The CREM gene is involved in genetic predisposition to inflammatory bowel disease in the Tunisian population

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A B S T R A C T

The identification of susceptibility genes for inflammatory bowel disease (IBD) is key to understanding pathogenic mechanisms. Recently, the results of genetic association studies have highlighted many loci that are shared among several autoimmune diseases. We aimed to study the genetic epidemiology of polymorphisms in specific genes previously associated with other autoimmune diseases, namely the CREM, STAT4, STAT5a, Stat5b, and IRF5 genes. Twelve polymorphisms in the CREM, STAT4, STAT5a, Stat5b, and IRF5 genes were genotyped in a cohort of 107 IBD patients (39 Crohn’s disease [CD] and 68 ulcerative colitis [UC]) and 162 controls from southern Tunisia. One CREM single nucleotide polymorphism (SNP) displayed evidence for genetic association with IBD (p = 8.7 × 10^{-4}, odds ratio [OR] = 2.84 [1.58; 5.09]). One STAT4 SNP (p = 0.026; OR = 1.65 [1.06; 2.58]) exhibited a marginal association with UC but not with CD. No significant association was observed with the SNPs in STAT5a, IRF5, and STAT5b. These results suggest that common variants of the CREM gene are involved in the genetic component conferring general susceptibility to IBD, whereas STAT4 appears to be more specifically associated with UC. This work provides motivation for studies aiming to replicate these findings in larger populations.

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1. Introduction

Crohn’s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the gastrointestinal tract that most commonly arise during the second and third decades of life. Incidence, family, twin, and phenotype concordance studies suggest that inflammatory bowel disease (IBD) is highly heritable, albeit complex, spurring an ongoing search for genetic factors that confer susceptibility to this disease [1,2]. The search for IBD-associated gene variants was particularly successful in CD, resulting in the identification of 3 major groups of susceptibility genes, including genes involved in bacterial recognition (e.g., NOD2/CARD15 [3,4]), T-helper 17 cell differentiation (e.g., IL23R [5,6]), and autophagy (e.g., ATG16L1 [7–9] and IRGM [10]). Recent research demonstrated that several risk loci are common to CD and UC (IL23R, IL12B, NOD2, STAT4, and MST1), whereas the autophagy genes ATG16L1 and IRGM, along with NOD2/CARD15, are specific for CD [11].

Some IBD susceptibility genes are shared with other autoimmune diseases [12], probably reminiscent of the higher risk of other immune-mediated conditions among IBD patients compared with controls [13]. Thus, genome-wide methodology revealed the genetic association of IRF5 and STAT4 genes in different autoimmune diseases (systemic lupus erythematosus [SLE], rheumatoid arthritis, and diffuse cutaneous systemic sclerosis) [14–16]. These transcription factors play a pivotal role in the development, differentiation, and function of the immune system. By contrast, STAT5a/b have been reported to play a crucial role in the development and maintenance of T regulatory (Treg) cells [17,18] and in the differentiation of T-helper 17 cells [19], which produce interleukin (IL)-17, an inflammatory cytokine implicated in various autoimmune diseases [20].

Furthermore, recent studies have established the importance of the cAMP response element modulator (CREM) as a negative regulator of IL-2 production in SLE T cells [21]. T cells from SLE patients express increased amounts of CREMα protein. CREMα acts as a transcriptional repressor and was proposed to be a key mechanism for decreased IL-2 production in SLE T cells [21–23]. CREMα can form homoduplexes or heteroduplexes with CREB and bind to the −180 site of the IL-2 promoter. This regulatory site is critical for IL-2 production because mutations of this site almost completely abolish IL-2 transcription [21,24]. T cells of mice that express a dominant negative form of CREB exhibit a marked decrease in IL-2 production [25].
Table 1
Clinical description of Crohn’s disease and ulcerative colitis patients

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
<th>Total (IBD)</th>
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<tr>
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<td>107</td>
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</tbody>
</table>

Sex (male/female) 24/15 33/35 57/50
Age (years), mean ± SD 39.4 ± 13.8 40.5 ± 12.4 40.1 ± 13.2
Pathology location
Ileum 14
Colon 11
Ileocolon 14
Pancolitis 35
Left-sided 26
Proctitis 7
Extraintestinal manifestations 18 13 31

2. Subjects and methods

2.1. Patients

Blood samples were obtained from 107 IBD patients (68 with UC, 39 with CD) diagnosed at the Department of Gastroenterology of Hedi Chaker University Hospital (Sfax, Tunisia) from March 2004 to May 2008. All patients were of Tunisian descent (north Africa). The diagnosis was based on standard clinical, radiographic, endoscopic, and histopathological criteria [26]. Table 1 summarizes their clinical data. Patients with CD were assessed according to the Montreal classification [27] based on age at diagnosis, localization, and behavior of disease. In patients with UC, anatomic localization was also determined according to the Montreal classification, using the criteria ulcerative proctitis, left-sided, and extensive. A total of 162 healthy Tunisian subjects with no history of digestive system disease, unrelated to each other or to the patients recruited in this study, were used as unaffected controls (HC). Unaffected controls were drawn from the same geographic region as the patients but sample control identifiers (including age and sex) were not available.

The study was approved by the local ethics committee and all enrolled patients gave their informed consent to participate.

2.2. Methods

2.2.1. Genotyping methods

Genomic DNA was extracted from whole blood samples using a standard proteinase K digestion and phenol/chloroform extraction procedure. Genotyping was performed using primer extension chemistry and mass spectrometric analysis (iPLEX assay, Sequenom, San Diego, CA) on the Sequenom Mass Array at the Instituto Gulbenkian de Ciência (Oeiras, Portugal). We genotyped 3 single nucleotide polymorphisms (SNPs) in CREM (rs1148247, rs17583959, and rs2384352), 1 SNP in STAT4 (rs7574865), 1 SNP in STAT5a (rs3198502), 2 SNPs in STAT5b (rs16967620 and rs4029774), and 5 SNPs in IRF5 (rs729302, rs2004640, rs752637, rs10954213, and rs2070197). SNP details are given in Table 2. All information about the selected SNPs was extracted from the public database db SNP built 126 found at http://www.ensembl.org (release 61). For quality control purposes these SNPs were also typed using a panel of HapMap samples.

Assay design was performed according to the manufacturer’s instructions, whereby the genomic sequence containing the SNP is amplified by multiplex polymerase chain reaction. The amplified product was cleaned using shrimp alkaline phosphatase and used for allele-specific primer extension reaction according to the MassEXTEND protocol. The reaction mixture was then spotted onto a SpectroCHIP microarray and subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. SpectroTyper software identifies the SNP-specific peaks and automatically assigns the genotype calls.

2.2.2. Data analysis

Quality control criteria excluded SNPs with a call rate lower than 80% in cases or controls. Genotype frequencies were in Hardy–Weinberg equilibrium in the Tunisian control population for all typed SNPs (p > 0.05). Case–control association analysis was performed for each SNP. Calculations of allelic and genotypic associations of SNPs with susceptibility to IBD were performed using a homemade program written in R language (http://www.r-project.org). The results were verified with the χ² test. Results were considered statistically significant when p < 0.05. Bonferroni correction was carried out when single SNP analysis yielded significant results. Relative risks were calculated as odds ratios (OR) using 2 × 2 contingency tables. Linkage disequilibrium (LD) maps were generated using Haplovie version 4.2.

3. Results

3.1. CREM

The rs1148247 polymorphism in CREM exhibited a marked increase in the frequency of the G allele in IBD patients (76.6%) compared with controls (64%; Table 3) and allelic association anal-
The rs3198502 polymorphism of STAT5a demonstrated a genotypic association (GG; \( p = 0.0480, \ OR = 1.66 \)) in IBD patients when compared with unaffected controls (Tables 3 and 4). However, statistical significance was lost after Bonferroni correction (\( p_{corr} = 0.14 \)). When stratified by disease type, only CD patients exhibited a significant difference in GG genotype frequency (\( p = 0.035; \ OR = 2.06 \)) compared with unaffected controls, but this result was not confirmed after Bonferroni correction (\( p_{corr} = 0.105; \ Table 4 \)). For STAT5b and IRF5, no significant differences in the frequencies of alleles and genotypes were detected between IBD patients and unaffected controls.

### 4. Discussion

This study examined the association of SNPs in several candidate genes to IBD susceptibility. Our results reveal a significant association of the SNP rs1148247 in the CREM gene with IBD, which raises the possibility that CREM is a susceptibility genetic factor for this complex disease. Consistent with our results, a recent case-control study reported an association of the CREM gene with CD in Italian patients [28]. Nevertheless, our results suggest that CREM may be associated with both UC and CD, supporting the notion that CREM is a general susceptibility factor in inflammatory bowel syndromes.

CREM is a transcription factor of the leucine-zipper family that also includes CREB, CREB-2, and ATF1, -2, and -3 [29]. All members share highly homologous structure in both the DNA-binding and the kinase-inducible domains. However, they differ from each other in the number of activation domains they contain. CREMa and CREMb, unlike CREB, do not encode the Q1 and Q2 activation domains; therefore, they function as transcriptional suppressors [30]. CREM binds to the CAMP response element either as a homodimer or as a CREM/CREB heterodimer. Binding of CREM to the IL-2 promoter in the anergic T cell line AET [31] and SLE T cells [32,33] limits the production of IL-2. IL-2 plays a key role in setting the balance between immunity and tolerance. This cytokine has a dual role as the regulator of the 2 main phases of the immune response (proliferative and suppressive). Likewise, activation-induced cell death and the induction and maintenance of Tregs are the tolerance mechanisms regulated by IL-2, which convey the link between IL-2 abnormalities and the development of autoimmune disorders [34].

In line with previous reports of significant associations of genetic variants in the STAT4 gene with the risk for autoimmune diseases, such as SLE, rheumatoid arthritis, and psoriasis [15,25–37], our study identified the STAT4 SNP rs7574865 as being marginally associated with UC susceptibility. However, our analysis did not detect an association with CD susceptibility. Similar to our

### Table 3

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findings, a recent Korean study reported no association of STAT4 SNPs with CD but a weak association of the STAT4 SNP rs925847 with susceptibility to UC (p = 0.025; OR = 0.63) [38]. In contrast, a Spanish study including 674 IBD patients reported a significant association of the STAT4 variant rs7574865 with CD susceptibility [39]. Another recent study from Spain reported no association of the STAT4 SNP rs7574865 with CD and UC [40], whereas an extended meta-analysis indicated a disease association of this SNP with UC and not CD in the Spanish population [41]. Considering the limited data of STAT4 gene variants in IBD patients and the conflicting results from the Spanish studies, the impact of STAT4 on IBD susceptibility seems limited, but a trend does exist supporting a possible role of STAT4 in UC susceptibility.

We have also observed that the SNP rs 3198502 in the STAT5a gene was weakly associated with IBD, whereas SNPs in the STAT5b gene failed to demonstrate an association. STAT5a and STAT5b play essential redundant and nonredundant roles in orchestrating immunoregulation and the development of immune cells. Notably, in the complete absence of STAT5, mice failed to develop T, B, and natural killer cells [41,42]. Mice that express N-terminally truncated STAT5 displayed less severe immunologic defects [43–45]. Recent studies have demonstrated that STAT5 is the critical link between the IL-2/15 and FOXP3 [46,18], the master regulator of Treg cells. It is conceivable that the reduced sample size in our study hampered the detection of existing weak associations and studies in larger samples are
required to more precisely define the role of STAT5a in IBD susceptibility.

In our study, we did not observe any association between IBD and the 5 IRF5 SNPs studied. Consistent with our results, Dideberg et al. [47] reported no association of these IRF5 SNPs with IBD patients from Leuven, Belgium. This study, however, included a second cohort of IBD patients from Wallonia, Belgium, with a significant association of IRF5 SNPs and the CCGGG indel polymorphism with IBD susceptibility. A strong association signal \( (p = 1.4 \times 10^{-8}, OR = 1.62 [1.37 ; 1.91]) \) with IBD from Leuven and Wallonia, Belgium, was observed for a 5-bp indel (CCGGG) polymorphism in the promoter region of the IRF5 gene. The association was also observed in CD patients \( (p = 3.3 \times 10^{-6}) \) and was particularly strong among the UC patients \( (p = 7.9 \times 10^{-10}, OR = 1.93 [1.56 ; 2.38]) \). The absence of association signals in our sample could be caused by the lack of statistical power or by the genetic makeup of the Tunisian population under study. In addition, we cannot rule out that the association tests may be influenced by unknown biases in age and sex of controls.

In conclusion, our study indicates a direct genetic association between CREM and IBD. The GC genotype at rs1148247 appears to contribute to Crohn’s disease susceptibility. However, further studies involving larger numbers of IBD patients should be performed before arriving at a definitive conclusion regarding the implications of the analyzed SNP in IBD susceptibility.

Acknowledgments

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