Objective. To determine if the mutations in the CARD15/NOD2 gene predisposing to Crohn’s disease (CD) contribute also to the genetic susceptibility to rheumatoid arthritis (RA).

Methods. The frequencies of the three commonest mutations of CARD15/NOD2 predisposing to CD (2104C > T, 2722G > C and 3020insC) were determined in 210 RA patients and 227 controls.

Results. Allelic frequencies of the CARD15/NOD2 mutations in RA patients (2104C > T, 2.8%; 2722G > C, 0.9%; and 3020insC, 2.4%) did not differ significantly from the controls (2104C > T, 5.3%; 2722G > C, 0.7%; and 3020insC, 1.1%).

Conclusion. There was no evidence of association between the commonest CD CARD15/NOD2 mutations and RA susceptibility.

KEY WORDS: Rheumatoid arthritis, Crohn’s disease, Genetic predisposition to disease, Autoimmune diseases.

Twin, family and HLA studies indicate that rheumatoid arthritis (RA) has a significant genetic component [1, 2]. The HLA genes account for 30–40% of RA genetic susceptibility [1–5]. The remaining 60–70% has an unknown molecular basis, although the involvement of some candidate genes has been suggested [2]. A fraction of the unexplained genetic susceptibility should be related to the loci linked to RA [3–6]. One of these loci, in chromosome 16q, has shown linkage in two genome-wide studies [3, 4] and overlaps with susceptibility loci to other diseases resulting from dysregulation of the immune system, including Crohn’s disease (CD), psoriasis, insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus, ankylosing spondylitis and asthma. This coincidence is indicative of a common disease gene [7–9] and it suggests that the mutations on the CARD15/NOD2 gene located on the chromosome 16q locus, which cause susceptibility to CD [10, 11], could also contribute to RA predisposition.

The CARD15/NOD2 protein, expressed almost exclusively in monocytes, mediates the activation of NF-κB in response to bacterial products [11, 12]. Disruption of the regulatory region of CARD15/NOD2 by the CD mutations alters the magnitude of NF-κB activation leading to an uncontrolled inflammatory response [11]. These characteristics are compatible with current models of RA pathology [13–15]. Therefore, both genetic and pathogenic evidence would argue for the participation of the CD CARD15/NOD2 mutations in RA susceptibility.

Patients and methods

Patients

We studied 210 patients with RA, according to the American College of Rheumatology (ACR) criteria [16], and 227 controls of homogeneous Spanish ancestry. The regional ethics committee (Comité Ético de Investigación Clínica de Galicia) approved the study, and written informed consent was obtained from all patients.

Genotyping

DNA was extracted from peripheral blood by conventional techniques. Three polymorphisms of the CARD15/NOD2 gene (3020insC, 2722G > C, 2104C > T) were typed by analysis of the melting curve after hybridization with FRET probes on a
|                       | 3020insC | 2722G>C | 2104>T | Total  
|-----------------------|----------|---------|--------|--------
| Controls (n = 454)    | 0.011    | 0.007   | 0.053  | 0.070  
| Cases (n = 420)       | 0.024    | 0.009   | 0.028  | 0.062  
| OR (95% CI)           | 2.2 (0.7–6.5) | 1.4 (0.3–6.5) | 0.5 (0.3–1.1) | 0.9 (0.5–1.5) 

*aJoint frequency of the three mutations. The three mutations occurred in independent chromosomes and there were no compound heterozygotes.

bNumber of studied chromosomes.

Table 1. Allelic frequencies of the three commonest CD CARD15/NOD2 mutations (3020insC, 2722G>C, 2104C>T) in RA patients and controls.

LightCycler (Roche Diagnostics, Barcelona, Spain) polymerase chain reaction (PCR) system. Primers and FRET probes were synthesized by TIB MOLBIOL (Berlin, Germany) and have been reported previously [17]. PCR reactions were as described previously [17]. Selected samples were sequenced on the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Madrid, Spain).

Statistical analysis
Allelic frequencies, odds ratios (OR), their confidence intervals (CI) and the χ²-test were calculated (http://home.clara.net/sisa/index.htm). The post-hoc power of the study was determined with the Gpower software (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/).

Homology search
An extensive search of CARD15/NOD2 (GenBank: NP_071445) homologues located in chromosome 16q was done with BLAST, FASTA3 and PSI-BLAST search algorithms. Homologue sequences were located on the NCBI sequence of the Human Genome (build 29).

Results and discussion
The possibility of the participation of genes predisposing to several autoimmune diseases in the aetiology of RA has been a recurrent theme in the genome-wide linkage studies [3–5]. Coincidences have been signalled with IDDM, SLE, ankylosing spondylitis, multiple sclerosis, asthma and CD. One of the RA loci, on chromosome 16q, found in two genome scans undertaken in American [4] and European [3] families, respectively, could be related to the mutations on the CARD15/NOD2 genes predisposing to CD. However, our study did not find any evidence of association between the three commonest CD CARD15/NOD2 mutations and RA susceptibility, taken individually or collectively (Table 1). Analysis of several disease features (gender, age of onset, rheumatoid factor, sensitivity to disease-modifying anti-rheumatic drugs) showed no correlation with the genotypes. We did not find any homozygote for 2104C>T or 2722G>C, nor any compound heterozygote, and only one homozygote for 3020insC among the RA patients. This patient, a 54-yr-old man, had no distinctive features.

The negative result found in this study was associated with a post-hoc power to detect association at the 0.05 level of 99.98% and 80% for attributable risks of 2 and 1.5, respectively. In this analysis, the three mutations were considered together owing to their independent origin and inheritance and because they seem to act through the same pathogenic mechanisms [10, 18–21]. Additionally, the frequencies of the three alleles in the control population were very similar to the frequencies that have been found in different European populations [10, 18–21], and the genotype frequencies of the three polymorphisms were distributed according to the Hardy–Weinberg equilibrium. This result confirms the lack of association reported by Steer et al. [21] between two of the mutations studied here (2104C>T and 3020insC) and RA in the British population and a smaller study published previously with French RA patients [22]. These results should be considered together with similar studies exploring the involvement of the CD CARD15/NOD2 mutations in susceptibility to other autoimmune diseases linked to chromosome 16q—psoriasis [23], ankylosing spondylitis and spondylarthropathies [17, 22, 24], and SLE (I. Ferreiros-Vidal et al., unpublished). All of them have failed to find evidence of association. Therefore, it seems that the CD CARD15/NOD2 mutations have an effect restricted to CD. This result was unexpected and it provokes more questions about the function of the CARD15/NOD2 protein and how the mutated forms contribute to CD pathology.

A possible alternative to a single gene underlying all the autoimmune diseases linked to a given region is a cluster of functionally related genes, each of them underlying susceptibility to a different disease [9]. There are many examples of this kind of gene cluster, some of them central in immune responses, such as the MHC, immunoglobulin and T-cell receptor gene complexes. Therefore, we have searched for CARD15/NOD2 homologues in other regions of the same chromosome. This analysis excluded a cluster of CARD15/NOD2-related genes on chromosome 16q as it found only a putative protein, derived from cDNA libraries, that is a distant homologue of CARD15/NOD2 and maps more than 7 Mb telomeric to it. Therefore, no candidate for the RA susceptibility locus on chromosome 16q can be proposed.

Conflict of interest
The authors have declared no conflicts of interest.
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