CONCISE REPORT

Asporin repeat polymorphism in rheumatoid arthritis

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Background: Asporin belongs to a family of proteins associated with the cartilage matrix.

Objective: To investigate the role of the functional polymorphism consisting of an aspartic acid (D) repeat polymorphism located in the ASPN gene in the susceptibility to and clinical outcome of rheumatoid arthritis.

Methods: A total of 803 Spanish Caucasian patients with rheumatoid arthritis and 904 controls of the same ethnic origin and matched for age and sex were included in the study. The asporin D repeat polymorphism was genotyped using polymerase chain reaction with a fluorescent primer.

Results: No significant differences were detected in the distribution of the 10 alleles found in our population on comparing patients with rheumatoid arthritis with control groups. Nevertheless, individuals bearing D14 produced rheumatoid factor more often than the rest (85.7% v 72.1%, $p=0.006$, odds ratio (OR) = 2.35, 95% confidence interval 1.21 to 4.50), and the mean (SD) onset age was higher in the group of individuals bearing D13 (50.09 (13.94)) compared with the rest (47.21 (14.31)), although the difference did not reach significance ($p=0.06$).

Conclusion: The results do not support a major role for asporin D repeat polymorphism in the susceptibility to rheumatoid arthritis. Nevertheless, they support the influence of this gene on the outcome of the disease.

The irreversible destruction of the cartilage, tendon and bone that comprise synovial joints is the hallmark of both rheumatoid arthritis and osteoarthritis. Tendon and bone are composed primarily of type I collagen, whereas cartilage is made up of proteoglycans and type II collagen. Rheumatoid arthritis is a systemic autoimmune disease in which numerous joints are affected throughout the body; in contrast, osteoarthritis develops in a small number of joints, usually resulting from chronic overuse or injury. In both diseases, inflammatory cytokines such as interleukin (IL)1 β and tumour necrosis factor α stimulate the production of matrix metalloproteinases, enzymes that can degrade all components of the extracellular matrix.¹ Asporin belongs to a family of leucine-rich repeat proteoglycans associated with the cartilage matrix. The name asporin reflects the unique aspartate-rich N terminus and the overall similarity to decorin.² The asporin gene (ASPN) maps probably within a leucine-rich repeat gene cluster in 9q21.3–q22,³ spans more than 26 kb and contains eight exons.² Kizawa *et al*⁴ investigated the potential role of asporin in the pathogenesis of osteoarthritis. They found a significant association between osteoarthritis and an aspartic acid (D) repeat polymorphism located in the N-terminal region of the protein in two independent populations of individuals with knee osteoarthritis. The ASPN allele D14 (14 D repeats) was over-represented in patients relative to the common allele D13

(13 D repeats), and its frequency increased with disease severity. The D14 allele was also over-represented in individuals with hip osteoarthritis. Asporin suppressed transforming growth factor (TGF) β -mediated expression of the aggrecan 1 and collagen type II α 1 genes and reduced proteoglycan accumulation in an in vitro model of chondrogenesis. The effect on TGF β activity was allele-specific, with the D14 allele resulting in greater inhibition than other alleles.⁴ In vitro binding assays showed a direct interaction between asporin and TGF β . Although the ASPN gene is not located in a chromosomal area of linkage with rheumatoid arthritis, its function leading to this polymorphism is being considered as a candidate for rheumatoid arthritis susceptibility. The aim of this study was to investigate the role of the D repeat polymorphism located in the ASPN gene in the susceptibility and clinical outcome of RA.

PATIENTS AND METHODS

A total of 803 (205 (25.5%) men and 598 (74.5%) women) patients meeting the American College of Rheumatology 1982 revised criteria for rheumatoid arthritis⁵ were included in the study. Patients were recruited from four Spanish hospitals: Hospital Universitario Virgen del Rocío (Seville), Hospital Virgen de las Nieves (Granada), Hospital Xeral-Calde (Lugo) and Hospital de La Paz (Madrid). The mean (standard deviation (SD)) age of onset was 49.23 (14.8) years. Patients were positive for the presence of rheumatoid factor in 75.2% of the cases, rheumatic nodules were observed in 18.3%, extra-articular manifestation in 25.3%, and human leucocyte antigen shared epitope was present in 60.6% of the patients. A total of 904 blood bank and bone marrow donors were included as controls. All the subjects, cases and controls were of Spanish Caucasian origin matched for age and sex. Samples were obtained from subjects after they provided written informed consent. The study was approved by all local ethics committees of the corresponding hospitals.

Asporin D repeat genotyping DNA from patients and controls was obtained from peripheral blood using standard methods. The D repeat region was amplified using the forward primer 5'-TCCTAGACTGGTCTTCTACT-3' and the reverse primer 5'-TCTGAGCAATGTACAACCTCGTG-3'. The polymerase chain reaction (PCR) was carried out with a mixture consisting of 0.05 μ g of genomic DNA, 1 μ l of 10 $\times$ PCR buffer, 200 μ mol/l of each dNTP, 500 μ mol/l of MgCl₂, 2.5 pmol of each primer, 0.25 U of Taq DNA polymerase and double distilled water up to a final volume of 10 μ l. The forward primer was 5' labelled with WellRED D4-PA. The amplification protocol used was: initial denaturation at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 5 min. After PCR, alleles were discriminated using a Beckman Coulter CEQ 8000

Abbreviations: ASPN, asporin gene, PCR, polymerase chain reaction; TGF, transforming growth factor

Genetic Analysis System and the Fragment Analysis Module software (Beckman Coulter, Fullerton, California, USA).

Genotypic and allelic frequencies were obtained by direct counting. Statistical analysis to compare distributions was performed by the χ^2 test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated according to Wolf's method. The software used was the Statcalc program (Epi Inpo 2002; Centers for Disease Control and Prevention, Atlanta, Georgia, USA). *p* Values <0.05 were considered significant. *p* Values were corrected by the number of variables included in the outcome study (*n* = 6). Means were compared by analysis of variance using the same software. Statistical power was calculated using the software Statistical Power Calculator (Percentages, Two Samples) available at http://www.dssresearch.com/toolkit/spcalc/power_p2.asp

RESULTS

No significant differences in the frequencies of the different alleles were observed among patient groups with rheumatoid arthritis or among control groups from the four different cohorts. Hence we combined all these four cohorts to form a rheumatoid arthritis case-control group, which was used in further analyses. The control study population was found to be in Hardy-Weinberg equilibrium for the polymorphism studied.

Table 1 shows the distribution of alleles of the D repeat of the asporin gene in patients with rheumatoid arthritis and controls. We detected 10 alleles containing 11–20 residues, as in Japanese and UK populations^{4,6}; the most common allele was D13, with a frequency in our control population of 45.5%. The other two alleles with a frequency >10% in our control population were D14 and D15. No significant differences were observed in the distribution of the alleles when comparing patients with rheumatoid arthritis and controls (alleles with a frequency <5% in the control population were grouped and a 2×6 contingency table analysis was performed, *p* = 0.34). The frequency of the D13 allele was lower in patients with rheumatoid arthritis (42.3% *v* 45.5% in controls) but the difference did not reach significance (*p* = 0.07).

Subsequently, ASPN repeat polymorphism was analysed taking into account the following variables: sex, rheumatoid factor, human leucocyte antigen shared epitope, nodules, extra-articular manifestation and age of onset. Production of rheumatoid factor was significantly higher in patients bearing D14 (85.7%) than in the rest of the patients (72.1%, *p* = 0.006, *p*_(c) = 0.036, OR = 2.35, 95% CI 1.21 to 4.50), and the mean (SD) onset age was higher in patients bearing D13 (50.09 (13.94)) than in the rest (47.21 (14.31)), although the difference did not reach significance (*p* = 0.06, *p*_(c) = 0.36). Also, among homozygotic patients, the latest onset

corresponded to D13D13 (52.22 (14.08)) and the earliest to D14D14 (44.75 (8.86)).

DISCUSSION

This work was the first to investigate the potential role of asporin, an extracellular matrix component, in the susceptibility and clinical outcome of rheumatoid arthritis. Association between a polymorphism consisting of a variable number of aspartic acid (D) repeats and osteoarthritis has been reported. The allele D14 was found to be over-represented in Japanese patients with osteoarthritis, and the same trend was observed in a group of Caucasian patients with osteoarthritis from the UK, but only in men who had undergone hip replacement.^{4,6} Frequencies of the three most common alleles in the Spanish control population (D13: 45.5%; D14: 13.6%; D15: 22.1%) are quite similar to those described in the UK Caucasian control population (D13: 50.3%; D14: 12.7%; D15: 19.3%)⁶ and different from those described in the Japanese control population (D13: 64.0%; D14: 4.8%; D15: 4.5%).⁴ The statistical power of our study to detect an association with an OR of 1.5 was higher than 85% for the three most common alleles. Results obtained in our study support the idea that the ASPN repeat polymorphism does not have a major influence on susceptibility to rheumatoid arthritis. However, an influence cannot be completely discounted because the increase in the age of onset of patients bearing D13 approached significance and patients bearing D14 produced rheumatoid factor significantly more often than the rest. These results would support a role for D13 and D14 in the outcome of rheumatoid arthritis.

Data reported by Kizawa *et al*⁸ suggest that asporin acts as a negative regulator of TGF β signalling, and according to their results, the D14 variant has the strongest inhibitory effect. TGF β is a multifunctional cytokine involved in essential biological processes, including development, extracellular matrix synthesis, cell proliferation and differentiation and tissue repair. These authors propose that the asporin may play a major part in cartilage homeostasis as a modulator of cell and extracellular material regeneration and as a crucial modulator of TGF β ; too much inhibition of TGF β activity by the susceptibility allele (D14) would lead to more rapid progression of osteoarthritis. There are several studies supporting the involvement of molecules that modulate the initiation and progression of cartilage degradation in both osteoarthritis and rheumatoid arthritis.^{7–9} Approaches aimed at multiple steps of the pathogenesis of arthritis may be needed to break the chronic cycle of joint destruction.

In conclusion, our results do not support a major role for the asporin repeat polymorphism in susceptibility to rheumatoid arthritis, but they support some influence of this gene in the outcome of the disease.

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Table 1 Frequencies of the asporin D repeat alleles in Spanish patients with rheumatoid arthritis and controls

Alleles	RA		Controls	
	2n = 1606	%	2n = 1808	%
D11	3	0.2	0	0.0
D12	95	5.9	120	6.6
D13	680	42.3	822	45.5
D14	223	13.9	245	13.6
D15	394	24.5	399	22.1
D16	114	7.1	117	6.5
D17	62	3.9	71	3.9
D18	24	1.5	13	0.7
D19	8	0.5	20	1.1
D20	3	0.2	1	0.05

RA, rheumatoid arthritis.

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ECHO.....

Biliary cirrhosis associated with systemic sclerosis

Please visit the *Annals of the Rheumatic Diseases* website [www.annrheumdis.com] for a link to the full text of this article.

Patients with primary biliary cirrhosis (PBC) and systemic sclerosis (SSc) incurred a slower progression in liver disease compared with those who had PBC alone, a study has found. The study at London's Royal Free Hospital set out to compare survival and time to liver transplantation and to occurrence of complications in patients with both PBC and SSc, compared to those with PBC alone.

A PBC database of 580 patients identified 43 patients with clinically evident PBC and SSc. Two patients with PBC alone were matched to each patient with PBC-SSc for serum bilirubin concentration at the initial visit. During follow up, nine patients (21%) with PBC-SSc died, six because of SSc related causes and two with liver related causes. Of the patients with PBC alone, nine (11%) died, six as a result of liver related causes and two with extrahepatic malignancy.

Risk of needing a transplant, or death from time of diagnosis, was significantly lower in PBC-SSc patients—a liver transplant rate of 16% in the group with PBC-SSc compared to 21% in the group with PBC alone. Patients with PBC and SSc had a slower rate of bilirubin increase compared with patients with PBC alone.

The reasons for the difference in progression are not known but one may argue that some immunological interactions in the presence of an additional disorder may influence the clinical picture and favour a better outcome of the liver disease. It would be useful if the data are confirmed in a larger group of patients in a multicentre study.

▲ Rigamonti C, et al. *Gut* 2006;**55**:388–94