Subclinical Intestinal Inflammation and Sacroiliac Changes in Relatives of Patients With Ankylosing Spondylitis

INGVAR BJARNASON,* KRISTJAN O. HELGASON,† ÁRNI J. GEIRSSON,† GUDMUNDUR SIGTHORSSON,* INGA REYNISDOTTIR,§ DANIEL GUDBJARTSSON,§ ANNA S. EINARSDOTTIR,§ ROY SHERWOOD,* KRISTLEIFUR KRISTJANSSON,§ ÓLAFUR KJARTANSSON,† and BJARNI THJODLEIFSSON†

*Department of Medicine, Guy's, King's, St. Thomas' Medical School, London, England; †Department of Medicine, University Hospital of Iceland, Reykjavik; and §deCODE Genetics, Reykjavik, Iceland

Background & Aims: **It has been suggested that subclinical intestinal inflammation plays a pathogenic role in the spondylarthropathy of ankylosing spondylitis (AS). We assessed the possible presence and inheritance pattern of subclinical intestinal inflammation in first-degree relatives of patients with AS. The relationship between this inflammation and the subjects' HLA-B27 genotype as well as computerized tomographic sacroiliac abnormalities was also assessed.** ***Methods:*** **A total of 124 of 213 (58%) available first-degree relatives of 47 patients with AS in Iceland underwent investigation for intestinal inflammation (fecal calprotectin concentration), HLA-B27 genotyping, and computerized tomography of the sacroiliac joints.** ***Results:*** **A total of 41% of the first-degree relatives had subclinical intestinal inflammation, whereas 15 of 17 spouses were normal. Variance components analyses suggest that the inheritance pattern of this inflammation is affected by a major additive gene. Some sacroiliac changes, suggestive of early AS, differed significantly between subjects with and without subclinical intestinal inflammation (mean diameter of subchondral cysts [2.9 vs. 1.2 mm; $P = 0.026$] and blurring of joint margins [9 of 44 (20%) vs. 1 of 41 (2%); $P = 0.02$]). Intestinal inflammation and sacroiliac changes did not relate to the subjects' HLA-B27 status.** ***Conclusions:*** **Many first-degree relatives of patients with AS appear to have an inherited abnormality that leads to subclinical intestinal inflammation. The association between the presence of this inflammation and the sacroiliac changes suggests that it may play a pathogenic role in the spondylarthropathy of AS.**

In the past century, an interesting link has been found between some intestinal diseases and arthritis.[1] It is most obviously seen in the inflammatory bowel diseases (IBD) ulcerative colitis and Crohn's disease (CD). These are associated with 3 patterns of arthritis,[2] one of which is a spondylarthropathy. This spondylarthropathy (requiring both clinical and radiologic features) is found in 1%–6% of patients with classic IBD, whereas radiologic sacroiliitis is more common and evident in 18% of patients with classic IBD[3] and as many as 53% are affected when modern techniques are used.[4] Clinically, this spondylarthropathy is almost identical to that of idiopathic ankylosing spondylitis (AS). However, there is a difference in the prevalence of HLA-B27 in the 2 spondyloarthropathies; the prevalence in IBD, while still high, is significantly lower than in AS.[5]

Idiopathic AS usually occurs without overt signs of intestinal inflammation, but ileocolonoscopy studies[6–9] show a high prevalence of asymptomatic intestinal inflammation. This usually involves the ileum, although some reports also show a microscopic colitis.[10,11] There seems to be a close relationship between this subclinical intestinal inflammation and the inflammation seen in patients with CD. Hence, the prevalence (AS, 20%–80%; CD, 35%–80%) and type of inflammation (presence of giant cells, granulomas, and fissures: AS, 7%–19%; CD, 8%–17%) seen on ileal biopsy are almost identical in AS and CD.[11] Furthermore, about 10% of patients initially diagnosed with AS associated with this subclinical ileitis develop classic IBD when restudied some years later.[12,13] These findings have led to 2 important suggestions about the nature and importance of the intestinal inflammation in AS.[11] First, it is suggested that the intestinal inflammation in AS may represent subclinical CD, that is, it might share a pathogenic event (genetic or environmental) that does not usually by itself progress to the full phenotype of CD. Second, it is suggested that the intestinal inflammation may play a pathogenic role in the arthropathy of AS. One proposed

Abbreviations used in this paper: AS, ankylosing spondylitis; CT, computerized tomography.

0016-5085/03/$30.00
doi:10.1053/j.gastro.2003.08.035

mechanism associates the inflammation with increased intestinal permeability to luminal antigens and/or bacteria with an HLA-B27–dependent immune response to elements of fibrocartilage resulting in the arthropathy.[11]

There are other similarities between the 2 diseases that strengthen these suggestions. Patients with AS and CD have a high prevalence of increased intestinal permeability.[14,15] Moreover, the prevalence of increased intestinal permeability in first-degree relatives of patients with AS (10%–60%) and CD (10%–54%) is similar.[11,14,15] In the case of relatives of patients with CD, it is suggested that the intestinal permeability changes are consequent to subclinical intestinal inflammation.[16] The pattern of this subclinical inflammation conforms to an autosomal additive trait, suggesting an inherited underlying genetic susceptibility (a risk factor) that requires an environmental factor for the full phenotype expression of CD.[16] If the intestinal inflammation associated with AS truly represents subclinical CD, it would seem possible that the 2 diseases share this genetic risk factor.

The purpose of this study was to assess the prevalence and possible inheritance pattern of subclinical intestinal inflammation in first-degree relatives of patients with AS. In a subgroup of relatives, we assessed if the presence of intestinal inflammation related to the subjects' HLA-B27 genotype or skeletal changes suggestive of early AS.

Patients and Methods

This study was performed in Iceland, which has a particularly well-characterized homogeneous population of 280,000. Icelanders are mostly of Scandinavian origin and, until recently, have lived in relative isolation for 1100 years; therefore, they are ideally suited for genetic studies.

This was a 2-part study. First, we studied the prevalence and possible mode of inheritance of subclinical intestinal inflammation in first-degree relatives of patients with AS. Second, we studied the possible consequences of this subclinical intestinal inflammation by assessing the prevalence of skeletal abnormalities that are suggestive of early AS by computerized tomography (CT). These findings were also assessed in relation to the subjects' HLA-B27 genotype status.

Altogether, 53 of 54 patients with AS who were approached for this study by mail and telephone participated. This represented all of the patients with AS under the care of the rheumatologists at the Reykjavik University Hospital and just >25% of all patients in Iceland with AS. The diagnosis of AS had been established at least 1 year before this study, and all patients met the diagnostic criteria for definite AS as defined by the modified New York criteria.[17] There was clustering of cases in 9 families with 2 first-degree relatives with AS; in one family, there were 4 first-degree relatives with AS.

Intestinal studies in patients with AS are complicated by the fact that these patients are treated with a number of drugs that cause or modify intestinal inflammation.[18] All of the patients with AS in this study were receiving or had received conventional nonsteroidal anti-inflammatory drugs (NSAIDs) for prolonged periods, which can lead to intestinal inflammation (NSAID enteropathy).[18] At the time of this study, 7 were not receiving any treatment, 41 were taking NSAIDs, and 21 were taking sulfasalazine (which may reduce the intestinal inflammation associated with AS[19] as well as that due to NSAIDs[20]). Furthermore, 4 were taking prednisolone and 3 were taking methotrexate, both of which may reduce intestinal inflammation in patients with IBD.

Table 1. Demographic Details of Patients With AS Studied, Their First-Degree Relatives, and Their Spouses

	Patients with AS	First-degree relatives	Spouses of patients with AS
No.	47	124[a]	17
Sex (M/F)	37/10	51/73	3/14
Age, yr (mean ± SD)	40 ± 12	45 ± 16	38 ± 11

[a]A total of 213 first-degree relatives, which excludes those younger than 16 and older than 80 years of age, were potentially available for study.

The first-degree relatives of the 53 patients who participated were approached for the first part of the study. Six patients had no relatives available for study because the relatives lived abroad or in remote places in Iceland. The remaining 47 patients had 213 first-degree relatives potentially available for study (excluding those who were younger than 16 years and older than 80 years), and 124 (58%) participated. Eighty-nine relatives (42%) did not participate because they had predetermined exclusion criteria, were living abroad, or declined participation because of frailty or various other reasons. The predetermined exclusion criteria for the patients and relatives were age younger than 16 years and older than 80 years; severe neurologic, psychiatric, endocrine (including diabetes mellitus), cardiovascular, pulmonary, hepatic, or renal diseases; malignancy; and pregnancy. Relatives with established gastrointestinal disease were excluded, as were those misusing alcohol (n = 14) and those taking NSAIDs (n = 8).[14,21] Low-dose aspirin (≤300 mg/day) was not an exclusion criteria (n = 5) because, unlike conventional NSAIDs, aspirin does not cause small bowel inflammation.[11,14,21]

Seventeen spouses also underwent studies for intestinal inflammation; if they were affected similar to the patients or first-degree relatives, this would suggest that environmental rather than genetic factors might be responsible for the inflammatory changes. The couples had been living together for 4–33 years (mean, 8 years). The spouses were subject to the same exclusion criteria as the first-degree relatives and were not taking aspirin or NSAIDs.

Table 1 shows the demographic details of the patients, first-degree relatives, and spouses studied. Of the 124 first-degree relatives studied, 100 were randomly invited to participate in the CT study. Fifteen declined (mostly those younger

than 25 years and concerned about the radiation); therefore, 85 (32 men [38%] and 53 women [62%]) underwent CT of the sacroiliac joints to assess the possible consequence of the subclinical intestinal inflammation. All but 4 of these underwent HLA-B27 genotyping.

Participants attended an investigational unit at the Icelandic National University Hospital. A medical and drug history was taken, all were questioned on back symptoms, and physical examination was performed with emphasis on spinal mobility (Schober test), chest expansion, and signs of enthesitis. Blood samples were taken for HLA-B27 genotyping, and subjects were provided with a container to collect a stool sample.

All subjects provided written informed consent. The studies were approved by the National University Hospital ethical committee, the Radiation Protection Institute, and the Data Protection Authority of Iceland. All subjects were aware of the relatively high dose of radiation (2.65 millisieverts) received during the CT scan.

Intestinal Inflammation

Intestinal inflammation was assessed by measurement of calprotectin in feces. Calprotectin is a neutrophil-selective protein that is also present in small quantities in other polymorphonuclear white cells. Its presence in feces relates quantitatively to the neutrophil flux to the gastrointestinal tract, that is, it is proportional to the degree of acute inflammation.[21–23]

Subjects provided a stool sample within 3 days of visiting the investigational unit. The samples were usually received within 8 hours of passing the stool, and 20-g portions were frozen and stored at −20°C. Calprotectin is resistant to bacterial degradation and is stable in feces at room temperature for at least 1 week.[22] After thawing, 5-g aliquots were processed for quantitative measurements by a sensitive and specific enzyme-linked immunosorbent assay as previously described.[21] The within-assay coefficient of variation was 1.2%, and the between-assay variation was 15%.

The normal range of fecal calprotectin excretion and concentration was established in 163 healthy Icelandic volunteers during these studies, mostly from health care professionals and their immediate families (88 men and 75 women; mean age, 46 ± 8 years; range, 19–72 years). None of the controls had a first-degree relative with IBD or a chronic arthritic condition (excluding osteoarthritis).

HLA-B27 Genotyping

Genomic DNA was isolated from whole blood according to standard protocols, and HLA-B27 status was determined by polymerase chain reaction as previously described.[24]

Assessment of Inheritance

Variance component analysis was performed[25] to identify possible factors controlling the calprotectin concentrations in first-degree relatives of patients with AS. This modeling allows for a single major gene affecting the trait as well as the trait being affected by environmental influences or an additive polygenic component. The polygenic and environmental influences are not distinguishable in the type of analysis, and their effect will hereafter be referred to as polygenic. The variance of trait measurement is partitioned into an additive variance (s_a), a dominance variance (s_d), a polygenic variance (s_p), and a residual variance (s). The variance of a measurement is the sum of these components, and the covariance between 2 individuals is $k\,s_a + z\,s_d + p\,s_p$, where k is the kinship coefficient of the 2 (the expected proportion of alleles they share identically by descent), p is the number of alleles shared identically by descent at the major gene, and z is 1 if 2 alleles are shared identically by descent at the major gene and 0 otherwise. These models/hypotheses will be considered.

H_0 $s_a = s_d = s_p = 0$ No genetic factor

H_p $s_a = s_d = 0, s_p \geq 0$

No major gene but a polygenic factor

H_a $s_d = 0, s_a \geq 0, s_p \geq 0$

A major additive gene and a polygenic factor

H_d $s_a = 0, s_d \geq 0, s_p \geq 0$

A major dominance gene and a polygenic factor

H_{ad} $s_a \geq 0, s_d \geq 0, s_p \geq 0$

A major additive and dominance gene and a polygenic factor

Three classes of tests were performed. To establish that there is a familial effect, H_0 was tested against all the other hypotheses. To establish that there is a major gene affecting the trait, H_p was tested against H_a, H_d, and H_{ad}. Finally, to compare how the major locus dominance and additive components fit the data, H_a and H_d were tested against H_{ad}. Testing was performed using a likelihood ratio test, assuming multivariate normality. Note that the estimate of polygenic variance component under H_p is an estimate of the heritability of log-calprotectin levels.

CT Imaging

The sacroiliac joints were examined with CT (CT Lightspeed; General Electric, Milwaukee, WI), and 3.75-mm contiguous scans were taken over the sacroiliac joint with the subjects in a supine position and the gantry angled craniocaudally parallel to the sacrum and the sacroiliac joints. The estimated effective radiation dose equivalent received during this procedure was 2.65 millisieverts. All images were read independently by 2 radiologists with no knowledge of the clinical findings. Numeric data were generated as the mean from the 2 observers. Overall, there was excellent agreement between the 2 observers, with only a single interobserver variation that was resolved by discussion.

A widely accepted scoring system for the evaluation of sacroiliitis with CT is not available. For the purpose of this study, a system was devised based on a conventional radiography scoring system that describes 6 stages of arthritis from grade 0 (normal) to grade 5 (bony ankylosis in the sacroiliac joints).[26] The CT score in the relatives fell within grade 0 (normal) and 1 (suspicious for early AS changes), and none had grade 2 (definite AS changes) or higher. Grade 1 included the presence, number, and size of subchondral cysts/erosions and blurring of joint margins. The predetermined grade 1 criteria also included subchondral sclerosis (sacral/iliac), osteitis condensans ilii, joint space narrowing (<2 mm wide), and air streak in the sacroiliac joint, but these were so infrequently encountered that reliable statistical analysis between groups was not possible.

Statistics

A Shapiro–Wilks W prime test showed that the fecal calprotectin data from the patients with AS and their relatives were not all normally distributed. Values for these are therefore presented as median and ranges, with the upper limit of normal for measurement of fecal calprotectin concentrations defined as below the 95th percentile of the median. We used the Welch modified 2-sample t test to test the difference in means of the relatives of patients and of controls, assuming the variance in the relatives and the controls was unknown and not necessarily the same in the 2 groups.

Radiologic data are presented as confidence intervals calculated by the t test and, where appropriate, the Mann–Whitney test and Fisher exact test. Kendall's τ was used for correlations.

Results

Intestinal Inflammation

The median fecal calprotectin value from controls was 3 mg/L (range, 0.1–15.5 mg/L; upper 95% confidence limits, 10.8 mg/L). Figure 1 shows the fecal calprotectin concentrations from the study groups. Significantly increased fecal calprotectin concentrations ($P < 0.0001$) were observed in patients with AS (median, 21.5 mg/L; range, 2–600 mg/L) and their first-degree relatives (median, 8.6 mg/L; range, 0.7–305 mg/L). Fecal calprotectin concentrations in the spouses of patients with AS did not differ significantly from controls (median, 4 mg/L; range, 0.1–21.5 mg/L), with 15 of 17 having values within the reference range. By comparison, 78% and 41% of the patients with AS and their first-degree relatives, respectively, had fecal calprotectin concentrations above the reference range.

HLA-B27 Genotype

Of the 47 patients with AS who underwent the fecal calprotectin studies, 42 (89%) were HLA-B27 positive. Of the 93 first-degree relatives, 53 (57%) and 40 (43%) were HLA-B27 positive and negative, respectively. The median fecal calprotectin concentrations did not differ significantly ($P > 0.6$) between the HLA-B27–positive (median, 11.9 mg/L) and HLA-B27–negative (median, 8.1 mg/L) relatives.

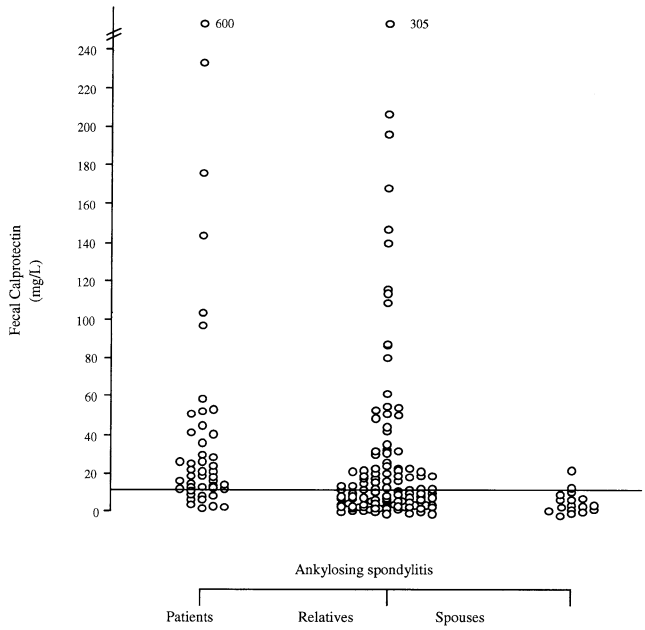

Figure 1. Fecal calprotectin concentrations in patients with AS, first-degree relatives, and spouses. The *horizontal line* indicates the upper normal limit of fecal calprotectin concentrations. A total of 78% of patients with AS and 41% of their first-degree relatives have increased calprotectin concentrations, representing intestinal inflammation. Two spouses had evidence of low-grade intestinal inflammation.

Assessment of Inheritance

We used the log-transformed calprotectin measurements in the analyses, because these transformed values are less skewed than the original values. Table 2 shows the results of variance component analyses for the first-degree relatives of patients with AS. The observed heritability is 57% and is significantly different from 0 ($P = 2 \times 10^{-15}$ when testing H_p against H_0). There is strong evidence for the presence of a major additive gene influencing calprotectin levels, and its additive variance component is estimated as 73% ($P = 0.002$ when testing H_a against H_p). Interestingly, in the presence of the additive variance component, the polygenic component is estimated as 0, showing the strength of the effect of the estimated gene effect. There is no evidence for a dominance component; adding a dominance component does not fit the data better than having only a polygenic component ($P = 0.4$ when testing H_d against H_p). Also, adding a dominance component to a model with a polygenic and an additive component does not fit the data better ($P = 1$ when testing H_{ad} against H_a).

Table 2. Results of Variance Components Analysis

Model	*df*	Additive component (%)	Dominance component (%)	Polygenic component (%)	*P* vs. H_0	*P* vs. H_p	*P* vs. H_{ad}
H_p	1			57	2×10^{-15}		
H_a	2	73		0	2×10^{-16}	0.002	1
H_d	2		42	55	8×10^{-15}	0.4	0.003
H_{ad}	3	73	0	0	6×10^{-16}	0.007	

NOTE. Estimates of variance components are reported as percentages of the total variance. *P* values are for testing the given model against H_0 and against H_p and for testing H_{ad} against the given model. The *df* of each model is given and in the first set of tests is compared with the 0 *df* of H_0.

Figure 2 shows the pedigrees (families in which 50% or more eligible relatives were studied) where relatives are identified as having increased or normal calprotectin concentrations.

CT Imaging

CT of the sacroiliac joints was grade 0 in 50 (59%) of the first-degree relatives of the patients with AS, whereas 35 (41%) had grade 1 changes; the most consistent changes were the presence of subchondral cysts (n = 33) and blurring of joint margins (n = 10).

Table 3 shows the overall results that are presented separately according to the subjects' HLA-B27 genotype and the presence of subclinical intestinal inflammation. There was no significant difference ($P > 0.6$) in the prevalence or type of sacroiliac CT changes between the HLA-B27–positive and HLA-B27–negative subjects.

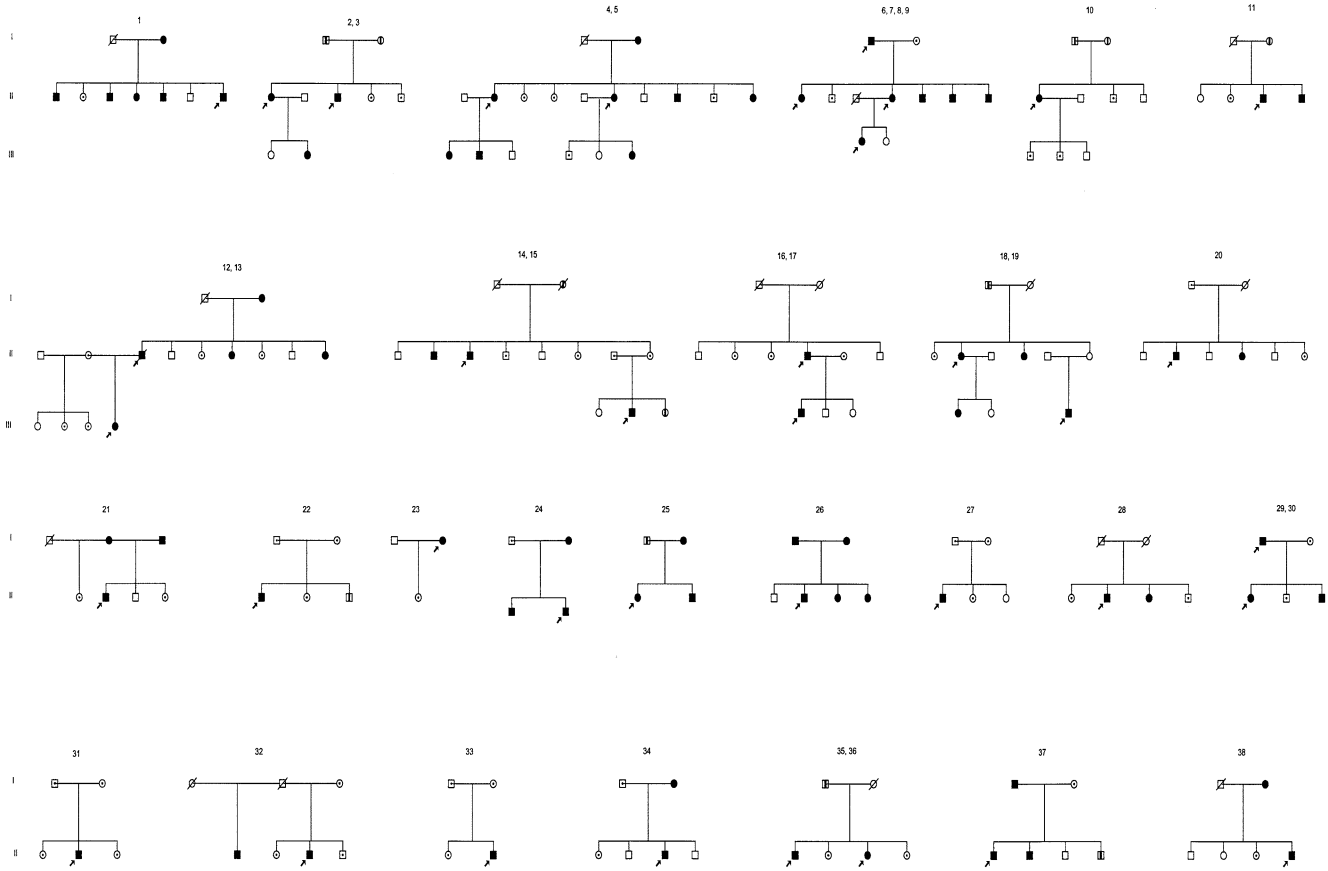

Figure 2. The pedigrees of 38 patients with AS. A *box* represents a male, and a *circle* represents a female. *Blank boxes* and *circles* indicate that the subject was not studied. *Shaded boxes* and *circles* indicate subjects with increased fecal calprotectin concentrations. Those with a *dot* indicate subjects with normal concentrations. *Arrows* identify index cases of AS. Deceased subjects have an *oblique line* through the box or circle, and a *vertical line* indicates that the subject was outside the age limits for the study. Analyses of the inheritance pattern (text) of the subclinical intestinal inflammation among the first-degree relatives of the patients with AS show a pattern consistent with an additive trait.

Table 3. Sacroiliac CT Changes, HLA-B27 Genotype, and Fecal Calprotectin

	HLA-B27 positive (n = 45)	HLA-B27 negative (n = 36)	Increased fecal calprotectin concentration (n = 44)	Normal fecal calprotectin concentration (n = 41)
Subjects with subchondral cysts (%)	19/45 (42)	13/36 (36)	21/44 (48)	12/41 (29)
No. of subchondral cysts (average no. of cysts/relatives)	45 (1.0)	39 (1.1)	59 (1.3)	29 (0.7)
Mean total cystic diameter (*mm*) of subchondral cysts (95% confidence interval)	2.0 (1.1–2.5)	2.1 (1.3–3.0)	2.9 (1.7–4.2)	1.2[a] (0.4–2.0)
Blurring of joint margins (%)	5/45 (11)	5/36 (14)	9/44 (20)	1/41 (2)[b]

NOTE. Of the 10 subjects with blurring of joint margins, 7 were men and 3 were women. Five each were HLA-B27 positive and HLA-B27 negative.
[a]Differs significantly ($P = 0.026$) from relatives with increased fecal calprotectin concentrations.
[b]Differs significantly ($P = 0.02$) from relatives with increased fecal calprotectin concentrations.

The total number and average number of subchondral cysts per subject did not differ significantly ($P = 0.076$ and $P = 0.058$, respectively) between first-degree relatives with and without subclinical intestinal inflammation. However, those with subclinical intestinal inflammation had significantly larger cysts ($P = 0.026$). Nine of the 10 subjects ($P = 0.02$) with blurring of the joint margins had evidence of subclinical intestinal inflammation. All subjects with blurring of joint margins had subchondral cysts apart from one with subclinical intestinal inflammation and the one subject without the inflammation. All of these CT changes were independent of age and sex.

Fecal calprotectin concentrations in the first-degree relatives who had CT changes (median, 12.2 mg/L) did not differ significantly ($P = 0.87$) from those without (median, 8.0 mg/L) abnormality. A further analysis was performed to assess if fecal calprotectin concentrations in subjects with CT changes differed according to whether they were HLA-B27 positive (18.6 mg/L; range, 0.80–86.2 mg/L) or HLA-B27 negative (9.3 mg/L; range, 1.8–50.1 mg/L), but this did not differ significantly ($P = 0.11$).

Symptoms and signs of spinal disease (back pain, spinal mobility, enthesitis, morning stiffness) did not differ significantly ($P > 0.4$) between the 2 groups (HLA-B27 positive vs. HLA-B27 negative; normal vs. increased fecal calprotectin concentrations).

Discussion

Our results show a high prevalence of subclinical intestinal inflammation among first-degree relatives of patients with AS. Of the models tested, the pattern of inheritance is most consistent with an additive model, suggesting that there is a genetic susceptibility to the development of the subclinical intestinal inflammation. Furthermore, the association of the subclinical intestinal inflammation with some of the CT abnormalities suggests that the intestinal inflammation may play a pathogenic role in the sacroiliac bone changes.

The intestinal manifestations of CD and AS share certain features, which suggests that they share a common etiology and pathogenesis.[7–9,11] In the first-degree relatives of patients with CD, a persuasive case can be made for the idea that the underlying cause of the subclinical intestinal inflammation (found in 50%) is genetically determined,[16] perhaps involving defective neutrophil function, and that the increased intestinal permeability[14] is consequent to this inflammation.[16] In the current study, we show that patients with AS have significantly increased fecal concentrations of calprotectin comparable to patients with CD.[16,23] However, the interpretation of this finding is hampered by the fact that all of the patients with AS were receiving or had received NSAIDs, which can cause an enteropathy.[14] However, a similar proportion of first-degree relatives of patients with AS (who had not received NSAIDs) had subclinical intestinal inflammation, as in relatives of patients with CD and of comparable severity.[16] Furthermore, the pattern of inheritance among both groups of relatives is consistent with the presence of a major additive gene when assessed with established and accepted techniques that are commonly applied to segregation analysis of quantitative traits.[27] Fifteen of 17 spouses of patients with AS had normal calprotectin concentrations, which further suggests but does not exclude that genetic rather than external factors are of pivotal importance in the development of this inflammation. Although the precise localization and nature of the subclinical intestinal inflammation in the first-degree relatives of patients with AS is uncertain, it did not relate to their HLA-B27 genotype. Collectively, these findings suggest that first-degree relatives of patients with CD and AS share a common genetic risk factor for the development of subclinical intestinal inflammation. The full phenotype expression of CD might only be brought about by the

cumulative effects of additional genetic and/or possibly environmental interactions under these circumstances.[28]

The clinical course and radiographic appearances of the spondylarthropathy associated with IBD and idiopathic AS are often indistinguishable.[2,29] The high prevalence of intestinal inflammation in patients with AS and certain other spondyloarthropathies has suggested that it plays a pathogen role in the arthropathy.[9,11,19] This intestinal-spondylarthropathy interaction hypothesis is strengthened experimentally by the finding that transgenic rats, which strongly express the human HLA-B27 and β_2-microglobulin genes, develop colonic inflammation and arthritis.[11,30] The activity of the colitis and arthritis in these rats is dependent on the presence of certain intestinal bacteria. This intestinal bacterial-host interaction is of interest because a similar mechanism is implicated in the pathogenesis of CD[28] and modification of the enteric bacterial flora is currently being exploited for therapeutic purposes in CD and various other diseases.[31]

In the present study, the intestinal-spondyloarthropathy interaction was studied by assessing the presence of sacroiliac bone changes in the first-degree relatives and correlating the findings with the results of the calprotectin test. The CT studies are complicated by at least 2 related factors. First, there are no large population studies from Iceland or elsewhere that define "normality" or "nonpathologic" age-related changes. Second, although the CT diagnostic criteria for clinically evident AS (bony ankylosis of the sacroiliac joints) are straightforward and widely recognized, there is no consensus as to the most sensitive CT criteria for the early evolving bony lesions of AS. In an attempt to overcome these problems, we extrapolated an accepted radiologic scoring system for the early changes suggestive of AS over to the CT method. The CT scans would be expected to be somewhat more sensitive than radiology and thus explain our higher prevalence of skeletal abnormalities in the first-degree relatives of patients with AS than previous (radiographic) studies.[32,33] The strength of the scoring system is that it does not include common age-related changes such as that associated with osteoarthritis and osteoporosis. The main drawback is that subchondral cysts/erosions and blurring of joint margins are not pathognomic for early AS. Nevertheless, there was excellent agreement between the radiologists in their interpretation of the CT findings, neither of who had any knowledge of the clinical or laboratory results. For the whole group of relatives, 41% had grade 1 changes without any significant association/correlation with HLA-B27 status, sex, or age. This lack of association is in keeping with there being a significant genetic susceptibility to the severity of AS that is largely conferred by complex genes other than HLA-B27.[34,35] However, there was a statistically nonsignificant tendency for an increased prevalence and number of subchondral cysts in relatives with as opposed to without subclinical intestinal inflammation. Furthermore, the mean cyst diameter was significantly greater (the larger the cysts, the more likely they are believed to represent a pathologic process) than in those with normal calprotectin concentrations, and there was a significant clustering of cases with abnormal joint margins in the high calprotectin group, most of whom also had cysts. These findings, although not specific for early AS, add some further weight to an intestinal-spondylarthropathy interaction. The reason why not all of the relatives with subclinical intestinal inflammation develop evidence of sacroiliac bone changes or why those that develop it do not progress to the full AS phenotype may again be that such progression requires additional interacting risk factors (genetic or environmental).

The complex inheritance pattern of AS is in common with many chronic diseases. AS falls best within the "complex disease trait" category of inheritance in which various environmental and additive genetic combinations (risk factors) need to be present, with the HLA-B27 genotype particularly important, before a "threshold" is reached that leads to the full phenotype of AS. The results from this study lead us to 2 main conclusions. First, the subclinical intestinal inflammation that we have identified in the first-degree relatives of patients with AS may represent the consequence of inherited genetic defects. This inflammation (frequency and inheritance pattern) closely resembles that found in first-degree relatives of patients with CD[16] and provides further evidence that the 2 diseases share common pathogenic factors. Second, this subclinical intestinal inflammation may play a pathogenic role in the sacroiliac changes on CT that have certain features that might represent the early stages of AS.

References

1. Bywaters EGL. Historical aspects of the etiology of rheumatoid arthritis. Br J Rheumatol 1988;27(suppl II):110–115.
2. Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. Gut 1998;42:387–391.
3. Wright V, Watkinson G. Articular complications of ulcerative colitis. Am J Proctol 1966;17:107–115.
4. de Vlam K, Mielants H, Cuvelier C, De Keyser F, Veys EM, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease: prevalence and HLA association. J Rheumatol 2000;27:2860–2865.
5. Russell AS, Percy JS, Schlaut JS, Sartor VE, Goodhart JM, Sher-

baniuk RW, Kidd EG. Transplantation antigens in patients with Crohn's disease: the linkage of associated ankylosing spondylitis with HLA-W27. Am J Dig Dis 1995;20:359–361.
6. Leirisalo-Repo H, Repo H. Gut and spondyloarthropathies. Rheumatol Dis Clin North Am 1992;18:23–35.
7. Mielants H, Veys EM, Cuvelier C, De Vos M, Botelberghe L. HLA B27 related arthritis and bowel inflammation: Part 2. Ileocolonoscopy and bowel histology in patients with HLA B27 related arthritis. J Rheumatol 1985;12:294–298.
8. Mielants H, Veys EM, De Vos MM, Cuvelier C. Gut inflammation in the pathogenesis of idiopathic forms of reactive arthritis and in the peripheral joint involvement of ankylosing spondylitis. In: Mielants H, Veys EM, ed. Spondylarthropathies. Involvement of the gut. Amsterdam: Excerpta Medica, 1987:11–21.
9. Mielants H, Veys EM, Cuvalier C, De Vos M. Ileocolonoscopic findings in seronegative spondyloarthropathies. Br J Rheumatol 1988;27(suppl II):95–105.
10. Leirisalo-Repo H, Turunen U, Stenman S, Helenius P, Seppala K. High frequency of silent inflammatory bowel disease in spondylarthropathy. Arthritis Rheum 1994;37:23–31.
11. Smale S, Natt RS, Orchard TR, Russell AS, Bjarnason I. Inflammatory disease and spondylarthropathy. Arthritis Rheum 2001; 44:2728–2736.
12. De Vos M, Cuvelier C, Mielants H, Veys E, Barbier F, Elewaut A. Ileocolonoscopy in seronegative spondylarthropathy. Gastroenterology 1989;96:339–344.
13. Mielants H, Veys EM, Cuvelier C, De Vos M, Goemaere S, De Clercq L, Schatteman L, Gyselbrecht L, Elewaut D. The evolution of spondyloarthropathies in relation to gut histology: II. Histological aspects. J Rheumatol 1995;22:2273–2278.
14. Bjarnason I, Macpherson AJM, Hollander D. Intestinal permeability: an overview. Gastroenterology 1995;108:1566–1581.
15. Martinez-Gonzales O, Cantero-Hinojosa J, Paule-Sastre P, Gomez-Magan JC, Salvatierra-Rios D. Intestinal permeability in patients with ankylosing spondylitis and their healthy relatives. Br J Rheumatol 1994;33:644–647.
16. Thjodleifsson B, Sigthorsson G, Cariglia N, Reynisdottir I, Gudbjartsson DF, Kristjansson K, Meddings JB, Gudnason V, Wandall JH, Andersen LP, Sherwood R, Kjeld M, Oddsson E, Gudjonsson H, Bjarnason I. Subclinical intestinal inflammation: an inherited abnormality in Crohn's disease relatives? Gastroenterology 2003;124:1728–1737.
17. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for the modification of the New York criteria. Arthritis Rheum 1984;27:361–368.
18. Bjarnason I, Hayllar J, Macpherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine. Gastroenterology 1993;104:1832–1847.
19. De Vos M, Mielants H, Cuvelier C. Ileitis in the spondylarthropathies. In: Allan RN, Rhodes JM, Hanauer SB, Keighley MRB, Alexander-Williams J, Fazio VW, eds. Inflammatory bowel disease. New York: Churchill Livingstone, 1997:451–460.
20. Hayllar J, Price AB, Smith T, Macpherson A, Gumpel MJ, Bjarnason I. Nonsteroidal antiinflammatory drug-induced small intestinal inflammation and blood loss: effect of sulphasalazine and other disease modifying drugs. Arthritis Rheum 1994;37:1146–1150.
21. Tibble J, Sigthorsson G, Foster R, Scott D, Roseth A, Bjarnason I. Faecal calprotectin: a simple method for the diagnosis of NSAID-induced enteropathy. Gut 1999;45:362–366.
22. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating calprotectin in feces: a methodologic study. Scand J Gastroenterol 1992;27:793–798.
23. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M, Bjarnason I. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;47:506–513.
24. Calvert GT, Shore EM. Human leukocyte antigen B27 allele is not correlated with fibrodysplasia ossificans progressiva. Clin Orthop 1998;346:66–70.
25. Abney M, McPeek MS, Ober C. Estimation of variance components of quantitative traits in inbred populations. Am J Hum Genet 2000;66:629–650.
26. Dale K. Radiographic grading of sacroiliitis in Bechterew's syndrome and allied disorders. Scand J Rheumatol 1980;9(suppl 32):92–97.
27. Cardon LR, Garner C, Bennett ST, Mackay IJ, Edwards RM, Cornish J, Hegde M, Murray MAF, Reid IR, Cundy T. Evidence for a major gene for bone mineral density in idiopathic osteoporotic families. J Bone Miner Res 2000;15:1132–1137.
28. Shanahan F. Crohn's disease. Lancet 2002;359:62–69.
29. Orchard TR, Thiygaraja S, Welsh KI, Wordsworth BP, Gaston JHS, Jewell DP. Clinical phenotype is related to HLA genotype in the peripheral arthropathies in inflammatory bowel disease. Gastroenterology 2000;118:274–278.
30. Rath HC, Ikeda JS, Linde HJ, Schomerich J, Wilson KH, Sartor RB. Varying cecal bacterial loads influences colitis and gastritis in HLA-B27 transgenic rats. Gastroenterology 1999;116:310–319.
31. Farrell RJ, LaMont JT. Microbial factors in inflammatory bowel disease. Gastroenterol Clin North Am 2002;31:41–62.
32. Contu L, Capelli P, Sale S. HLA B27 and ankylosing spondylitis: a population and family study in Sardinia. J Rheumatol 1977; 3(suppl):18–23.
33. Konieczna B, Stawarz M, Mackiewicz S. Occurrence of HLA antigens in patients with ankylosing spondylitis and in their families. Arch Immunol Ther Exp (Warsz) 1983;31:153–160.
34. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, Taylor A, Calin A, Wordsworth P. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–1828.
35. Hamersma J, Cardon LR, Bradbury L, Brophy S, van der Horst-Bruinsma I, Calin A, Brown MA. Is disease severity in ankylosing spondylitis genetically determined? Arthritis Rheum 2001;44: 1396–1400.

Received April 19, 2003. Accepted August 21, 2003.

Address requests for reprints to: Ingvar Bjarnason, M.D., M.Sc., F.R.C.Path., F.R.C.P.(Glasg), D.Sc., Guy's, King's, St. Thomas' Medical School, Bessemer Road, London SE5 9PJ, England. e-mail: ingvar.bjarnason@kcl.ac.uk; fax: (44) 20-7346-6474.

Supported in part by a Biomedical Research Collaboration grant from the Wellcome Trust (ref. 048461) and the Arthritis Research Campaign, United Kingdom (ref. B0627).