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The influence of N-acetyltransferase 2 polymorphism on rheumatoid arthritis activity

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ABSTRACT

Objective. *The N-acetyltransferase polymorphism is involved in the metabolism of many xenobiotics, as well as in susceptibility to some diseases such as rheumatoid arthritis (RA). The aim of this study was to investigate the influence of NAT 2 polymorphism on disease activity in RA patients.*

Methods. *70 with RA were enrolled in the study. As a measure of disease activity, the number of swollen and tender joints, the duration of morning stiffness, ESR and CRP as well as disease activity based on a global physician's assessment were evaluated. The NAT2 polymorphism was determined by a polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).*

Results. *The mean number of swollen and tender joints, as well as the ESR and CRP values, did not differ significantly with the acetylation genotype. Erosive RA was diagnosed in 74.5% of the slow and 40% of the fast acetylators. The risk for the development of erosive RA was 4.39 time greater in slow acetylators than in fast acetylators.*

Conclusion. N*AT2 polymorphism may be a genetic risk factor for joint destruction.*

Introduction

Rheumatoid arthritis (RA) is a multifactorial disease with a genetic background. Multiple genes are thought to be involved in disease susceptibility, whereas others could be more important as modulators of disease severity (1, 2). As such they may account for the spectrum of clinical expression ranging from the mild, non-destructive form to severe and rapidly debilitating disease.

HLA DRB_1 is associated with RA and to some extent with disease severity, but probably accounts for only about one-third of the genetic component of RA (3, 4). Even when combined with other indicators of destruction such as rheumatoid factor (RF), the specificity and sensitivity of HLA DRB alleles as prognostic factor for destructive arthritis are frequently considered to be low and of limited value to clinicians (5). Other genetic markers of disease severity would therefore be better, especially in the light of the present tendency to treat patients with severe disease earlier and more aggressively.

Recently the effect of environmental factors on RA susceptibility has been considered (6-8). RA may be induced by one or more environmental factors acting on a specific genetic background. However, xenobiotics that could initiate development of the disease have yet to be defined. Some of them are believed to be the activating factors involved in the initiation of the disease and disease progress. Many investigators have proposed that RA be considered a semi-malignant process triggered by oncogene activators (9, 10).

Several studies have shown that N-acetyltransferase 2 (NAT2) is important in the metabolism of aromatic and heterocyclic amine carcinogens (11, 12). Individual differences in NAT2 activity determine therapeutic efficacy and side effects of a drug metabolized via acetylation, as well as the susceptibility to some diseases (13-16). Humans exhibit genetic polymorphism in NAT2, resulting in fast or slow acetylation. One allele coding fast acetylation and several mutated alleles coding slow acetylation are known (17). Different mutations of the NAT2 gene have been identified, five of which lead to changes in the encoded protein. About 50% of Caucasians show decreased activity of the enzyme (slow acetylators). The subjects with normal N-acetyltransferase 2 activity are called fast acetylators (18).

In a previous study we analyzed the possible correlation of NAT2 polymorphism with the predisposition to RA (16). A statistically significant prevalence of slow acetylators was found among RApatients in comparison with healthy subjects. We consider that NAT2 polymorphism may be a risk factor for the development of RA. The aim of the present study was to evaluate the association of this polymorphism with disease activity parameters and with joint damage in RApatients.

Materials and methods

We examined 70 patients (50 women, 20 men; age 20-75 years, mean 52.5) with RA diagnosed according to the

criteria of the American College of Rheumatology. The disease duration was 2-16 years (mean 6.9 years). Patients were recruited from the outpatient and inpatient populations of the Department of Rheumatology, University Hospital in Szczecin, Poland. All subjects were Caucasian from the Pomeranian region of Poland.

Subjects enrolled in the study underwent routine biochemical blood analysis and, when required, assays for anticardiolipin antibodies, antinuclear antibodies and immunologic complexes. X-rays of the chest, hands and feet (erosive or non-erosive RA) were obtained in all patients, and when required, radiographs of other joints. These were interpreted by two different expert radiologists. The evaluation of subjects included physical examination with particular focus on the pattern of joint involvement, the presence of nodules and other extra-articular features (such as vasculitis, anaemia, sicca syndrome, amyloidosis, organ involvement), and laboratory features such as rheumatoid factor (RF).

Disease activity was determined on the basis of defined parameters and a global physician's assessment. The number of swollen and tender joints, duration of morning stiffness, ESR and CRP were measured several times over a one-year period and the mean values were used. 50/70 patients were classified as having active disease based on the global physician's assessment and the finding during at least one examination of: a swollen joint count > 3, an ESR >25 mm/h and a duration of morning stiffness > 0.5 h (19). All patients were treated with low dose methotrexate and glucocorticosteroids.

Genomic DNAwas extracted manually (precipitation with trimethylammonium bromide salts) from leukocytes contained in 450 ml of venous blood with ethylenediaminetetraacetic acid as anticoagulant. The DNA was then precipitated in 95% ethanol, dissolved in distilled water, and stored at -20ºC until analysis (20). The chemicals for the extraction were purchased from Sigma Chemical Company (St Louis, MO).

The mutations at positions 481T, 803G, 590Aand 857Aof the NAT2 gene were determined by a polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) (21). The wild-type NAT*4 allele was considered to be the fast allele and NAT2*5, NAT2*6 and NAT2*7 the slow alleles. NAT2 genotypes were divided into 3 groups: homozygous and heterozygous fast acetylators (individuals with 2 wild-type alleles or 1 wild-type and 1 mutated allele) and homozygous slow acetylators (individuals with 2 mutated alleles).

Statistical analysis

Statistical analysis of investigated parameters was performed using the Mann-Whitney test.

Results

The patients in the two groups – slow (n = 55) versus fast (n = 15) acetylators – did not differ in terms of sex, age, or disease duration (slow acetylators: 16/39 M/F, mean age 52.9 years, mean disease duration 6.9 years; fast acetylators: 4/11 M/F, mean age 51.1 years, mean disease duration 6.8 years). Rheumatoid factor was diagnosed in 34 (61.8%) of slow and 9 (60.0%) of fast acetylators. The mean number of swollen and tender joints was 5.8 and 7.9 in slow acetylators and 4.3 and 6.4 in fast acetylators, respectively (Table I). Although the number of swollen and tender joints was higher in slow acetylators, these differences were not statistically significant. Morning stiffness lasted a mean of 0.9 h in fast acetylators, and 1.4 h in slow acetylators. This difference was statistically significant $p < 0.05$. The mean values of ESR and CRPwere 62.1 mm/h and 69.5 mg/L in slow acetylators and 60.4 mm/h and 57.7 mg/L in fast acetylators; the differences were not statistically significant. Active RA was diagnosed in 40 (72.7%) slow and 10 (66.7%) fast acetylators. The risk of developing active RA was 1.33 times greater for slow acetylators in comparison with fast acetylators (odds ratio 1.33; 95% confidence interval 0.33 – 5.26). This difference was not statistically significant. Erosive RA was diagnosed in 41 (74.5%) slow and 6 (40%) fast acetylators. The risk of developing erosive RA was 4.39 time greater in slow acetylators than in fast acetylators (odds ratio 4.39; 95% confidence interval 1.15 – 17.33). This difference was statistically significant ($p < 0.02$) (Table I).

Discussion

Understanding how interactions between genes and the environment contribute to the development of arthritis is a central issue for understanding the etiology of RA, as well as for eventual efforts to prevent the disease. In this study we evaluated the influence of NAT2 polymorphism on parameters determining disease activity as well as joint damage. These parameters were compared between two groups: slow

Table 1. The values of parameters of disease activity in RApatients in relation to acetylation genotypes (mean values ± SD).

Patients	No. of swollen joints	No. of tender joints	Morning stiffness (h)	OB (mm/h)	CRP (mg/l)	Patients with active RA (n)	(%)	Patients with erosive RA (n)	(%)
Fast acetylators	4.3 (± 0.91)	6.4 (± 1.42)	0.9 (± 0.22)	60.4 (± 22.4)	57.7 (± 25.4)	10	66.7	6	40.0
Slow acetylators	5.8 (± 0.98)	7.9 (± 1.56)	1.4 (± 0.47)	62.1 (± 29.7)	69.5 (± 34.9)	40	72.7	41	74.5
p value*	NS	NS	<0.05	NS	NS	NS		< 0.02	

and fast acetylators, matched for age, sex, and disease duration. There were no significant differences in most of the parameters of disease activity: number of swollen and tender joints, ESR, CRP. However, the number of patients with erosive RAwas significantly higher among slow acetylators. The percentage of joint erosions is often higher after a mean disease duration of 7 years than in the patients included in our study. All our patients were placed on methotrexate when the RA diagnosis had been established, a therapy that has been demonstrated to attenuate the progression of joint erosions (22). The higher number of patients with erosive RA among the slow acetylators suggests an influence of xenobiotics metabolized via acetylation not only on the predisposition to RA but also on the process of joint destruction. Nevertheless the question remains open as to what extent xenobiotics and other environmental factors determine individual susceptibility to RAor disease progression.

Recently efforts have been directed towards the investigation of interactions between genes and the environment in the pathogenesis of RA (6-8). The polymorphic genes determining the phenotype of arthritis in animals have been studied, and a number of gene regions have been identified that influence susceptibility to arthritis and the destructiveness and severity of the disease (23, 24). An interesting aspect is that the same genetic context that predisposes for the development of adjuvant arthritis induced by single compounds (mineral oil, squalene) also predisposes to the development of collagen-induced arthritis, in which the additional presence of certain MHC class II genes is mandatory for the development of disease (25-27). It thus appears that a certain set of genes may determine the response of the innate immune system to adjuvants, sometimes resulting in arthritis without the addition of further stimuli. The addition of specific immunity to self-antigens such as collagen will in these cases make the arthritis more severe and destructive (28). Our present knowledge of environmental influences on the development of RAis of two kinds. The first is derived from twin studies which all indicate that the genetic influence is important but probably less so than environmental effects. The second derives from case-control or cohort studies on the influence of distinct agents or events on RA, in which exposure to a number of agents have been reported to be associated with RA (29). Such agents include smoking, silica, and mineral oils (30-32). It is possible that the arylamines metabolized via acetylation are also involved in the development and progression of RA and NAT2 polymorphism may be just one of many genetic risk factors for RA susceptibility and joint destruction.

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