subjects without WG and no personal or family history of an autoinflammatory disease and were recruited by all 8 centers from geographically matched populations. The Institutional Review Boards at each of the 8 study sites approved the study protocol. Written informed consent was obtained from all study subjects.

For each patient with WG, comprehensive cumulative demographic, clinical, and laboratory data were collected by means of a structured questionnaire. Eligible control subjects were asked to complete a questionnaire to collect information on age, sex, and ethnicity. For both cases and controls, ethnicity was self-declared.

In light of the known variability in α_1AT genotype frequencies among different racial and ethnic groups (14), the present study was limited to non-Hispanic Caucasian subjects, which is the predominant ethnic background in WG (2). We therefore selected among all subjects included in the repository the 436 case subjects and 426 control subjects of "Caucasian, non-Hispanic" ancestry. Genotyping of the α_1AT gene was unsuccessful in 3 cases and in 5 controls, leading to our final analyzed tally of 433 cases and 421 controls.

Genotyping procedures

For each case and control subject, DNA was extracted in a central laboratory using a Puregene genomic DNA extraction kit (Qiagen) from blood collected in Vacutainer tubes containing EDTA. The genotyping procedures were performed in an international reference laboratory for α_1AT deficiency (Alpha-1 Foundation, University of Florida, Gainesville). Genotyping for α_1AT Z and S was performed using an allele discrimination assay on an ABI Prism 7500 Fast unit (Applied Biosystems) (15). Assays were run in a 96-well format according to the recommendations of the manufacturer. By default, alleles that were not found to be Z or S alleles were considered to be the common "wild-type" M variant. In each plate, one DNA sample of known SZ genotype was included as quality control.

Statistical analysis

To evaluate whether the α_1AT genotype distributions were identical in cases and controls, we first performed contingency table analysis using Pearson's chi-square test or, when appropriate, Fisher's exact test. These analyses were done for the 6 possible combinations of the M, S, and Z alleles (i.e., MM, MS, MZ, SS, ZZ, and SZ). In addition, to assess the potential differential effects of the Z and S alleles, we performed subgroup analyses by comparing the genotypes within the restricted cohorts that excluded cases and controls carrying ≥1 Z allele or ≥1 S allele, respectively. To assess the potential influence of covariates, we also examined whether the age (stratified by median or quartile values calculated for the combined case and control sample) and sex distributions differed between cases and controls, using Pearson's chi-square tests. The assumption of Hardy-Weinberg equilibrium was tested among the controls by comparing the genotype distribution with that expected on the basis of the observed allele frequencies and random mating using a one-way chi-square goodness-of-fit test with 3 degrees of freedom (df) (16).

To quantify the influence of α_1AT genotypes on the risk of WG, we performed unconditional logistic regression analyses that used WG as the dichotomous dependent variable and the α_1AT genotypes as the independent variable. Genotypes were assigned indicator variables with the use of the MM genotype as the reference group. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed using exact methods (to allow for contingency cell counts with small or zero values). In addition, we performed sensitivity analyses with adjustment for potential confounding variables from age (dichotomous stratified by median age in the combined case and control sample) or sex.

The primary analyses were based on a general codominant genetic model that assigned different risks to different strata (2 parameters). Subsequently, we also evaluated other patterns of inheritance, i.e., according to a codominant multiplicative model (linear increase on a log OR scale with each additional deficient allele; 1 parameter), a dominant model (with at least 1 deficient allele at increased risk; 1 parameter), and a recessive model (individuals with 2 deficient alleles at increased risk; 1 parameter). To decide which of these 4 patterns of inheritance best fit the data, we used Akaike's information criterion (AIC) (17) to compare the fits of non-nested models of inheritance. The AIC measure assesses the relative fits of different models, with smaller values reflecting a better fitting model. These analyses were done within the entire data set and, to further assess the individual effects of Z or S, in the restricted cohort that excluded all Z or S (case and control) carriers, respectively (as described above).

We used the population attributable risk (PAR) statistic to estimate the proportion of WG that can be attributed to α_1AT deficiency genotypes. The PAR was calculated on the basis of the formula PAR = ([relative risk − 1]/relative risk) × proportion of exposed. The relative

risk of WG in this formula is estimated by the OR. The proportion exposed is the proportion of participants with WG carrying the respective α_1AT genotype (12).

Finally, we looked for a potential effect of α_1AT genotype on WG phenotype with respect to the following parameters: age, sex, clinical manifestations (ear, nose, and throat; pulmonary; renal; musculoskeletal; skin; ophthalmologic; neurologic; gastrointestinal; and cardiac involvement), ANCA pattern, length of followup, and percentage of cases with severe WG (18). These characteristics were compared among cases of WG with and without α_1AT deficiency using Student's *t*-test or, where appropriate, Kruskal-Wallis nonparametric test for continuous variables, and with Pearson's chi-square test or, where appropriate, Fisher's exact test for categorical variables.

All statistical tests were 2-sided, and the level of significance was set at $\alpha = 0.05$. We performed all of the statistical analyses using SAS, version 9.1 (SAS Institute).

RESULTS

Characteristics of the study population

The characteristics of the cases are summarized in Table 1. Controls had a mean ± SD age of 49.8 ± 16.0 years and included 170 males (41.2%). Comparisons of demographic characteristics between cases and controls showed an increased proportion of males among cases ($P < 0.001$); differences were also detected in age distribution when age was analyzed in 2 or 4 groups stratified by the median or quartile values in the overall sample ($P = 0.01$ for both comparisons).

Genotyping results

The numbers of occurrences of the 6 possible genotypes among cases versus controls were as follows: for MM, 353 (81.5%) versus 371 (88.1%); for MS, 44 (10.2%) versus 32 (7.6%); for MZ, 26 (6.0%) versus 18 (4.3%); for SS, 4 (0.9%) versus 0; for SZ, 2 (0.5%) versus 0; and for ZZ, 4 (0.9%) versus 0. The corresponding allele frequencies of Z and S were 4.16% and 6.24%, respectively, among the 433 case subjects and 2.14% and 3.80%, respectively, for the 421 control subjects. The genotype frequencies differed in a statistically significant manner between case and control subjects (5 df, $P = 0.01$). Statistically significant differences in genotype distributions were also found when restricting these analyses to the M and Z genotypes (MM, MZ, ZZ) (2 df, $P = 0.045$) or to the sole M and S genotypes (MM, MS, SS) (2 df, $P = 0.03$). The distributions of α_1AT genotypes observed in controls were in Hardy-Weinberg equilibrium when compared with those predicted by allele frequencies ($\chi^2 = 1.68$, 3 df, $P = 0.64$). The observed allele frequencies in controls were slightly higher than published summary statistics for the Caucasian population in the US, i.e., 1.45% and 3.08% for the Z and S alleles, respectively (14).

Table 2 presents the risks of WG for the α_1AT genotypes in the entire cohort and in the 2 subcohorts evaluated for the effect of Z and S alleles specifically. Under the general 2-parameter codominant model, the combined MS and MZ genotypes were associated with an OR of 1.47 (95% CI 0.98–2.22; $P = 0.06$), and the combined ZZ, SS, and SZ genotypes were associated with a statistically significantly elevated OR of 14.58 (95% CI 2.33–∞; $P = 0.002$), both as compared with the MM genotype. ORs of similar direction and magnitude were observed for subgroups that consisted of Z alleles only or S alleles only, although the differences from the MM genotype were not statistically significant (Table 2). Adjustment for age groups or sex did not change the risk estimates significantly (data not shown). These analyses therefore suggested that both alleles contribute to the risk of WG, consistent with a dose-response effect.

The existence of such a dose-response effect was also supported by the search for the best-fitting genetic model. Of all of the models tested, the general 2-parameter codominant model yielded the best fit to the data (AIC = 1172.24), followed by the recessive (AIC = 1174.03), multiplicative (AIC = 1176.97), and dominant (AIC = 1180.46) models. In sensitivity analyses, the 2-parameter codominant model also proved to be the best fit within the restricted cohort that excluded the S carriers. In contrast, the analyses based on the restricted cohort that excluded the Z carriers and in the whole-group model adjusted for age (dichotomous) and sex indicated slightly lower AIC for the recessive models, followed by the 2-parameter codominant model (data not shown).

PAR

Based on these estimates, the PAR of WG for α_1AT Z and/or S polymorphisms was 7.32% (i.e., 5.17% for heterozygous carriers and 2.15% for homozygous/compound heterozygous carriers).

Subgroup analyses

Subgroup analyses stratified by ANCA status are shown in Table 3. These analyses showed a similar genotype risk pattern for the 339 anti–PR3 ANCA–positive and the 39 anti–PR3/antimyeloperoxidase (MPO) ANCA–negative cases. In contrast, among the 44 anti–MPO ANCA–positive cases, no increased risk of WG was identified in relation to α_1AT deficiency.

Correlates between α_1AT genotype and WG phenotype

Characteristics of WG stratified by α_1AT genotype are presented in Table 1. Carriage of the Z and/or S allele was associated with a lower frequency of WG with severe phenotype (P = 0.045); no other between-group differences were detected for any of the other selected demographic, clinical, or ANCA characteristics. When comparing these variables among the 10 individuals with ZZ, SS, or SZ genotypes versus the remainder of the cases with WG, no statistically significant differences were found (data not shown).

DISCUSSION

This genetic case–control study provides further support for the association between WG and α_1AT deficiency. These findings confirm previous observations of overexpression of the Z polymorphism of the α_1AT gene among patients with WG while suggesting that the S polymorphism of α_1AT is also overexpressed among patients with WG. Additionally, this is the first study to demonstrate that susceptibility to WG is most strongly determined by the subset of homozygous (ZZ, SS) or compound heterozygous (SZ) genotypes, which increase the risk by a factor of 14.6, whereas heterozygous carriage (MZ or MS) is associated with a much smaller (1.5-fold) increase in risk. Thus, although this study emphasizes α_1AT deficiency as the most consistent genetic susceptibility factor identified for WG to date, these data also indicate that α_1AT deficiency accounts for, at most, 7% of all cases of WG.

WG should definitely be added to the list of α_1AT deficiency–related diseases, of which emphysema and liver cirrhosis are the 2 most prominent conditions. Alpha$_1$-antitrypsin is a major inhibitor of the proteolytic enzyme elastase. In the context of α_1AT deficiency, development of emphysema is attributed to the unopposed elastase activity on connective lung tissue. Because α_1AT deficiency is inherited in a codominant pattern, and since the Z polymorphism especially compromises the hepatic synthesis of this protein, α_1AT deficiency usually segregates into “severe,” “moderate,” and “weak” categories for individuals with the ZZ, SZ/SS, and MZ/MS genotypes, respectively. Accordingly, the risk of emphysema is stratified by the genotype and is highest in individuals with severe α_1AT

deficiency, whereas the risk is only moderate or low in the remainder of people with α_1AT deficiency. In contrast, α_1AT deficiency–associated cirrhosis is due to intrahepatocytic polymerization of a structurally abnormal protein and exclusively affects individuals with the ZZ genotype (19,20).

Several mechanistic hypotheses have been proposed to explain the association of α_1AT deficiency with the development of WG (19,20). Because α_1AT is also a major inhibitor of PR3, the PR3–α_1AT imbalance may lead to increased levels of circulating PR3 and possibly trigger the synthesis of anti–PR3 ANCA. This theory would imply that the association of WG with α_1AT deficiency is restricted to anti–PR3 ANCA–positive cases and is not present in the more uncommon occurrences of anti–MPO-positive or ANCA-negative cases of WG. Alternatively, it has been postulated that patients with WG and α_1AT deficiency have a reduced ability to bind PR3 released by previously activated neutrophils, thus promoting PR3-mediated proteolytic vessel damage; this theory is supported by findings that α_1AT-deficient patients with WG have a more severe disease course than non–α_1AT-deficient patients (4,21).

Taken together, the findings of the present study and of previous studies strengthen the evidence that the association of α_1AT deficiency with WG might be causal rather than a reflection of linkage disequilibrium. While it is possible that the causative variant (or variants) is in linkage disequilibrium with α_1AT deficiency, i.e., that there is confounding by location with the true “WG gene” being in the vicinity of the α_1AT gene, several lines of evidence support a causal role for α_1AT variation in genetic susceptibility to WG. The replication of this association in varied geographic regions (Table 4) and the strong effect size, with an up to 15-fold risk increase, favor the idea of causality (22). Moreover, the finding that, similar to the risk of emphysema, the α_1AT deficiency–WG association follows a codominant genetic model (i.e., a dose-response relationship) implies a causal effect (22). In retrospect, this genotype risk stratification is also suggested by the fact that several of the previous studies included numbers of individuals with ZZ, SS, or SZ genotypes that appeared higher than expected from the total number of patients with WG identified with α_1AT deficiency (Table 4).

Our study provides additional insights into the pathophysiologic mechanisms involved in α_1AT deficiency predisposing to WG. The theory that α_1AT deficiency determines a more severe WG phenotype is challenged by our finding of the smaller proportion of severe WG cases associated with α_1AT deficiency (Table 1). Moreover, our results support the findings of previous studies (8,9) suggesting that the risk of anti–MPO ANCA–positive WG is not linked to α_1AT deficiency, possibly favoring the hypothesis that α_1AT deficiency triggers WG by means of anti–PR3 ANCA autoimmunity. However, in our analysis, the few ANCA-negative cases also appeared to be associated with α_1AT deficiency, and no anti–PR3 ANCAs were detected in 191 individuals with established severe (ZZ) α_1AT deficiency (23). Because α_1AT also has other properties, including antiinflammatory characteristics (20), the effect of α_1AT deficiency on WG could be mediated by other mechanisms.

There are direct implications of the results of the present study for the understanding of the etiology of WG and for clinical practice. Consistent with observations that first studies tend to overrate the effect of gene–disease associations (24), the proportion of carriers of α_1AT deficiency alleles among patients with WG in the present study was at the lower end of the range reported by previous investigations. The calculated PAR of α_1AT was 7%, highlighting that α_1AT deficiency is only one among multiple etiologic factors at work in the development of WG. Our data also lend support to published guidelines recommending diagnostic genetic testing for α_1AT deficiency in patients with WG (19,20). Whether

manifestations of vasculitis among patients with WG and severe α_1AT could benefit from α_1AT augmentation therapy is unknown.

Our study has several limitations to consider. Despite the inclusion of a multisite sampling method and the large case sample size for a rare disease, we recognize that cases of the most severe, rapidly fatal forms of WG may have been underrepresented in our sample and that our analyses still had limited power to detect small risk increases and limited precision in the risk estimates for the rare ZZ, SS, and SZ genotypes. In light of the genetic diversity of Americans of European descent (25), the restriction of cases and controls to those with a Caucasian background does not definitely eliminate confounding by population stratification, and this possibility was not further addressed by methods such as genotyping of ancestral informative markers. Because the frequencies of Z and S alleles appear to follow an inverse gradient across European populations (26), population stratification would have distorted our effect size estimates for the Z allele and the S allele in opposite directions. Since the genotyping technique tested only for the most common polymorphisms of the α_1AT gene, we acknowledge the possibility that additional allele variants, e.g., null or other rare deficient variants which account for only 2–4% of the α_1AT deficiency alleles in the general population (26), were mislabeled as wild-type M alleles. Conversely, we do not believe that the lack of measurements of serum α_1AT levels is a drawback because there are wide variations of serum concentrations in settings of systemic inflammation that may lead to falsely normal values (19).

This study substantially strengthens the evidence that α_1AT deficiency predisposes to WG and further establishes this polymorphism as the strongest genetic risk factor thus far discovered for WG. However, the specific role of α_1AT deficiency in the pathophysiology of WG requires further study. Some insight might come from the data suggesting that this genetic association might not apply to the subset of patients with WG who are anti–MPO ANCA–positive, which calls for further studies in large samples of anti–MPO ANCA–positive WG and of other forms of ANCA–associated vasculitis, i.e., microscopic polyangiitis and Churg-Strauss syndrome.

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Table 1

Demographic, clinical, and ANCA characteristics of the 433 Caucasian patients with WG in the entire cohort and stratified by presence or absence of the α_1AT-deficiency alleles Z and S*

Variable	Entire cohort	Patients without Z and/or S alleles	Patients with Z and/or S alleles	*P*
Sample size	433	353	80	
Age, mean ± SD years	53.2 ± 15.7	53.1 ± 15.9	54.0 ± 14.8	0.65
Males, no. (%)	229 (52.9)	191 (54.1)	38 (47.5)	0.29
Manifestations, no. (%)				
ENT	379 (87.9)	307 (87.5)	72 (90.0)	0.53
Pulmonary	311 (72.2)	257 (73.2)	54 (67.5)	0.30
Renal	254 (58.7)	210 (59.5)	44 (55.0)	0.46
Skin	160 (37.1)	132 (37.6)	28 (35.0)	0.66
Eye	111 (25.6)	87 (24.7)	24 (30.0)	0.32
Neuropathy	119 (27.6)	94 (27.8)	25 (31.3)	0.42
Gastrointestinal	10 (2.4)	8 (2.3)	2 (2.6)	0.32
Cardiac	13 (3.2)	12 (3.6)	1 (1.3)	0.91
ANCA status, no. (%)				
PR3 ANCA positive	339 (80.7)	277 (81.2)	62 (78.5)	0.58
MPO ANCA positive	44 (10.7)	38 (11.3)	6 (7.8)	0.37
PR3/MPO ANCA negative	39 (9.4)	27 (8.0)	12 (15.4)	0.047
Severe WG, no. (%)	315 (73.9)	263 (76.0)	52 (65.0)	0.045
Followup, mean ± SD years†	5.5 ± 5.6	5.4 ± 5.6	5.9 ± 5.8	0.51

*Percentages account for missing data. ANCA = antineutrophil cytoplasmic antibody; α_1AT = α_1-antitrypsin; ENT = ear, nose, throat; PR3 = proteinase 3; MPO = myeloperoxidase.

†Time from date of diagnosis of Wegener's granulomatosis (WG) to enrollment in this study.

Table 2

Results of Z and/or S allele carriage determining α_1AT deficiency (under a codominant model) and association with WG*

Genotype	Cases, no. (%)	Controls, no. (%)	OR (95% CI)	*P*
All genotypes				
MM	353 (81.5)	371 (88.1)	1 (reference)	–
MS and MZ	70 (16.2)	50 (11.9)	1.47 (0.98–2.22)	0.06
SS, ZZ, and SZ	10 (2.3)	0 (0)	14.58 (2.33–∞)	0.002
S genotypes excluded				
MM	353 (92.2)	371 (95.4)	1 (reference)	–
MZ	26 (6.8)	18 (4.6)	1.52 (0.79–2.99)	0.24
ZZ	4 (1.0)	0 (0)	5.53 (0.69–∞)	0.11
Z genotypes excluded				
MM	353 (88.0)	371 (92.1)	1 (reference)	–
MS	44 (11.0)	32 (7.9)	1.44 (0.87–2.41)	0.16
SS	4 (1.0)	0 (0)	5.53 (0.69–∞)	0.11

*OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).

Table 3

Results of Z and S allele carriage determining α_1AT deficiency and association with WG according to ANCA pattern*

Genotype	Cases, no. (%)	Controls, no. (%)	OR (95% CI)	*P*
Anti–PR3 ANCA positive				
MM	277 (81.7)	371 (88.1)	1 (reference)	–
MS and MZ	54 (15.9)	50 (11.9)	1.45 (0.94–2.24)	0.10
SS, ZZ, and SZ	8 (2.4)	0 (0)	14.72 (2.26–∞)	0.002
Anti–MPO ANCA positive				
MM	38 (86.4)	371 (88.1)	1 (reference)	–
MS and MZ	6 (16.6)	50 (11.9)	1.17 (0.39–2.99)	0.88
SS, ZZ and SZ	0 (0)	0 (0)	NA	NA
Anti–PR3/MPO ANCA negative				
MM	27 (69.2)	371 (88.1)	1 (reference)	–
MS and MZ	10 (25.6)	50 (11.9)	2.74 (1.12–6.28)	0.03
SS, ZZ, and SZ	2 (5.1)	0 (0)	31.81 (2.45–∞)	0.01

*OR = odds ratio; 95% CI = 95% confidence interval; NA = not applicable (see Table 1 for other definitions).

Table 4

Summary of studies of α_1AT deficiency in WG*

Author, year (ref.)	Country	Vasculitis type	Sample size, cases/controls	No. (%) Z allele carriers		No. (%) S allele carriers		No. of cases with SS, ZZ, and SZ genotypes
				Cases	Controls	Cases	Controls	
Elzouki et al, 1994 (4)	Sweden	WG	66/NR	15 (22.7)	NR (4.7)	Not tested	Not tested	0
Lhotta et al, 1994 (5)	Austria	cANCA	32/868	5 (15.6)	24 (2.8)	1 (3.1)	39 (4.5)	2 ZZ
Savige et al, 1995 (6)	Australia	Anti–PR3 ANCA	31/NR	3 (9.7)	NR (1.5)	4 (12.9)	NR (8.3)	1 ZZ
Baslund et al, 1996 (7)	Sweden/Denmark	WG	44/NR	8 (18.2)	NR (4.7)	Not tested	Not tested	0
Griffith et al, 1996 (8)	UK	cANCA	99/2,310	10 (10.1)	83 (3.6)	9 (9.1)	209 (9.0)	1 SS, 1 ZZ
Callea et al, 1997 (9)	Italy	cANCA	38/200	2 (5.3)	3 (1.5)	2 (5.3)	NR	0
Esnault et al, 1997 (10)†	France	cANCA/anti–PR3 ANCA	37/1,310	10 (27.0)	50 (3.8)	9 (24.3)	196 (12.7)	1 SS, 3 ZZ, 2 SZ
Borgmann et al, 2001 (11)	Germany	WG	79/752	7 (8.9)	19 (2.5)	Not tested	Not tested	1 ZZ
Present study	US	WG	433/421	32 (7.4)	18 (4.3)	50 (11.5)	32 (7.6)	4 SS, 4 ZZ, 2 SZ

*NR = not reported; cANCA = cytoplasmic ANCA (see Table 1 for other definitions).

†Includes cases previously reported in ref. 3.