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## SHORT REPORT

## Relationship between the gut and the spine: a pilot study of first-degree relatives of patients with ankylosing spondylitis

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**INTRODUCTION**

Patients with ankylosing spondylitis (AS) have a high frequency of asymptomatic acute and chronic gut inflammation resembling inflammatory bowel disease (IBD), with as many as 60% displaying evidence of microscopic gut inflammation in the absence of gastrointestinal (GI) symptoms.<sup>1</sup> Further, there is considerable overlap in susceptibility loci between AS and IBD but this pleiotropic phenomenon likely only explains part of the clinical co-occurrence.<sup>2,3</sup> Animal model data investigating molecular mechanisms point to a critical relationship between the gut and AS and the potential role of the gut in the pathogenesis of AS.<sup>4,5</sup> Several markers are utilised in clinical practice to determine the likelihood that a patient has IBD, the most common of which is the faecal calprotectin which has an estimated sensitivity and specificity of upwards of 80% for identification of patients with IBD and thus serves as a very useful marker of gut inflammation.<sup>6</sup> Similarly, various IBD-related antibodies including anti-*Saccharomyces cerevisiae* antibodies (ASCA), antineutrophil cytoplasmic antibodies (ANCA), anti-I2 (associated with anti-*Pseudomonas* activity), anti-*Escherichia coli* outer membrane porin C (anti-OmpC) and anti-flagellin antibodies (anti-CBir1) have been shown to have very good utility in distinguishing individuals with IBD from healthy controls, particularly when these tests are used in combination and thus serve as useful serological biomarkers for IBD.<sup>7</sup>

Using the above biomarkers along with advanced imaging studies, this pilot study investigated if first-degree relatives (FDRs) of patients with AS, who themselves do not carry a diagnosis of AS or IBD, have evidence of mucosal dysregulation, subclinical gut

or sacroiliac joint inflammation that could potentially precede the development of overt IBD or AS. We postulate that this group will provide insights into the relationship between the gut and AS.

**METHODS**

The investigators collaborate in a large AS and IBD consultation and research centre where patients with AS and IBD are seen regularly for clinical care and participate in longitudinal outcome studies. For purposes of this pilot study, we approached a convenience sample of patients with AS (all fulfilling New York criteria for the diagnosis of AS, including X-ray evidence of definite sacroiliitis) from our voluntary research registry and those seen for clinical care at our institution who had an FDR without a known clinical diagnosis of either AS or IBD. Thirty-two healthy FDRs between the ages of 18 and 57 years were subsequently recruited for this study.

FDRs were excluded if known or previously diagnosed AS, IBD, inflammatory arthritis or other concomitant rheumatic disease was present. The study protocol was approved by the Institutional Review Board (IRB) at Cedars-Sinai Medical Center.

Blood (20 cc) was collected from each FDR and tested by ELISA for the presence of IBD-related antibodies: ASCA, ANCA, anti-I2, anti-OmpC and anti-CBir1 as previously described.<sup>8-11</sup> A stool sample (10 cc) was also collected to measure faecal calprotectin (PhiCal Calprotectin ELISA, Quest Diagnostics) and a stool aliquot was saved for subsequent investigations of the microbiome.<sup>12,13</sup>

**Table 1** Demographic and clinical characteristics of the 32 FDR subjects

Demographic and clinical characteristics	Number of patients (%)
Age 18–29 years	8
Age 30–50 years	22
Age >50 years	2
Female	16 (50%)
Relationship to proband=sibling	25
Relationship to proband=offspring	4
Relationship to proband=both sibling and offspring	3
HLA-B27 positive	13

FDR, first-degree relative; HLA-B27, human leucocyte antigen B27.

The FDRs received questionnaires examining the presence of AS or other spondyloarthritis (SpA) phenotypes. Clinical data about AS symptoms and signs were obtained using the Toronto Axial Spondyloarthritis Questionnaire in IBD<sup>14</sup> and all subjects were queried about the presence of any GI symptoms. Additionally, all participants were asked if they experienced back pain for greater than 6 weeks. If they answered yes, they were subsequently questioned about their non-steroidal anti-inflammatory drug use.

A musculoskeletal pelvic MRI was obtained on all subjects using Short Tau Inversion Recovery and T1 sequences to examine for the presence of axial SpA. These images were independently read and interpreted by a trained and certified musculoskeletal radiologist (TJL).

### Statistical analyses

Two-sided *p* values comparing faecal calprotectin levels between two groups were calculated using the Wilcoxon rank-sum test. The faecal calprotectin levels were evaluated in subjects with sacroiliitis compared with those subjects without sacroiliitis. The calprotectin levels were also evaluated in serology positive as compared with serology negative subjects. *p* Values less than 0.05 were considered statistically significant.

## RESULTS

### Demographic characteristics

Thirty-two FDRs without a known diagnosis of AS or IBD were recruited; demographic characteristics of FDRs are shown in [table 1](#). Subjects ranged from 18 to 57 years in age with equal numbers of males and females. Twenty-five of the subjects were siblings of patients diagnosed with AS, four were children of affected patients and three had both an affected sibling and an affected parent. Of the 32 recruited FDRs, 13 subjects (41%) were human leucocyte antigen (HLA)-B27+. Three FDRs had both an affected sibling and parent, only one of which (subject

12) was HLA-B27+, and this subject also had an MRI indicating mild active sacroiliitis. All of the documented probands of these three FDR subjects were HLA-B27+. Seven probands were HLA-B27– as were their FDRs. Of the 25 HLA-B27+ probands, 13 FDRs were negative for HLA-B27.

### Clinical characteristics

In our FDRs, one subject had a history of eye inflammation and one had a history of psoriasis. None of the FDRs had GI symptoms suggesting GI inflammation, although one reported a remote history of gastric ulceration. Nine subjects reported low back pain of any kind for at least 6 weeks in a row.

The GI and rheumatological manifestations of the FDRs are shown in [table 2](#). Seven (~22%) of the 32 FDRs were positive for at least one IBD-associated serological marker. Six were anti-CBir1 positive and two had a positive ASCA antibody. None of the FDRs were positive for ANCA, anti-I2 or anti-OmpC. Of the seven found to have a positive IBD antibody, two were HLA-B27+ (29%) compared with 11 of the 25 (44%) who were antibody negative. None of the FDRs with a positive IBD antibody had a history of inflammatory back pain. One of the seven patients with a positive IBD antibody had an abnormal MRI with evidence of sacroiliitis ([table 2](#), Subject 27). None of the seven patients with a positive IBD antibody had existing GI symptoms.

Five FDRs out of the total cohort had MRI evidence of sacroiliitis, as read and interpreted by a musculoskeletal radiologist ([table 2](#)). One FDR (Subject #2) had minimal bone marrow oedema at the right sacroiliac joint, which was not present on consecutive slices and should not be considered positive. Of the five FDRs with MRI evidence of sacroiliitis, three were HLA-B27+. One of the patients with a positive IBD antibody also had evidence of bilateral sacroiliitis. Three of the nine FDRs who endorsed back pain had evidence of sacroiliitis by MRI; six did not have imaging evidence of sacroiliitis.

The faecal calprotectin value was numerically, but not statistically, higher in subjects with MRI findings of sacroiliitis (74.65 mcg/g) as compared with those subjects who did not have MRI evidence of sacroiliitis (40.85 mcg/g) (*p*=0.18). The difference between the faecal calprotectin values in subjects with IBD antibodies (54.64 mcg/g) as compared with those without IBD antibodies (42.69 mcg/g) was numerically higher but not statistically significant (*p*=0.63). The mean calprotectin value in patients without IBD antibodies and without MRI findings of sacroiliitis was 37.06 mcg/g ([table 3](#)).

## DISCUSSION

Our pilot study revealed that a substantial proportion of otherwise healthy FDRs of patients with AS displayed a positive IBD antibody profile, specifically with respect to anti-CBir1 and without clinical evidence of IBD, findings which are suggestive of mucosal dysregulation. This

**Table 2** Evidence of subclinical gut inflammation, mucosal dysregulation or MRI evidence indicative of sacroiliitis in FDRs

FDR	ASCA IgA	ASCA IgG	CBir1	fCal (mcg/g)	MRI findings	HLA-B27 status
Subject 2	Negative	Negative	Negative	109.4 (ULN)	Minimal bone marrow oedema of right sacroiliac joint present solely on a single slice; not considered positive	Positive
Subject 4	Negative	Positive	Negative	<15.6 (Negative)	Not present	Negative
Subject 6	Negative	Negative	Negative	<15.6 (Negative)	Bilateral sacroiliitis with inflammatory bone marrow oedema	Negative
Subject 8	Positive	Positive	Positive	157.3 (ULN)	Not present	Negative
Subject 11	Negative	Negative	Positive	<15.6 (Negative)	Not present	Positive
Subject 12	Negative	Negative	Negative	76.6 (Negative)	Mild active right sacroiliitis	Positive
Subject 15	Negative	Negative	Positive	<15.6 (Negative)	Not present	Negative
Subject 16	Negative	Negative	Negative	No sample available	Small area of sclerosis of left sacroiliac joint	Negative
Subject 18	Negative	Negative	Positive	81 (Negative)	Not present	Negative
Subject 20	Negative	Negative	Positive	28 (Negative)	Not present	Negative
Subject 27	Negative	Negative	Positive	69.4 (Negative)	Active sacroiliitis left inferior sacroiliac joint, subtle changes right sacroiliac joint suggest chronic changes related to sacroiliitis but no active inflammation	Positive
Subject 28	Negative	Negative	Negative	137 (ULN)	Advanced bilateral sacroiliitis	Positive

ASCA, anti-*Saccharomyces cerevisiae* antibodies; CBir1, anti-flagellin antibodies; fCal, faecal calprotectin; FDR, first-degree relative; HLA-B27, human leucocyte antigen B27; Ig, immunoglobulin; ULN, upper limit of normal.

is meaningful as the specificity of the majority of IBD antibodies is upwards of 80%.<sup>15</sup> Our data is in agreement with previously published data with regard to the presence of mucosal dysregulation in patients with established AS.<sup>9 16 17</sup> Approximately, 6.5% of patients with AS will develop overt clinical IBD during their lifetime and as many as 60% have evidence of microscopic gut inflammation without GI symptoms.<sup>1</sup> A recent study found that a substantial proportion of patients with AS had evidence of clinical IBD, although analysis of the temporal pattern was not consistent with a causal relationship.<sup>18</sup>

**Table 3** Mean faecal calprotectin values in FDRs according to IBD antibody and radiographic status

FDR status	Mean fCal (mcg/g)	p Value
Positive for sacroiliitis (SI) findings in MRI	74.65	0.18
Negative for sacroiliitis (SI) findings in MRI	40.85	
Positive for IBD antibodies	54.64	0.63
Negative for IBD antibodies	42.69	
Negative for SI and IBD antibodies	37.06	

fCal, faecal calprotectin; FDR, first-degree relative; IBD, inflammatory bowel disease.

A variety of antibodies directed against commensal flora are clinically relevant and characteristic of subgroups of IBD.<sup>19</sup> These include ASCA, ANCA, anti-I2, anti-OmpC and anti-CBir1 antibodies.<sup>20</sup> In prior studies from our group examining biomarkers of intestinal inflammation in AS subjects without clinical evidence of IBD, median anti-I2 response was greater in patients with AS, and abnormal faecal calprotectin levels were observed at a significantly higher frequency.<sup>9 16</sup> Further, levels of anti-CBir1 and ANCA were elevated in patients with AS without clinical IBD. All of these studies point to the presence of mucosal dysregulation and subclinical gut inflammation in patients with AS.<sup>17</sup>

There is strong evidence that IBD serologies exist prior to the clinical onset and overt development of IBD, pointing to a prodromal state.<sup>19 21 22</sup> Examining this prodromal period in patients with SpA is important for disease prevention and will require additional studies.

A substantial proportion of our FDR cohort, 34.3% (n=11) had evidence of either subclinical IBD or MRI findings indicative of SpA. In addition, 15.6% (n=5) of FDRs had MRI evidence of SpA, suggesting that subclinical AS occurs in a healthy cohort of people at risk of AS that may never reach clinical significance. Our study which focused on the presence of IBD markers does extend and enhance the recent observations that many



FDRs of HLA-B27+ probands exhibit clinical and/or imaging abnormalities suggestive of SpA.<sup>23</sup>

We observed that nearly one-quarter of healthy FDRs had evidence of a positive IBD antibody suggestive of mucosal dysregulation. In addition, the mean faecal calprotectin value, a marker of gut inflammation, was numerically but not statistically significantly higher in subjects with MRI findings of sacroiliitis than the mean faecal calprotectin value in patients without MRI findings of sacroiliitis (74.65 mcg/g vs 40.85 mcg/g). There was little overlap between our FDR study subjects with positive gut and those with sacroiliac joint abnormalities; neither group was limited solely to HLA-B27+ subjects.

Limitations exist in the interpretation of our data. Our cohort was small, limited to a convenience sample and did not examine all FDRs of each family. Conclusions of exact risk cannot be drawn. Much larger cohorts of patients examined in a cross-sectional manner would be needed to generate hypotheses to further test in longitudinal studies. In addition, the lack of a control group with healthy age-matched controls is a shortcoming of the study. However, this is a pilot study and national data regarding the prevalence of sacroiliac joint MRI abnormalities in the normal healthy population is limited. One small study did show minor abnormalities, usually limited to one slice, in approximately 20% of healthy normal subjects.<sup>24</sup> In the current study, the frequency of positive at risk findings (either musculoskeletal or gut) in undiagnosed FDRs was most certainly high and justifies further studies to enable preventative interventional strategies.

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