Role of the PXR Gene Locus in Inflammatory Bowel Diseases

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Background: The pregnane X receptor gene (PXR/NR1I2) has been recently associated with an increased risk for inflammatory bowel disease (IBD), although a subsequent case-control study failed to replicate the original association in an independent population. This nuclear receptor regulates genes involved in the detoxification process in the liver and intestine, like ABCB1/MDR1. PXR expression was significantly reduced in the colon of patients with ulcerative colitis (UC), but remained unaffected in Crohn’s disease (CD) patients. Considering previous results, we aimed at investigating the impact of this locus on IBD predisposition in the Spanish population.

Methods: Three PXR polymorphisms, including the 1 more strongly correlated with IBD risk in the initial study at −25385C/T (rs3814055) and the 6 haplotypes conformed by them, were analyzed in 365 UC and 331 CD patients and compared with 550 ethnically matched controls.

Results: The overall haplotypic distribution showed a significant difference between UC and CD patients (P = 0.05; χ² = 10.84). Among UC patients a significant difference was seen between those with extensive colitis and controls (P = 0.004; χ² = 17.04), mainly due to the presence of a risk haplotype (rs3814055*T/rs6784598*C//rs2276707*C: /H11005P = 0.001; odds ratio [OR] = 1.66, 95% confidence interval [CI] 1.20–2.30). Patients with extensive UC carrying the −25385T allele showed increased susceptibility compared with left-sided colitis patients and with healthy subjects. In patients with extensive UC a significantly different distribution of genotypes of the MDR1 G/A change located in intron 3 (rs3789243) was observed between carriers/noncarriers of the −25385T risk allele (P = 0.005).

Conclusions: Our data seem to support the association of the PXR locus with extensive UC and the interaction between PXR and MDR1 genes.

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Key Words: pregnane X receptor gene, ulcerative colitis, Crohn’s disease, susceptibility

The pregnane X receptor (PXR) is a member of the nuclear receptor family of ligand-regulated transcription factors that includes the steroid, retinoid, and thyroid hormone receptors and also orphan receptors with as-yet unidentified physiological ligands. Like many nuclear receptors, it contains both DNA and ligand binding domains and binds to response elements in the regulatory regions of target genes as a heterodimer with the retinoid receptor (RXRalpha). However, a difference between PXR and the majority of nuclear receptors is its broad response to a wide variety of chemically distinct xenobiotics and endobiotics. Therefore, instead of a specialized sensor of a physiological ligand, PXR has evolved into a promiscuous receptor protecting the body against xenobiotic insult.¹ The PXR/RXR complex binds to a variety of response elements in the promoter regions of an entire set of genes that are important in xenobiotic metabolism and the detoxification process (for an excellent review on PXR, see Kliewer et al²).

Inflammatory bowel disease (IBD) is a complex trait derived from the interaction of genetic and environmental factors, most probably part of the luminal bacterial flora, leading to uncontrolled immune activation and chronic inflammation.³ The mucosa of the intestine is the largest body surface in contact with the world. Studies with animal models of colitis and IBD patients have shown that an intact detoxification is critical for preserving the integrity of the intestinal barrier.⁴–¹⁰ A search for transcriptional mechanisms mediating the coordinate downregulation of detoxification genes in IBD colon based on microarray analysis led to the description of the key role of PXR.¹¹ Messenger RNA expression of PXR was significantly reduced in the colon of patients with ulcerative colitis (UC), but was unaffected in patients with Crohn’s disease (CD). PXR binds to promoters of the majority of the detoxification genes including those involved in oxidation (phase 1), conjugation (phase 2), and transport (phase 3) of xenobiotics. Mice lacking a functional Pxr gene developed and reproduced normally; however, these PXR-null mice did not respond normally to xenobiotic treatment, and the transgenic animal expressing human PXR exhibited growth retardation, hepatomegaly, and liver toxicity, suggest-
ing a deleterious effect of \textit{PXR} sustained activation.\textsuperscript{12} Moreover, \textit{Pxr} knockout mice showed significantly increased inflammation of the small bowel due, as recently discovered, to reciprocal inhibition between NF-κB and \textit{PXR}.\textsuperscript{13}

The human \textit{PXR} (\textit{NR1I2}) gene was cloned 5 years ago and regulatory sites in its promoter have been recently identified.\textsuperscript{14} The gene mapping on 3q11-13 presents multiple polymorphisms through its 38 kb, some of them related with altered regulation of \textit{PXR} target genes involved in the metabolism and elimination of xenobiotics. A recent study in an Irish population showed association of this gene with susceptibility to IBD.\textsuperscript{15} Replication in an independent population was warranted to establish the real impact of this gene in susceptibility to these polygenic disorders. A subsequent study performed in a Scottish population reported lack of association of the gene with IBD.\textsuperscript{16} Therefore, we aimed at testing \textit{PXR} polymorphisms and the haplotypes conformed by them in our well-powered cohorts of UC and CD patients and compared their frequencies with those observed in healthy controls. Moreover, IBD has been associated with genetic variations in the \textit{ABCB1}/\textit{MDR1} gene, whose expression is dependent on \textit{PXR} and encodes a transporter critically involved in the protection of the intestinal barrier.\textsuperscript{17} We pursued to study the epistatic interactions between both genes.

\section*{MATERIALS AND METHODS}

Three hundred and thirty-one white, unrelated, Spanish CD (51% women) and 365 UC (40% women) patients consecutively recruited from a single center (Hospital Clínico, Madrid) and 550 ethnically matched healthy controls (51% women), mainly blood donors and staff, were included in a case-control study. Diagnosis of IBD patients was based on standard clinical, radiologic, endoscopic, and histologic criteria.\textsuperscript{18} The mean age at onset for UC patients was 38 years; 41% with pancolitis, 47% presented extraintestinal manifestations, and 13% underwent colectomy. CD patients were classified according to the location of the lesions in ileal (L1, 48%), colonic (L2, 16%), ileocolonic (L3, 32%), and upper gastrointestinal tract (L4, 3%) and according to the disease behavior in inflammatory (B1, 43%), stricturing (B2, 15%), and perforating (B3, 42%). Only 20% of the CD patients presented after the age of 40. All patients were included in the study after informed consent and the study was approved by the Hospital Ethics Committee.

The genotyping of the \textit{PXR} polymorphisms at −25385 and 8055 was performed by using TaqMan Assays-on-Demand (Applied Biosystems, Foster City, CA; C—27504984–30 and C—15882324–10 corresponding to rs3814055 and rs2276707, respectively) and an Assay-by-Design for the rs6784598. The 5-μL polymerase chain reaction (PCR) reaction with 1× TaqMan Universal Master Mix, 1× probe and primers assay mix and 10 ng of genomic DNA was performed in 384 well-plates with a 7900HT Fast Real-Time PCR system under the conditions recommended by the manufacturer (Applied Biosystems). These polymorphisms conformed to Hardy–Weinberg equilibrium in the control population. Discrepancies in the final number of subjects analyzed for each polymorphism are due to a small unsuccessful genotyping rate.

Statistical analysis was performed with a standard statistical package (Epi Info v. 6.02; World Health Organization, Geneva, Switzerland). Phenotype and genotype frequencies in patients and controls were compared by chi-square test or Fisher’s exact test. Strength of association was given as odds ratio (OR) with a 95% confidence interval (CI). Haplotype frequencies were estimated using the Expectation-Maximization algorithm implemented in the Arlequin v. 2.000 software\textsuperscript{19} with the number of iterations set at 5000 and initial conditions at 50, with an epsilon value of 10⁻⁷.

\section*{RESULTS}

The \textit{PXR} promoter polymorphism located at −25385C/T (NM—003889, rs3814055) showed the strongest nominal association with IBD overall and with both clinical subgroups in an Irish population, in comparison with other single-nucleotide polymorphisms (SNPs) examined.\textsuperscript{15} The analysis of this variant in our Spanish cohort did not show significant differences between either CD or UC patients versus controls or between the combined CD and UC cohorts compared to controls (Table 1). When the clinical subphenotypes were studied, carriers of the −25385T allele showed an increased susceptibility for patients with extensive UC compared with patients with extensive UC compared with left-sided colitis (99/38 versus 113/70: OR = 1.61, 95% CI 0.97–2.68; \(P = 0.049\)) and with healthy subjects (99/38 versus 331/219: OR = 1.72 95% CI 1.12–2.66; \(P = 0.01\)), although these comparisons did not withstand Bonferroni correction.

\begin{table}[h]
\centering
\caption{Genotype Distribution of the 3 Polymorphisms Tested within the \textit{NR1I2} Gene} \\
\begin{tabular}{lllll}
\hline
SNP & Genotype & Controls \((n = 550)\) & CD Patients \((n = 331)\) & UC Patients \((n = 365)\) \\
\hline
rs3814055 & CC & 219 (0.40) & 112 (0.35) & 126 (0.34) \\
 & CT & 242 (0.44) & 154 (0.48) & 182 (0.50) \\
 & TT & 89 (0.16) & 56 (0.17) & 57 (0.16) \\
rs6784598 & CC & 173 (0.32) & 101 (0.31) & 112 (0.31) \\
 & CG & 275 (0.50) & 173 (0.52) & 177 (0.49) \\
 & GG & 98 (0.18) & 57 (0.17) & 72 (0.20) \\
rs2276707 & CC & 356 (0.65) & 202 (0.63) & 238 (0.66) \\
 & CT & 175 (0.32) & 110 (0.34) & 111 (0.31) \\
 & TT & 18 (0.03) & 10 (0.03) & 12 (0.03) \\
\hline
\end{tabular}
\end{table}
In order to analyze the frequencies of the estimated haplotypes, 2 additional polymorphisms located in introns 5 and 6, changes 7158C/G and 8055C/T, respectively, were tested (Table 1). No independent association of each genetic marker with either CD or UC, or their subphenotypes, could be identified. Interestingly, the genotypic distributions of the C/T change at 8055 (rs2276707) were significantly different between controls from both Caucasian populations, Irish and Spanish (252/88/5 versus 356/175/18; P = 0.001; OR (95% CI): 1.66 (1.20-2.30)); this comparison would admit an ample correction, while those patients suffering from left-sided colitis did not significantly differ from healthy controls (P = 0.75).

Given the induction of the \textit{ABCB1}/\textit{MDR1} gene by \textit{PXR}, the interaction of both genes was studied and no association was detected between \textit{MDR1} variants G2677TA, rs3789243, was observed in patients with extensive UC stratified by the presence/absence of the minor allele at −25385C/T (Table 3A). Patients with extensive UC displaying the combined presence of risk alleles in both genes showed significantly increased risk than the rest of extensive UC patients with either 1 or none of the risk alleles (Table 3B).

**DISCUSSION**

These findings seem to corroborate in an independent population the association between the \textit{PXR}/\textit{NR1I2} gene locus and IBD previously reported in Ireland, although some distinctions must be emphasized. A functional role was previously attributed to the −25385C/T polymorphism, rs3814055; however, while the minor allele frequency was significantly reduced in CD and UC patients versus healthy controls in Ireland, no similar trend was observed in Spain. Moreover, 1 haplotype carrying the minor allele at this site had significantly reduced frequency in patients and conferred protection to CD and UC Irish patients, whereas the UC risk haplotype found in our population also carries the minor allele at that position. Therefore, no etiological value can be confidently ascribed to this specific polymorphism, which seems to act as a mere genetic marker. Most probably, some other as-yet

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**TABLE 2.** Frequencies of the \textit{PXR} rs3814055//rs6784598//rs2276707 Haplotypes in the Spanish Population

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls ((n = 1078))</th>
<th>CD ((n = 606))</th>
<th>UC ((n = 692))</th>
<th>Extensive UC ((n = 256))</th>
<th>Left-sided UC ((n = 350))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>410 (0.38)</td>
<td>229 (0.38)</td>
<td>233 (0.34)</td>
<td>78 (0.30)</td>
<td>128 (0.37)</td>
</tr>
<tr>
<td>TCC</td>
<td>202 (0.19)</td>
<td>119 (0.20)</td>
<td>156 (0.23)</td>
<td>71 (0.28)*</td>
<td>71 (0.20)</td>
</tr>
<tr>
<td>CGC</td>
<td>168 (0.15)</td>
<td>82 (0.14)</td>
<td>123 (0.18)</td>
<td>45 (0.17)</td>
<td>62 (0.18)</td>
</tr>
<tr>
<td>CGT</td>
<td>93 (0.09)</td>
<td>45 (0.07)</td>
<td>58 (0.08)</td>
<td>18 (0.07)</td>
<td>29 (0.08)</td>
</tr>
<tr>
<td>TGC</td>
<td>91 (0.08)</td>
<td>52 (0.08)</td>
<td>56 (0.08)</td>
<td>12 (0.05)</td>
<td>30 (0.09)</td>
</tr>
<tr>
<td>TGT</td>
<td>114 (0.11)</td>
<td>79 (0.13)</td>
<td>65 (0.09)</td>
<td>32 (0.13)</td>
<td>29 (0.08)</td>
</tr>
</tbody>
</table>

*Extensive UC vs. left-sided UC: \(P = 0.032; OR (95\% CI): 1.51 (1.02-2.24)\). Extensive UC vs. controls: \(P = 0.001; OR (95\% CI): 1.66 (1.20-2.30)\).

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**TABLE 3.** Epistatic Interaction between \textit{NR1I2}/\textit{PXR} and \textit{ABCB1}/\textit{MDR1} Genes in Patients with Extensive UC

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Extensive UC</th>
<th>Observed Controls</th>
<th>Expected Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) (A^-B^-)</td>
<td>68 (57%)</td>
<td>166 (41%)</td>
<td>157 (39%)</td>
</tr>
<tr>
<td>(II) (A^-B^+)</td>
<td>101 (25%)</td>
<td>100 (27%)</td>
<td>110 (27%)</td>
</tr>
<tr>
<td>(III) (A^+B^-)</td>
<td>72 (18%)</td>
<td>81 (20%)</td>
<td>80 (20%)</td>
</tr>
<tr>
<td>(IV) (A^+B^+)</td>
<td>65 (16%)</td>
<td>56 (14%)</td>
<td>56 (14%)</td>
</tr>
</tbody>
</table>

\(A^-/A^+\) and \(B^-/B^+\) presence/absence of \textit{MDR1} intron3 allele A and −25385T, respectively.

\(I\) vs. \(IV\): \(p_{	ext{obs.}} = 0.071; OR_{	ext{exp.}} = 1.78 (0.91-3.50); OR_{	ext{exp.}} = 1.62 (0.82-3.22)\).

\(I\) vs. \((II + III)\): \(p_{	ext{obs.}} = 0.003; OR_{	ext{exp.}} = 1.97 (1.22-3.19); OR_{	ext{exp.}} = 2.30 (1.42-3.72)\).

\(I\) vs. \((II + III) + IV\): \(p_{	ext{obs.}} = 0.002; OR_{	ext{exp.}} = 1.91 (1.24-2.95); OR_{	ext{exp.}} = 2.10 (1.36-3.24)\).
unidentified variant in strong linkage disequilibrium with the risk haplotypes found in populations with different ethnicity would be responsible for the observed associations.

Langmann et al.\(^{11}\) identified that the amount of PXR transcripts in the colon of UC patients was strongly reduced, while no significant reduction was seen in patients with CD; therefore, an involvement of PXR would be expected mainly with UC. Analyses of the regulatory regions of the coordinately downregulated detoxification genes showed that the majority of these genes contain binding sites for PXR. In the original report by Dring et al.\(^{15}\) the \(P\) values for overall comparison of haplotypes between controls and CD and UC patients were \(P = 3.5 \times 10^{-6}\) and \(P = 0.013\), respectively, which would imply a stronger influence on IBD than that observed in our Spanish cohorts. In contrast, the subsequent replication in a Scottish population did not show an association with several PXR polymorphisms, including 1 at −24381 in strong linkage disequilibrium with the 1 at −25385, or with the haplotypes conformed using 4 additional polymorphisms.\(^{16}\) A different phenotypic composition of the cohorts may underlie the heterogeneous results obtained to date. Genotype–phenotype analyses following the Montreal Classification of Scottish IB patients did not apparently solve the discrepancy with the original report, but the statistical power after stratification might be compromised. Our data suggest a role of this PXR/NR1I2 locus on chromosome 3q increasing susceptibility to patients diagnosed with extensive UC, although opposite alleles of the −25385 variant are associated with UC risk in our cohorts and in those studied by Dring et al.\(^{15}\) Additional studies are warranted to fully elucidate the influence of this locus on the clinical phenotypes of IBD.

An epistatic interaction between the polymorphism rs3789243 in the intron 3 of ABCB1/MDR1 and the variant at −25385C/T in the PXR/NR1I2 gene has been found (Table 3A). The simultaneous presence of risk alleles in both positions increased predisposition in patients with extensive UC, as determined by phenotypic analyses (Table 3B).

Substantial progress has been made in understanding the IBD pathogenesis during the past years, pursuing the view that IBD could result from disturbances of the intestinal barrier and a pathologic activation of the intestinal immune response toward luminal antigens. PXR might contribute to a disturbed function of the epithelial barrier leading to imbalances in mucosal immunity and infiltration of luminal microflora that are involved in IBD pathogenesis.\(^{20}\) The defense mechanisms responsible for protecting the body from toxins are also involved in the metabolism of drugs. PXR mediates drug-induced changes by increasing transcription of genes that are involved in drug clearance and disposition.\(^{21}\) Therefore, one would expect PXR to have a role in IBD pharmacogenetics and further efforts in this direction are encouraged.

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