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Published in:
European Journal of Internal Medicine

Publication date:
2003

Document Version
Publisher's PDF, also known as Version of record

Link to publication in Tilburg University Research Portal

Citation for published version (APA):
Original article

Relationship between myeloperoxidase promotor polymorphism and disease severity in sarcoidosis

Snježana Rothkrantz-Kos\textsuperscript{a,d,f}, Marjolein Drent\textsuperscript{b,d,f,*}, Abraham Rutgers\textsuperscript{c}, Peter Heeringa\textsuperscript{c}, Jolanda De Vries\textsuperscript{c,f}, Marja P. van Dieijen-Visser\textsuperscript{a,f}, Jan W. Cohen Tervaert\textsuperscript{c,f}

\textsuperscript{a}Department of Clinical Chemistry, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands
\textsuperscript{b}Department of Respiratory Medicine, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands
\textsuperscript{c}Department of Immunology, University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands
\textsuperscript{d}Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht, The Netherlands
\textsuperscript{e}Department of Clinical Health Psychology, Tilburg University, Tilburg, The Netherlands
\textsuperscript{f}Sarcoidosis Management Center, Maastricht, The Netherlands

Received 5 November 2002; accepted 11 March 2003

Abstract

Background: Previously, we demonstrated that the number of polymorphonuclear neutrophils (PMNs) in bronchoalveolar lavage fluid (BALF) is useful in distinguishing sarcoidosis patients with a favorable outcome from those having a more severe course of disease. Neutrophils contain the oxidant-generating enzyme myeloperoxidase (MPO). Cellular levels of MPO can be influenced by functional promotor polymorphisms, \(-463G/A\) and \(-129G/A\), which may modify disease severity. Methods: In the present study, we investigated two MPO promotor polymorphisms in 110 sarcoidosis patients and in 191 ethnically matched controls. Pulmonary disease severity was evaluated by means of radiographic staging, HRCT scoring, lung function, and exercise capacity testing. Results: No significant differences were found between sarcoidosis patients and healthy controls with regard to either polymorphism. Nor was any association observed between \(-463 G/A\) and \(-129 G/A\) polymorphism and the severity of sarcoidosis. Conclusions: The functional MPO promotor polymorphisms \(-463G/A\) and \(-129G/A\) did not explain disease severity in the sarcoidosis population studied. Future studies are needed to identify predictive features useful in guiding therapeutic strategies and to determine difficult-to-treat cases.

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Keywords: Myeloperoxidase; Sarcoidosis; Neutrophils; Polymorphism; Granuloma

1. Introduction

Sarcoidosis, a disease predominantly affecting young adults, is the most common diffuse lung disease with a population prevalence of around 1:4000 [1,2]. Sarcoidosis is an immune-mediated inflammatory disease with unknown etiology, although genetic predisposition is likely to be important [2,3]. The main pathologic feature of the disease is a chronic inflammation resulting in non-caseating granuloma formation. The prognosis of sarcoidosis is rather variable. A great deal of uncertainty exists as to how to predict the natural course of the (untreated) disease and how to identify those individual cases who should be treated immediately. More important, as it is still not possible to identify the cause of sarcoidosis, therapy is directed at controlling the inflammatory reaction [4].

Bronchoalveolar lavage fluid (BALF) analysis appears to reflect the severity of sarcoidosis [5–9]. Notably, the number of polymorphonuclear neutrophils (PMNs) is
increased in advanced, chronic sarcoidosis [5–7,9]. In line with these findings, interleukin 8 (IL-8)—a potent chemoattractant and activator of neutrophils—in BALF appears to be highly elevated in sarcoidosis patients who show progressive disease [8,10]. Myeloperoxidase (MPO) is an abundant protein in PMNs and monocytes, stored in the azurophilic granules of neutrophils and released during phagocytosis [11,12]. MPO in BALF originates from lung PMNs and can be used to estimate the presence and/or activation of PMNs [13]. Indeed, a strong correlation between MPO level and PMN count in BALF has been demonstrated in patients with Wegener’s granulomatosis [14]. MPO concentrations appear to be associated with two described functional promotor polymorphisms, namely, the −463 G/A MPO promotor polymorphism [15,16] and the recently described −129 G/A promotor polymorphism [17]. Several diseases have been reported to be associated with the −463 G/A promotor polymorphism [16,18–22], whereas no association studies of −129 G/A have yet been reported.

MPO appears to be involved in tissue damage through production of the potent oxidant HOCl, which is able to oxidize many cellular compounds, such as thiols and NADPH, and to lead to chlorination of DNA bases and tyrosine residues in proteins [11,12,23–25]. Therefore, it can be assumed that decreased availability of MPO, related to functional MPO promotor polymorphisms, has a protective effect by decreasing the chance of tissue damage.

It has been hypothesized that the MPO polymorphism may contribute, at least in part, to disease severity in sarcoidosis and make it possible to determine those cases who might benefit from agents controlling the inflammatory reaction at an early stage. Hence, the incidence of two MPO promotor polymorphisms (−463 G/A and −129 G/A) was analyzed in a Dutch Caucasian sarcoidosis population, as was their possible relationship with disease severity.

2. Materials and methods

2.1. Study population

Caucasian sarcoidosis patients (n=110) who visited the Sarcoidosis Management Center of the University Hospital Maastricht, a Dutch referral center for sarcoidosis, between January 2000 and April 2001 were included in this study. Patients were diagnosed based on consistent clinical features and BALF analysis, according to the ATS/ERS/WASOG guidelines [2,26]. The diagnosis was histologically confirmed in 85% of the cases. The control population consisted of 191 healthy individuals. Informed consent was obtained from all participating subjects. Patient characteristics are summarized in Table 1.

2.2. Evaluating sarcoidosis pulmonary disease severity

Chest radiographs were graded according to the radiographic staging of DeRemee (0 to III), adding stage IV, the end stage of lung fibrosis [2,27]. With high-resolution computer tomography (HRCT), thin-section scans with 1-mm collimation were obtained at 10-mm intervals through the chest. The scanning parameters included 137 kVp, 255 mA, and 1-s scanning time.

Both mediastinal (width 400 HU; level 40 HU) and lung (width 1600 HU; level −800 HU) window images were obtained. The semiquantitive HRCT scoring system was used as described previously [28].

Lung function parameters, including the forced expiratory volume in 1 s (FEV$_1$) and forced vital capacity (FVC), were measured with a pneumotachograph. The diffusing capacity for carbon monoxide (DL$_{CO}$) was measured using the single-breath method (both Masterlab, Jaeger, Würzburg, Germany). Values were expressed as a percentage of those predicted [29].

Serum ACE (sACE) was measured using the colorimetric method (Fujirebio Inc., Tokyo, Japan, cat. nr. FU 116). ACE acts on a substrate $p$-hydroxybenzoyl-glycyl-$t$-hydroxybenzoyl-$L$-leucine and separates $p$-hydroxybenzoyl-glycine, which is converted in two consequent reactions in quinoneimine dye. The absorbance of quinoneimine dye is measured at 505 nm to evaluate the ACE activity.

Soluble interleukin-2 receptor (sIL2R) was measured on the IMMULITEAutomated Analyser, which is a two-site chemiluminescent enzyme immunoassay assay with a detection limit of 50 kU/l and a measuring range of 50–7500 KU/l (Diagnostic Product Corporation, Los Angeles, CA, cat no LKIP1).

2.3. Molecular studies

The −463 G/A polymorphism was determined as previously described [16,18,19]. The 129G/A polymorphism was determined as described by Hoy et al. [17]. The
most important features are presented in Table 2. When screening for 129 G/A polymorphism, we identified a 129G to A specific Apa1 restriction site within the 278 bp PCR product. PCR products were digested with 20 units of Apa1 (Roche, Mijdrecht, the Netherlands) for 2 h or overnight at 30°C, separated on a 2% agarose gel, and stained with ethidium-bromide.

2.4. Statistics

All analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, USA). Agreement with Hardy–Weinberg equilibrium and differences in allele and genotype frequencies between groups were tested using chi-square tests. Because multiple comparisons were performed, a probability value divided by the number of comparisons was considered statistically significant (Bonferroni’s correction). Univariate logistic regression analysis was used to calculate odds ratios with 95% confidence intervals. Group comparisons were performed by means of an Independent samples t-test or the Mann–Whitney U-test.

3. Results

The two functional MPO polymorphisms fulfilled Hardy–Weinberg expectations in both sarcoidosis and control subjects. Genotype distributions associated with the -463 G/A or -129 G/A polymorphism did not differ significantly between patients with sarcoidosis and healthy controls (Table 3). Furthermore, for both polymorphisms, sex-specific analysis showed no differences in allele or genotype frequencies (data not shown).

Baseline clinical data of the patients with sarcoidosis are presented in Table 4. We also tested whether any association could be found with MPO polymorphisms. For this purpose, for -463 G/A polymorphism, the GA and AA genotypes were grouped together and compared to the GG genotype. No relationship with age at diagnosis was found. Severity of the disease was then evaluated in relation to genotype. The results of this analysis are presented in Table 5. As can be seen, no significant differences in the severity, as presented by clinical data, could be found. This finding remained unchanged after correcting for prednisone use. The same analysis that is shown in Table 5 was...
Table 4
Evaluation of severity in a Dutch Caucasian sarcoidosis patient population

<table>
<thead>
<tr>
<th>Sarcoïdosis population</th>
<th>Female (n=55)</th>
<th>Male (n=55)</th>
<th>Total (n=110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sACE (9–25 U/l)</td>
<td>20.0 (17–26)</td>
<td>20.0 (15–28)</td>
<td>20.0 (16–27)</td>
</tr>
<tr>
<td>sIL2R (241–846 kU/l)</td>
<td>97.0 (84–166)</td>
<td>87.0 (63–99)</td>
<td>92.0 (69.8–103)</td>
</tr>
<tr>
<td>FEV₁ (% pred)</td>
<td>105 (94.2–115)</td>
<td>91.0 (77.5–102)</td>
<td>98.5 (84.5–110)</td>
</tr>
<tr>
<td>FVC (% pred)</td>
<td>89 (78–96)</td>
<td>85.5 (72.5–97.5)</td>
<td>87.0 (76.5–96.5)</td>
</tr>
<tr>
<td>DLco (%)</td>
<td>4/8/14/14/2</td>
<td>4/8/14/20/9</td>
<td>21/16/28/34/11</td>
</tr>
<tr>
<td>0/I/II/III/IV</td>
<td>4.0 (1–6)</td>
<td>6.0 (3–9)</td>
<td>5.0 (3–8)</td>
</tr>
</tbody>
</table>

Abbreviations: sACE, serum Angiotensin-converting enzyme; sIL2R, soluble IL2 receptor; FEV₁, forced expiratory volume in 1s; FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide; CXR, Chest radiographic stage; HRCTtot, high-resolution computer tomography total score.

Table 5
Relationship between the −463 G/A myeloperoxidase (MPO) promoter polymorphism and disease severity in a Dutch Caucasian sarcoidosis population (n=110)

<table>
<thead>
<tr>
<th>Diagnostic parameter</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=32)</td>
<td>GA/AA (n=23)</td>
</tr>
<tr>
<td>sACE (9–25 U/l)</td>
<td>20 (18–26)</td>
<td>20 (16–27)</td>
</tr>
<tr>
<td>sIL2R (241–846 kU/l)</td>
<td>511 (397–806)</td>
<td>732 (414–1581)</td>
</tr>
<tr>
<td>FEV₁ (% pred)</td>
<td>99 (88–107)</td>
<td>92 (71–106)</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>105 (96–115)</td>
<td>105 (88–114)</td>
</tr>
<tr>
<td>DLco (%)</td>
<td>89 (81–95)</td>
<td>87 (72–99)</td>
</tr>
<tr>
<td>CXR</td>
<td>11/8/6/7/0</td>
<td>6/0/8/7/2</td>
</tr>
<tr>
<td>HRCTtot</td>
<td>3 (1–5)</td>
<td>6 (2–7)</td>
</tr>
</tbody>
</table>

Abbreviations: sACE, serum Angiotensin-converting enzyme; CXR, Chest radiographic stage; FEV₁, forced expiratory volume in 1s; FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide; sIL2R, soluble IL2 receptor; HRCTtot, high-resolution computer tomography total score.

4. Discussion

This study showed that the distribution of two functional MPO promoter polymorphisms, −463G/A and −129G/A, was similar in both sarcoidosis patients and controls. No relationship was found between these polymorphisms and disease severity. To exclude a possible bias due to corticosteroid use, treated and untreated sarcoidosis patients were also considered separately. The results in both subgroups appeared to be similar to those in the total population, i.e. no relationship to MPO genotype (also after stratification for sex and age) was found.

Of the cells present in BALF, PMNs are considered to be a key mediator indicative of severity of the disease. In cases with more extensive long-term radiographic features, impaired lung function parameters, a poor prognosis, and sometimes corticosteroid-resistant chronic disease, PMNs were found to be increased in BALF [5–7,9]. Moreover, a
strong correlation exists between MPO level and PMN count in BALF [14].

Some could argue that the neutrophils might just be an epiphenomenon, appearing when disease is extensive and fibrotic. However, it was recently demonstrated that the number of neutrophils in BALF, even at first presentation, is indicative of prognosis and disease outcome [5,9].

The GG genotype of −463 G/A polymorphism was previously found to correlate with higher levels of MPO mRNA and higher protein levels, as shown by Western blotting, than the GA or AA genotype [15]. In contrast, when circulating levels of MPO were measured in serum by means of enzyme immunoassay, no such correlation was found with −463 G/A polymorphism, but only with −129 G/A polymorphism [17].

It was recently demonstrated that −463 G/A MPO promoter polymorphism influences diseases severity and expression in chronic granulomatous disease [22] and Wegener’s granulomatosis [20], both granulomatous diseases different from sarcoidosis. In the present study, however, no association of MPO promoter polymorphisms with sarcoidosis were found. One possible explanation could be that disease expression in chronic granulomatous disease and Wegener’s granulomatosis is characterized by infections with extensive PMN involvement. Indeed, cotrimoxazole, an antibacterial drug, positively influences the course of disease in both chronic granulomatous disease [30] and Wegener’s granulomatosis [31,32], whereas this has never been shown in sarcoidosis.

Granuloma formation in sarcoidosis appears to be a strictly T-cell-dependent process [33]. Since MPO was shown to inhibit T-cell proliferation [34,35], one can postulate that MPO promoter polymorphisms might also influence the T-cell-dependent disease process in sarcoidosis. Therefore, the role of MPO and its polymorphisms seem very complex and not completely defined in the different diseases that are characterized by granulomatous inflammation.

In conclusion, the PMN count in BALF appears to be useful in determining chronic and difficult-to-treat sarcoidosis patients. MPO plays a key role in the immunopathogenesis of several granulomatous disorders and MPO release appears to be related to MPO polymorphism(s). However, in the Dutch Caucasian sarcoidosis population studied, similar frequencies of functional MPO promoter polymorphisms (−463 G/A and −129 G/A) were found as compared to control subjects. Furthermore, no association between −463 G/A and −129 G/A MPO promoter polymorphisms and the severity of sarcoidosis, including radiographic features, impaired lung function, time since diagnosis and corticosteroid use, was found in our study population. As we are unable to identify the cause of sarcoidosis, therapy must be focused on controlling the inflammatory reaction. Future studies are needed to determine those cases with a more advanced chronic course of the disease who might benefit from early treatment of the inflammatory reaction.

Acknowledgements

The authors gratefully acknowledge Ruud Teunissen and Petal Wijnen for their assistance and Professor Dr J. Geraedts, clinical geneticist, for his suggestions.

References


