**EXTENDED REPORT**

**CARD15 gene polymorphisms in patients with spondyloarthritis identify a specific phenotype previously related to Crohn’s disease**

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**Background:** The association between spondyloarthritis and Crohn’s disease is well known. A risk for evolution to Crohn’s disease has already been shown in the subgroup of patients with spondyloarthritis associated with chronic gut inflammation.

**Objective:** To investigate whether the reported polymorphisms in the CARD15 gene, a susceptibility gene for Crohn’s disease, are associated with the presence of preclinical intestinal inflammation observed in spondyloarthropathies.

**Methods:** 104 patients with spondyloarthropathies were studied. All underwent ileocolonoscopy with biopsies between 1983 and 2004. The prevalence of three single nucleotide polymorphisms in the CARD15 gene (R702W, G908R, and 1007fs) was assessed using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR); the patients were compared with an ethnically matched Crohn’s disease population and a control population.

**Results:** The carrier frequency of R702W, G908R, or 1007fs variants in the spondyloarthritis population (20%) was similar to the control population (17%), but increased to 38% in the spondyloarthritis subgroup with chronic gut inflammation. This frequency was significantly higher than in the other spondyloarthropathy subgroups (p = 0.001) or the control group (p = 0.006), but not different from the Crohn’s disease group (49%) (NS). This indicates that CARD15 polymorphisms are associated with a higher risk for development of chronic gut inflammation.

**Conclusions:** CARD15 gene polymorphisms clearly identify a subgroup of patients with spondyloarthropathies associated with chronic intestinal inflammation.

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The spondyloarthropathies are a group of interrelated inflammatory diseases characterised by a pauciarticular, peripheral, asymmetrical arthritis with or without axial involvement, with ankylosing spondylitis as the prototype. Reported prevalences of spondyloarthropathy vary between 0.2% and 1.9%. Although an association with HLA-B27 is strong, recent genetic studies suggest a polygenic model of susceptibility.

In up to 60% of spondyloarthropathy patients, articular involvement is associated with subclinical histological evidence of chronic or acute gut inflammation in the ileum or colon. We described a long term evolution to overt Crohn’s disease in 13% of patients with initial chronic gut inflammation. The presence of chronic intestinal inflammation was not related to HLA-B27, but a weak association with HLA-B62 was found.

The observed immunological similarities between spondyloarthropathy with gut inflammation and Crohn’s disease support the concept that this subgroup of spondyloarthropathy patients can be considered a model for early immune alterations related to Crohn’s disease. An enrichment of gut mucosal T cell lines with αEβ7 integrin and an increased expression of its ligand, E-cadherin, is found in the intestine in Crohn’s disease as well as in spondyloarthropathy patients. Recirculation of gut primed T cells to synovial tissue is one potential mechanism whereby gut and synovial inflammation could be linked. This hypothesis is supported by an altered expression of β7 integrins, which are highly expressed within the gut, on synovial T cells from patients with spondyloarthropathies compared with rheumatoid arthritis. Another potential mechanism includes trafficking of antigen presenting cells between gut and joints. Consistent with this was the augmented infiltration of gut mucosa and synovium with CD163 positive macrophages (producing interleukin 1 (IL1) and tumour necrosis factor α (TNFa)) in both Crohn’s disease and spondyloarthropathy.

In 2001, a correlation was reported between polymorphisms in the CARD15 gene and an increased susceptibility to Crohn’s disease. Three independent single nucleotide polymorphisms (SNPs) in CARD15 are associated with Crohn’s disease in around 30–46% of patients (one frame shift mutation (1007fs (SNP13)) and two missense mutations (R702W (SNP8) and G908R (SNP12)). These variants increase the risk for Crohn’s disease by a factor of 3 for heterozygotes and by a factor of 38 or 44 for, respectively, homozygous or compound heterozygous individuals. Lower prevalences have been described in Crohn’s disease patients in Scotland, Ireland, and northern Europe, whereas no association could be found in Japan.

The CARD15 gene encodes for an intracellular protein, which is expressed in monocytes, granulocytes, and dendritic, epithelial, and Paneth cells, and has binding affinity for various cytokines and chemokines.
bacterial cell wall components such as muramylpeptides. The CARD15 gene is involved in NFKB activation and in apoptosis by two N-terminal caspase recruitment domains (hence the term CARD), although its precise pathogenic role in Crohn’s disease remains to be determined.20–23

CARD15 gene polymorphisms have also been linked to another related disorder, Blau’s syndrome, characterised by granulomatous inflammation of the uvea, skin, and joints.24

Several studies have been carried out to investigate the role of CARD15 polymorphisms in spondyloarthropathies. These studies did not show an association with spondyloarthropathies or ankylosing spondylitis in particular.34,35 An increased prevalence of CARD15 polymorphisms was found in psoriatic arthritis though not in psoriatic skin disease.36 However, a recent Italian study could not confirm this association.37 Nevertheless, this finding could emphasise the importance of investigating the possible role of these genetic variants in specific clinical subpopulations of patients. In Crohn’s disease, CARD15 polymorphisms also seem to be related to certain clinical phenotypes.38–46

In view of the apparent correlation between gut inflammation in spondyloarthropathies and their clinical evolution to Crohn’s disease, we investigated whether the presence of polymorphisms in this susceptibility gene for Crohn’s disease is associated with gut inflammation in spondyloarthropathy.

METHODS

Study population

This study included 104 white patients with spondyloarthropathies (according to the ESSG criteria47), who underwent an ileocolonoscopy with concomitant ileal and colonic biopsies between 1983 and 2004. This population consisted of 74 male and 30 female patients with a mean age of 46 years (range 21 to 77). Spondyloarthropathic patients were systematically referred by the rheumatologist for an ileocolonoscopy with biopsies, independent of the presence of gastrointestinal symptoms.

Patients with a diagnosis of clinical Crohn’s disease or psoriasis before the diagnosis of spondyloarthropathy were excluded from the study.

A subgroup of 54 patients with long term follow up since the time of their diagnosis of spondyloarthropathy (ranging from 17 to 49 years) was recently clinically reassessed. New follow up colonoscopies were not carried out.

The total spondyloarthropathy population consisted of 75 patients with ankylosing spondylitis according to the modified New York criteria48 and 29 with an undifferentiated form of spondyloarthropathy. Eighteen patients with ankylosing spondylitis had only axial involvement, while 52 also had peripheral disease (defined as the history or presence of peripheral arthritis, enthesitis, or both). Twenty five patients with undifferentiated spondyloarthropathy had peripheral disease and four only had axial involvement. These four patients had inflammatory low back pain and fulfilled the ESSG criteria but not the modified New York criteria for ankylosing spondylitis.

HLA-B27 status was known in 81 patients. In 53 patients both HLA-B27 and HLA-B62 status was known.

A population of 156 consecutive patients with Crohn’s disease proven on clinical, endoscopic, and histological grounds was also included. This cohort included 57 male and 99 female patients with a mean age of 38 years (range 18 to 80). Prevalences were also compared with those in a control population of 140 individuals.

The study was approved by the local ethics committee. All patients signed their informed consent.

Histological classification

A classification of histological lesions was used as reported in previous studies.49–51 Three subgroups were distinguished: patients with normal gut histology, and those with acute and chronic inflammation.52

In acute inflammatory lesions normal architecture was well preserved. A mucosal and epithelial infiltrate of neutrophils and eosinophils was present, without a significant increase in lymphocytes. Small superficial ulcers covered with fibrin and neutrophils overlying hyperplastic lymphoid follicles were occasionally observed. The lamina propria was oedematous and haemorrhagic and contained mainly polymorphonuclear cells. The pattern of inflammation was similar to that seen in acute self limiting bacterial enterocolitis.

The principal features of chronic inflammatory lesions were mucosal architectural alterations with crypt distortion and atrophy in the colon, and villous blunting and fusion in ileal mucosa. In both ileum and colon there was an increased mixed cellularity and formation of basal lymphoid aggregates in the lamina propria. Whenever one of several biopsies featured chronic lesions, regardless of acute or active inflammation in other fragments, a diagnosis of chronic inflammation was made.

Although non-steroidal anti-inflammatory drugs may induce intestinal disorders, we and others excluded these drugs as factors in the aetiology of reported chronic inflammation.53

CARD15 genotyping (R702W, G908R, and 1007fs), and HLA-B27 and HLA-B62 typing

Genomic DNA was extracted from whole blood using the Qiagen blood and cell culture DNA kit (Westburg BV, Leusden, Netherlands). All patients were genotyped for R702W, G908R, and 1007fs using restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR), followed by separation of the DNA fragments on a 2.5% agarose gel. The missense mutation R702W (GenBank accession number G67950) abolishes the restriction site for MspI, resulting in an intact 130 base pair (bp) band for mutant alleles compared with two bands of 54 and 76 bp for wild type alleles (forward primer: 5’-CAGCCCGCTCCCTCTGCTACATCG TA-3’; reverse primer: 5’-AGGCCGCCTCCTCCTGCTACATCG TA-3’). The missense mutation G908R (GenBank accession number G67951) creates a restriction site for HinP1I. The frameshift mutation 1007fs (GenBank accession number G67955) creates a restriction site for NheI. The presence of a mutant allele results in two bands of 219 and 41 bp, while the wild type allele produces a single 260 bp product (forward

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*χ²: p<0.001 (carrier frequency in Crohn’s disease v control population).
†χ²: p=0.5 (carrier frequency in general spondyloarthropathies v control population).
‡ χ²: p=0.001 (chronic inflammation in patients with CARD15 variant v chronic inflammation in patients without CARD15 polymorphism)
populations cytotoxicity test, according to Terasaki and McClelland.53 Typing of these markers was done using the microlymphocytoxicity test, according to Terasaki and McClelland.53

Probability (p) values of less than 0.05 were considered statistically significant. Statistical analysis Statistical significance was determined by the χ2 test and odds ratios using SPSS (SPSS Inc, Chicago, Illinois, USA). Multivariate analysis (logistic regression) was carried out to determine the odds ratios using SPSS (SPSS Inc, Chicago, Illinois, USA).

RESULTS
We subdivided our cohort into three groups according to gut histology. Forty patients (38%) had normal histology, 24 (23%) had acute gut inflammation, and 40 (38%) had chronic gut inflammation (table 1).

Univariate analysis
Prevalence of CARD15 polymorphisms in the various populations
The prevalences of CARD15 polymorphisms in the total spondyloarthropathy (20%), specific ankylosing spondylitis (21%), and undifferentiated spondyloarthritis (17%) populations did not differ significantly (table 1). All except one (homozygous for the 1007fs allelic variant) were heterozygous for at least one mutation. The prevalences of R702W, G908R, and 1007fs allelic variants in these spondyloarthropathy populations were 12%, 4%, and 5% respectively (table 2). No compound heterozygosity was found. All carriers of CARD15 polymorphisms in the spondyloarthropathy cohort had a history of peripheral disease (table 3). There were no significant differences in disease duration or the duration of the follow up period between the spondyloarthropathy patients carrying CARD15 polymorphisms and the patients without these polymorphisms (data not shown).

In the Crohn’s disease population, a carrier frequency of 49% (77 of 156 patients) was observed (table 1). Forty-three Crohn’s disease patients carried at least one R702W polymorphism, 14 carried at least one G908R polymorphism, and 27 carried at least one 1007fs polymorphism. Fourteen percent of patients carried two polymorphisms, of which seven were homozygous and seven were compound heterozygous (table 2).

In the control group, 24 individuals (17%) carried CARD15 polymorphisms (table 1). All except one (compound heterozygous for the R702W and 1007fs variant) were single heterozygotes (table 2).

The prevalence of polymorphisms in the spondyloarthropathy cohort (20%) was not different from that in the control group (17%) (p = 0.5; odds ratio (OR) = 1.22 (95% confidence interval (CI), 0.64 to 2.34)) and significantly lower than in our Crohn’s disease population (49%) (p<0.001; OR = 3.85 (95% CI, 2.17 to 6.83)).

ASSOCIATION BETWEEN CARD15 POLYMORPHISM AND INTESTINAL INFLAMMATION IN SPONDYLOARTHROPATHY PATIENTS
The carrier frequency in the subpopulation of spondyloarthropathy patients with chronic gut inflammation was 38% (15 of 40 patients) which was significantly higher than in the control population (p = 0.006; OR = 2.9 (95% CI, 1.33 to 6.30)) or the other spondyloarthropathy populations (p = 0.001; OR = 5.80 (2.02 to 16.68)) and not statistically different from that in our Crohn’s disease population (49%, p = 0.2; OR = 1.62 (0.80 to 3.31)) (table 1).

Of all spondyloarthropathy patients carrying CARD15 polymorphisms, 71% (15 of 21 patients) had chronic gut inflammation, none had acute inflammation, and 29% had normal histology (table 1). The only spondyloarthropathy patient carrying two CARD15 variants also had chronic gut inflammation. In contrast, only 25 of 83 patients with a wild type genotype (30%) had chronic gut inflammation, 29% had acute inflammation, and 41% had normal histology. Consequently, the presence of CARD15 polymorphisms was associated with a higher risk of the development of chronic gut inflammation.

There were no significant differences between the ankylosing spondylitis and the undifferentiated spondyloarthropathy groups for the prevalence of CARD15 polymorphisms in patients with normal gut (3/29 vs 3/11, respectively; p = 0.3; OR = 3.3 (95% CI, 0.5 to 19.4)), acute gut inflammation (0/13 vs 0/11), or chronic gut inflammation (13/33 vs 2/7; p = 0.7; OR = 1.6 (95% CI, 0.3 to 9.7)).
In the subgroup of 54 patients who were clinically reassessed, four had evolved from histological chronic gut inflammation towards clinically overt Crohn’s disease. Two of these four patients carried CARD15 polymorphisms. The other 22 patients with chronic gut inflammation in this group did not develop clinical Crohn’s disease.

**Association between CARD15 polymorphisms and HLA-B27 in spondyloarthropathy patients**

There was no significant association between the presence of these two genetic markers. Six of 34 HLA-B27 negative patients carried CARD15 polymorphisms \( \chi^2 = 13 \) of 47 HLA-B27 positive patients \( (p = 0.3; OR = 1.8 \text{ (95\% CI, 0.6 to 5.3).}) \).

**Multivariate analysis**

In the subgroup of 53 spondyloarthropathy patients in whom both HLA-B27 and HLA-B62 status was known, logistic regression was undertaken (with the presence of chronic gut inflammation as the dependent variable). This showed that the association between chronic gut inflammation and CARD15 polymorphisms \( (p = 0.01; OR = 17.3 \text{ (95\% CI, 2.0 to 152.3).}) \) was independent of HLA-B27 \( (p = 0.42; OR = 1.7 \text{ (0.5 to 6.0).}) \) and HLA-B62 \( (p = 0.28; OR = 2.3 \text{ (0.5 to 13.0).}) \).

**DISCUSSION**

In this study we describe a novel and remarkably strong association between variants in a host defence gene located on chromosome 16 (CARD15) and a chronic form of gut inflammation in patients with spondyloarthopathies. The prevalence of CARD15 polymorphisms in this subgroup of spondyloarthropathy patients was not significantly different from that seen in patients with Crohn’s disease.

Three single nucleotide polymorphisms have been associated with Crohn’s disease.21–23 One variant (1007Ts) encodes a truncated protein which results in altered activation of NFkB in response to bacterial stimuli.20–31 The two other single nucleotide polymorphisms (R702W and G908R) result in an amino acid substitution.

More recently, several groups assessed the linkage of CARD15 variants in Crohn’s disease to particular clinical phenotypes, but the results of these retrospective studies are disparate. The presence of two mutations has been linked to younger age at onset and preferential involvement of small bowel.32 Preference for ileal involvement was also reported by Cuthbert et al.33 and by Ahmad et al.34 Fibrostenosing disease was the dominant type in a study by Abreu et al.35 In these studies, no association of CARD15 variants with extraintestinal involvement could be shown.

Our findings confirm the previous reported clinical, therapeutic, and immunological links between spondyloarthopathies and Crohn’s disease and provide genetic proof for the association between these two disorders. As chronic gut inflammation in the majority of spondyloarthropathy patients remains asymptomatic, this might suggest that CARD15 polymorphisms could be linked to the development of (subclinical) chronic gut inflammation rather than to Crohn’s disease as such.

The underlying pathogenic mechanisms that could explain the phenotypic expression of CARD15 mutations in spondyloarthropathies need to be investigated. CARD15 encodes a cytosolic protein that could play a role in spondyloarthropathies by interference with transport of antigens by macrophages from mucosal surfaces to the joints.53 CARD15 seems to function as an intracellular receptor for bacterial components, where the C-terminal leucine-rich repeat domain (LRR domain) is crucial for responsiveness. The cellular response to bacterial products has been shown to be altered in HEK293T cells transfected with expression plasmids containing any of the three SNPs.29–31 Moreover, expression of CARD15 in myeloblastic and epithelial cells is enhanced by proinflammatory cytokines and bacterial components, through NFkB.30 31 32 This response is likely to mediate cytokine production including TNFα, suggesting that upregulation of CARD15 may be part of a positive regulatory loop and facilitate the response of the host to pathogens. A genetically determined disturbance of handling of bacterial products in the intestinal tract, leading to altered transport of antigens by macrophages to synovial tissue, is an interesting hypothesis that should be investigated in spondyloarthropathy. A further identification and characterisation of inflammatory cells involved in gut and joint inflammation may also lead to new therapeutic targets.

**Conclusions**

A distinct phenotype associated with the three main Crohn’s disease associated CARD15 variants is reported in patients with spondyloarthopathies. Our data show that the presence of CARD15 variants in spondyloarthropathy patients strongly predisposes to chronic intestinal inflammation, defining a population at risk for evolution to Crohn’s disease. However, the persistence of the subclinical character of the inflammation in a large proportion of patients may reflect the fact that Crohn’s disease is a multigenic disease or alternatively that the heterozygous carriage of CARD15 polymorphisms predisposes only to subclinical inflammation.
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