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Brief Report

No association of G-463A myeloperoxidase gene polymorphism with MPO–ANCA-associated vasculitis

Anette Fiebeler[1], Stefan Borgmann[3], Alexander Woywodt[2], Hermann Haller[2] and Marion Haubitz[2]

[1]Division of Nephrology, Franz-Volhard-Clinic, Berlin, HELIOS, [2]Division of Nephrology, Hannover Medical School, Hannover and [3]Division of Medical Microbiology University of Tübingen, Germany

Abstract

Background. The activation of neutrophils and monocytes by ANCA, resulting in the release of reactive oxygen species and proteases like myeloperoxidase (MPO), is essential to the pathogenesis of ANCA-associated vasculitis. As the A allele of the G-463A MPO gene polymorphism is associated with diminished activity of MPO, it is conceivable that the presence of this allele protects against MPO–ANCA-associated vasculitis.

Methods. Allelic frequencies of the G-463A polymorphism were studied in 119 ANCA-associated vasculitis patients, 48 with MPO–ANCA and 71 with proteinase 3 (PR3)–ANCA.

Results. Allelic frequencies of MPO G-463A promoter polymorphism did not differ between MPO–ANCA- and PR3–ANCA-associated vasculitis patients. Moreover, allelic distribution was similar to that of the normal population.

Conclusions. The data suggest that G-463A polymorphism does not seem to contribute to either MPO–ANCA- or PR3–ANCA-associated vasculitis formation.

Keywords: ANCA; microscopic polyangiitis; myeloperoxidase; polymorphism; vasculitis; Wegener's granulomatosis

Introduction

Genetic predisposition has been discussed as contributing to the formation of ANCA-associated vasculitis [1]. Polymorphism within the gene of proteinase 3 (PR3), the main target protein of c-ANCA, has been described [2] and allelic frequencies of PR3 gene polymorphisms were increased in patients with Wegener's granulomatosis (WG). As one of these disease-associated polymorphisms was located within the PR3 promoter region, alteration of PR3 protein expression might contribute to the formation of c-ANCA-associated vasculitis. Interestingly, PR3 membrane expression is genetically controlled [3], and elevated levels of membrane PR3 expression were found in patients with WG [4]. Moreover, within the group of WG patients, elevated levels were associated with an increased risk of relapses [4]. Therefore, promoter polymorphism of ANCA target genes, leading to elevated protein expression rates, might contribute to the formation of ANCA-associated vasculitis.

Conversely, the presence of alleles resulting in a reduced expression of ANCA target proteins might be beneficial in preventing the development of ANCA-associated diseases. Myeloperoxidase (MPO) is the main target antigen of p-ANCA found in the majority of patients with microscopic polyangiitis, and occasionally in patients with WG [5,6]. In the MPO promoter region a G-463A polymorphism has been described, which is characterized by a single base substitution of guanine to adenine. The A allele of this polymorphism is associated with reduced MPO activity and carriers of the less frequent A allele have a lower risk of lung cancer, presumably by virtue of reduced activation of carcinogens in tobacco smoke [7,8].

MPO–ANCAs have been demonstrated to activate primed granulocytes and monocytes, resulting in respiratory burst with the release of reactive oxygen species and proteases leading to endothelial damage [9,10]. However, MPO–ANCAs do not induce respiratory burst in leukocytes from MPO-deficient individuals [11]. It therefore appears intriguing to speculate that diminished activity of MPO due to A allele carriage of the G-463A polymorphism confers reduced susceptibility to the development of MPO–ANCA-associated vasculitis. In the present study we therefore analysed

Correspondence and offprint requests to: Marion Haubitz, MD, Department of Nephrology, Medical School Hannover, D-30623 Hannover, Germany. Email: haubitz.marion@mh-hannover.de

whether allelic frequencies of this polymorphism in MPO–ANCA-associated vasculitis patients differ from those of healthy controls or PR3–ANCA-associated vasculitis patients.

Subjects and methods

Allelic frequencies of the G-463A polymorphism within the MPO promoter were analysed in 119 Caucasian ANCA-associated vasculitis patients originating from the northern part of Germany. Forty-eight patients had MPO–ANCA-associated vasculitis and 71 patients had PR3–ANCA-associated vasculitis. Patients' characteristics are shown in Table 1. ANCA positivity and specificity had been documented at the time of active disease by indirect immunofluorescence and an ELISA for MPO–ANCA and PR3–ANCA.

A commercially available kit (Qiagen-RNeasy Mini Kit, Qiagen, Hilden Germany) was used to extract DNA from peripheral blood leukocytes. For genotyping procedure, the polymorphic site at position –463 of the MPO gene was amplified as described by Cascorbi *et al*. [7]. In brief, DNA was extracted from leucocytes with a standard phenol–chloroform protocol. The MPO-DNA including the polyphorphism locus 463 was amplified using the following primers: FW: 5′-CGGTATAGGCACACAATGGTGAG, RV: 5;-GCAATGGTTCAAGCGATTCTTC. The PCR product was digested with Acil (New England Biolabs) resulting in four different fragments depending on the genotype. To visualize the results, the fragments were separated with gel electrophoresis and stained with ethidium bromide.

Differences between the groups were compared using χ^2-test (Bonferroni correction was performed for multiple comparison) and Monte Carlo simulation (empirical error rate for small patient numbers [12]). A P-value < 0.05 was considered significant.

Results

Frequencies of the G and A allele carriage of MPO G-463A polymorphism were investigated in MPO–ANCA- and PR3–ANCA-associated vasculitis. Hardy–Weinberg equilibrium was observed in both patient groups. Allelic frequencies of the MPO G-463A polymorphism did not differ significantly between the patient groups. The frequency of the A allele was 15.6% in MPO–ANCA and 22.5% in PR3–ANCA patients. As shown in Table 2 no statistical difference of the genotype frequencies between the two patient groups was observed, either. Moreover, MPO G-463A polymorphism genotype frequencies of both ANCA patients groups were similar to those observed in various Caucasian reference populations. Interestingly, like the patients analysed in the present study, one of the reference populations originated from the northern part of Germany. In addition, no differences were observed after ANCA-associated vasculitis patients had been stratified to gender (Table 2), organ manifestations or diseases (WG or microscopic polyangiitis, data not shown).

Table 1. Patients' characteristics

Vasculitis	MPO–ANCA-associated ($n = 48$)	PR3–ANCA-associated ($n = 71$)
Age (years)	21–87 (median 59.5)	13–89 (median 51)
Male/female	27/21	40/31
WG/MPA	5/43	58/13
Organ manifestation		
Kidney	44	46
Lung	17	47
Nervous system	12	17
Ear/nose/throat	9	62
Arthralgia	21	41
Eye	4	14
Other organs	8	16

Discussion

In ANCA-associated vasculitis, reactive oxygen species play an important role in tissue injury and the inflammatory reaction. Therefore, A allele carriage of G-463A MPO gene polymorphism, leading to reduced MPO activity with the presumable consequences of reduced production of radicals, might be protective against the development of an ANCA-associated vasculitis. However, our results demonstrate that allelic as well as genotype frequencies did not differ between MPO–ANCA- and PR3–ANCA-associated vasculitis patients. Furthermore, the frequencies of ANCA-associated vasculitis patients observed were comparable with those previously published in various Caucasian reference populations [7,8,13].

In contrast to the findings of Reynolds *et al*. [13], no differences were observed when the ANCA-associated vasculitis patients were stratified for gender or organ manifestation. As the data of Reynolds *et al*. were not available at the time when our study was planned, no study design concerning the results of Reynolds *et al*. was made. Using the allele frequencies of ethnically matched controls of Reynolds *et al*. and a significance of <0.05, the number of patients included in our population could be sufficient to find at least a tendency in female patients. However, since the number of patients

Table 2. Genotype frequencies of MPO G-463A polymorphism

Population	G/G	G/A	A/A
MPO–ANCA	33 (69%)	15 (31%)	0
Male	19 (70%)	8 (30%)	0
Female	14 (67%)	7 (33%)	0
PR3–ANCA	42 (59%)	26 (37%)	3 (4%)
Male	24 (63%)	13 (34%)	1 (3%)
Female	18 (55%)	13 (39%)	2 (6%)
Reference populations			
Northern Germany (7)	61%	35%	4%
Caucasian (8)	61%	31%	8%
Dutch (13)	64%	33%	3%

studied is very small, the power to find a significant statistical difference in our patient group is only 30%.

In the study of Reynolds *et al*., the frequency of G/G genotype was increased in female, but not in male, MPO–ANCA-associated vasculitis patients. Therefore, the presence of the A allele appeared to prevent formation of MPO–ANCA-associated vasculitis in females. In the same study, however, patients with the A allele had in an earlier onset of MPO–ANCA-associated vasculitis. Furthermore, the presence of the A allele was associated with a higher frequency of nasal, sinus and/or ear involvement and a shorter relapse-free period.

The contrasting results of the present study and the investigation by Reynolds *et al*., might be explained by the fact that reliable data are difficult to obtain in small patient groups, particularly when polymorphism is used for multiple comparisons. In the study of Reynolds *et al*., *P* values were not corrected for multiple comparison. The subgroups were very small ($n = 16$ for patients with nose/sinus involvement, $n = 6$ for ear involvement). Moreover, it seems unlikely that, on the one hand, the presence of A allele mediates protection against the disease and, on the other hand, implies the higher susceptibility to special organ involvement and the development of relapses.

In conclusion, our data suggest that G-463A MPO polymorphism contributes neither to MPO–ANCA- nor PR3–ANCA-associated vasculitis. However, a marginal impact of genetic polymorphism of the MPO gene regarding a trend towards decreased frequency of the A allele in MPO–ANCA positive compared with PR3–ANCA positive vasculitis disease would have to be assessed in a large study group.

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Conflict of interest statement. None declared.

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