Lack of Association of Ankylosing Spondylitis with the Most Common NOD2 Susceptibility Alleles to Crohn’s Disease

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ABSTRACT. Objective. To investigate whether the 3 most common mutations in the NOD2 gene that confer susceptibility to Crohn’s disease (CD) are also associated with ankylosing spondylitis (AS).

Methods. DNA from 112 patients with AS and 168 controls of homogenous Spanish ancestry were studied. The frequencies of the pathogenic alleles of NOD2 (3020insC, 2722G>C, and 2104C>T) were determined by analysis of the melting curves after hybridization with FRET probes on a Light Cycler real-time polymerase chain reaction (PCR) system.

Results. NOD2 allelic frequencies in controls (3020insC, 0.009; 2722G>C, 0.009; 2104C>T, 0.042) did not significantly differ from patients with AS (3020insC, 0.009; 2722G>C, 0.004; 2104C>T, 0.031).

Conclusion. The 3 most common CD NOD2 mutations do not contribute to disease susceptibility to AS, and therefore do not explain the susceptibility locus for AS in chromosome 16q. (J Rheumatol 2003;30:102–4)

Key Indexing Terms: ANKYLOSING SPONDYLITIS GENETIC PREDISPOSITION TO DISEASE CROHN’S DISEASE AUTOIMMUNE DISEASES

Different autoimmune and inflammatory diseases share common features: clinical manifestations, epidemiological distribution, and characteristics of pathogenesis. Some coincide in the same individual or, more often, in different members of the same family, leading to the hypothesis that these diseases have common genetic susceptibility factors. Genetic studies on a multiplicity of autoimmune and inflammatory diseases have lent support to this hypothesis: many of the susceptibility loci overlap. The overlap is much more frequent than would be expected by random distribution or than is observed with nonautoimmune diseases. The existence of these clusters of susceptibility loci is indicative of common disease genes, and the gene predisposing to a disease of this group may also be a susceptibility gene for the other diseases in the same cluster.

A particular cluster has been found in chromosome 16q. The diseases showing linkage with this cluster include Crohn’s disease (CD), ankylosing spondylitis (AS), systemic lupus erythematosus, psoriasis, rheumatoid arthritis, insulin dependent diabetes mellitus, asthma, and a murine model of multiple sclerosis. Recently, it has been found that some independent mutations on the regulatory region of the NOD2 gene cause the susceptibility locus for CD. These mutations account for a large fraction of CD heritability. The NOD2 gene, expressed almost exclusively in monocytes, mediates the activation of NF-κB, a key transcription factor in the induction of many inflammatory genes, in response to bacterial products. Disruption of the regulatory region of NOD2 alters the magnitude of NF-κB activation, leading to an uncontrolled inflammatory response to enteric bacteria.

This observation prompted investigations of the susceptibility factors for the autoimmune and inflammatory diseases clustering in chromosome 16q. AS has the highest heritability among them, with a recurrence risk for first-degree relatives 82 times higher than the risk in the general population. The genetic component in AS is not limited to the HLA-B27 allele: B27 positive patients with AS have a recurrence risk 5.6 to 16 times higher than unrelated B27 positive individuals. Genome-wide linkage studies have confirmed this with the identification of up to 7 additional loci causing susceptibility to AS. The stronger non-MHC locus was found in the cluster of chromosome 16q, in the vicinity of NOD2, with a LOD score of 4.7. The likelihood that this locus is caused by NOD2 mutations is reinforced by the special relationship
between AS and CD. Some CD patients have symptoms or signs characteristic of AS and vice versa. AS and CD also overlap in pathogenic mechanisms: in both diseases, gastrointestinal bacteria seem to trigger an inappropriate immune response mediated by macrophages and T cells. Tumor necrosis factor-α (TNF-α) plays a central role, and treatment with TNF-α blocking agents causes a marked improvement in patients with CD or AS. Also, there is an increased incidence of inflammatory bowel diseases in first-degree relatives of patients with AS, and the HLA-B27 positive first-degree relatives of patients with CD have an increased incidence of developing AS. Collectively, these observations suggest that the AS susceptibility locus on chromosome 16q could pertain to the same NOD2 mutations predisposing to CD.

MATERIALS AND METHODS

Patients. We studied 112 patients with AS, according to the New York criteria, and 168 controls of homogenous Spanish ancestry. The study was approved by the regional Ethical Committee, and written informed consent was obtained from all patients.

Genotyping. DNA was extracted from peripheral blood with the Puregen kit (Genta Systems, Minneapolis, MN, USA) following the manufacturer’s protocol. Three alleles of the NOD2 gene (3020insC, 2722G>C, and 2104C>T) were typed by analysis of the melting temperature of the hybrids formed between the polymerase chain reaction (PCR) products and specific fluorochrome labeled oligonucleotides. Detection of the hybridization signal was based on fluorescence resonance energy transfer (FRET) on a Light Cycler (Roche) real-time PCR system. Primers and fluorescent labeled probes for FRET were synthesized by TIB Molbiol (Berlin, Germany). The 3020insC allele was amplified with forward primer 5′-TCTTCTTTFCCAGGTTGTGC-CAA-3′ and reverse primer 5′-TGGAGTTCCAGAGCTTTAACACG-3′. In addition, an anchor probe, 5′-CCACCTCTGGAAGTCTGGTAAGGCCp-3′, labeled at the 5′ end with fluorescein (X), was used. The melting temperature of this probe is 64°C for the wild-type allele and 60°C for the mutated allele. The 2722G>C allele was amplified with forward primer 5′-GCACATATCAGTCTACTGCTGACT-3′ and reverse primer 5′-TTACCTGAGCCACCTCAAGC-3′. The anchor probe 5′-CTGAAAGGGCCAAAAAGTACTGCAACGAp-3′ was labeled at the 5′ end with LC-Red 640, and phosphorylated at the 3′ end. The sensor probe 5′-CCACTCTGTGTCGCGCAAX-3′ was labeled with fluorescein. The melting temperature for the wild-type allele is 67°C and 61°C for the pathogenic allele. The primers to amplify the 2104C>T alleles in the same reaction capillary; the 3 mutations we studied. We investigated whether the 3 mutations we studied.

Table 1. Allelic frequencies of the 3 mutations of NOD2 in patients with AS and controls.

<table>
<thead>
<tr>
<th>Allele</th>
<th>3020insC</th>
<th>2722G&gt;C</th>
<th>2104C&gt;T</th>
<th>Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 336)**</td>
<td>0.009</td>
<td>0.009</td>
<td>0.042</td>
<td>0.059</td>
</tr>
<tr>
<td>Cases (n = 224)</td>
<td>0.009</td>
<td>0.004</td>
<td>0.031</td>
<td>0.045</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1 (0.2–6.0)</td>
<td>0.5 (0.1–4.9)</td>
<td>0.7 (0.3–1.9)</td>
<td>0.7 (0.3–1.6)</td>
</tr>
</tbody>
</table>

* Joint frequency of the 3 mutations. No homozygous or compound heterozygous mutations were found.
** Number of studied chromosomes.
NOD2 mutations associated with CD, while the remaining 19% are due to 27 rare mutations. Given the post-hoc power of our study and the strength (LOD 4.7) of the chromosome 16q AS susceptibility locus, our results show that the most common CD-associated NOD2 mutations are not the basis for the susceptibility locus for AS. This is significant because the NOD2 mutations we investigated, unlike many other gene variants analyzed in association studies, have functional consequences affecting monocyte-dependent responses through NF-κB activation in response to Gram negative bacteria. Similarly to CD, a link of immune response to enteric bacteria and AS has been proposed. Lack of influence of the NOD2 mutations on AS indicates that enteric bacteria do not act in the same way in both diseases. Differences might include the bacteria involved, the type of response elicited, or the absence of this kind of trigger for AS.

Coincident mapping of susceptibility loci as a guide for selecting a candidate gene for the chromosome 16q AS locus has not been successful. A priori, a specific cluster of susceptibility loci could be due to a single gene underlying all the loci, a cluster of functionally or evolutionarily related genes, each playing a role in different diseases, or to poor precision mapping. Either of the latter 2 alternatives might explain our results.

Finally, our results point to the weakness of reasoning from clinical or pathogenic similarities, or even from coinheritance in families, as guides to genetic investigation. Although these features are still appealing and indicate a genetic link, our current understanding of disease mechanisms does not allow sound inferences about specific genetic factors. The similarities between AS and CD may be due to other pathogenic mechanisms related to common susceptibility genes different from NOD2 or to other unexplored NOD2 mutations. A similar interpretation could be given to the recent description of the lack of association of one of the NOD2 variants, 3020insC, with susceptibility to psoriasis, another of the diseases with a susceptibility locus in the same region of chromosome 16 sharing pathogenic and clinical features with CD.


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REFERENCES