No association between the functional cannabinoid receptor type 2 Q63R variants and inflammatory bowel disease in Turkish subjects

ABSTRACT

Background/Aims: The endocannabinoid system can exert beneficial effects on gastrointestinal inflammation, and cannabinoid receptor-2 (CB2) agonists may represent a new therapeutic approach in inflammatory bowel disease (IBD). A functional CB2 Q63R polymorphism (rs35761398) in the CNR2 gene has been shown to affect the immunomodulating properties of the CB2 receptor. We sought to investigate whether the functional CB2 Q63R polymorphism (rs35761398) is associated with IBD susceptibility in a Turkish clinical sample.

Materials and Methods: A total of 202 IBD patients, comprising 101 Crohn’s disease (CD) patients and 101 ulcerative colitis (UC) patients, and 101 healthy controls were included in the study. The CB2 Q63R polymorphism was genotyped using real-time PCR.

Results: There were no significant differences in the genotype frequencies of the three study groups. The odds ratio of the minor Q allele for CD relative to the common R allele was not significant (OR =1.02, 95% CI =0.67-1.56, p=0.99). Similarly, the odds ratio of the minor Q allele for UC relative to the common R allele did not reach statistical significance (OR =1.10, 95% CI =0.72-1.68, p=0.75). Moreover, the genotype frequencies did not show any significant association with the disease extent in either CD (p=0.71) or UC patients (p=0.59).

Conclusion: These pilot findings suggest that CB2 Q63R polymorphism does not play a major role in genetic susceptibility to IBD or in its disease phenotypes among Turkish subjects.

Keywords: Crohn’s disease, ulcerative colitis, cannabinoid receptor-2, polymorphism

INTRODUCTION

Chronic inflammatory bowel diseases (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are one of the most common causes of chronic gastrointestinal disease and represent a major cause of morbidity worldwide (1-3). CD is characterized by patchy transmural inflammation affecting any part of the gastrointestinal tract, from mouth to anus; over time, CD almost inevitably progresses, often leading to stricturing or fistulizing complications (4,5). In contrast, UC is characterized by continuous mucosal inflammation that always involves the rectum while extending to involve a variable extent of the colon proximally (6,7). Several distinct genetic and environmental factors contribute to the pathogenesis of IBD, which is probably modulated by their complex interaction (8,9). Initial evidence for a genetic predisposition to the development of IBD stems from the observation of differences in the incidence of IBD between different ethnic groups (10). There is well-documented evidence that Caucasians in Europe and North America have the highest rates of IBD, followed by Afro-Americans and then Asians (11). Moreover, one of the greatest risk factors for the development of IBD is having an affected family member, with approximately 5-10% of all affected individuals with IBD reporting a positive family history (12).

Cannabinoid receptors (CBs) are seven-transmembrane domain G protein-coupled receptors that can bind to both exogenous and endogenous cannabinoids (13). Two distinct types of CBs are known to date, namely cannabinoid receptor-1 (CB1) and cannabinoid receptor-2 (CB2) (14). The CB1 receptor is abundantly expressed in the central nervous system (CNS) (15).
whereas the CB2 receptor can be predominantly found outside the CNS, including the gut mucosa (16). Mounting evidence suggests that gut inflammation is accompanied by a hyperexpression of cannabinoid receptors (17). In particular, UC is characterized by increased intestinal levels of the endocannabinoid anandamide (18). Importantly, Wright et al. (19) have shown that the epithelium of colonic tissue characteristic of IBD displays an increased CB2 receptor immunoreactivity, which is more prominent in the epithelium at the crypt fissures where ulcerations can be found. Experimental data obtained in colonic epithelial cell lines also suggest that the pharmacological engagement of the CB2 receptor is protective against gut inflammation (20), mainly via the direct suppression of the proinflammatory molecules involved in IBD pathogenesis. The CB2 receptor is encoded by the Cnr2 gene, which maps on 1p36 (21), a genetic locus that has been implicated in IBD susceptibility through linkage studies (22). Previous studies (23-25) have identified a common Q63R polymorphism (rs35761398) in the Cnr2 gene that has a significant functional effect on the CB2 receptor. In particular, the presence of the homozygous RR genotype has been associated with a significantly lower functional response of the CB2 receptor after its engagement by endocannabinoids, compared with the homozygous QQ genotype (24).

To clarify the potential role of CB2 in the genetic susceptibility to IBD, we herein investigated whether the CB2 functional Q63R variant is associated with CD and UC in a Turkish clinical sample.

**MATERIALS AND METHODS**

The local Ethics Committees approved the protocol, and written informed consent was obtained from all participants. The study was conducted in compliance with the tenets of the Declaration of Helsinki.

**Study participants**

The IBD sample consisted of 202 subjects, including patients with CD (CD cases, n=101) and patients with UC (UC cases, n=101). The diagnosis of IBD was based on standard clinical, endoscopic, radiological, and histological criteria. A total of 101 unrelated, healthy volunteers recruited from the general population with no symptoms and no family history of IBD formed a control group. All volunteers were questioned regarding IBD symptoms, as well as for symptoms of other immune mediated diseases, such as rheumatologic conditions and psoriasis. The control subjects had no lower abdominal symptoms, diarrhea, or hematochezia. All patients and controls were confirmed to have parents and grandparents born in Turkey to ensure ethnicity. Consequently, the possible confounding effects of the inclusion in the study of members of different ethnic groups have been minimized.

**Genotyping**

Venous blood samples (2 mL) were collected from the study participants into EDTA-containing tubes and stored at +4°C until analysis. Genomic DNA was isolated from peripheral blood leukocytes using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) within one week of blood sampling. Genotyping of the CB2 Q63R polymorphism was performed by real-time PCR using fluorescence melting curve detection analysis on a Light Cycler 1.5 System (Roche Diagnostics, Mannheim, Germany). Primers and hybridization probes were designed using the GenBank reference sequence (NM_001841.2).

**Data analysis**

All statistical calculations were performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) for Windows. Continuous variables are expressed as means ± SD and were compared between the study groups using one-way ANOVA. We tested the Hardy-Weinberg equilibrium using online resources (http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm) (26). Genotype frequencies and categorical variables were compared using the χ2 test, with α set at 0.05 (two-tailed). We used multivariable logistic regression analysis to analyze the association between the functional CB2 Q63R polymorphism and IBD after adjusting for age and sex. Results were expressed as odds ratios (OR) with 95% confidence intervals (CI). The StatMate program version 2.0 (GraphPad Software, San Diego, CA, USA) was used to calculate the study’s power.

**RESULTS**

**The CB2 Q63R polymorphism and susceptibility to IBD**

The mean age was significantly lower in controls (35.5±11.0 years, p<0.001) and CD patients (38.7±12.1 years, p<0.001) than in those with UC (46.8±13.9 years). However, the sex distribution (males/females) did not differ significantly among controls (47/54), CD patients (43/58, p=0.67), and UC cases (43/58, p=0.67). Table 1 displays the distribution of the CB2 Q63R polymorphism in CD patients, UC patients, and healthy controls. The genotype frequencies in our sample followed the Hardy-Weinberg equilibrium in all three of the study groups (CD patients; p=0.70; UC patients; p=0.71; healthy controls; p=0.69). There were no significant differences in the genotype frequencies in the three study groups. The odds ratio of the minor Q allele for CD relative to the common R allele was not significant (OR =1.02, 95% CI =0.67-1.56, p=0.99). Similarly, the odds ratio of the minor Q allele for UC relative to the common R allele did not reach statistical significance (OR =1.10, 95% CI =0.72-1.68, p=0.75). These results did not change appreciably after adjusting for age and sex in multivariable regression analysis (OR =1.04, 95% CI 0.65-1.50, p=0.84 for CD; and OR =1.08, 95% CI 0.61-1.72, p=0.81 for UC).

**The CB2 Q63R polymorphism and clinical characteristics of IBD**

We next analyzed the distribution of the CB2 Q63R polymorphism in relation to the extent of the disease in each group.
of IBD patients (Table 2). However, the genotype frequencies did not show any significant association with disease extent in either CD (p=0.71) or UC patients (p=0.59). Similarly, when we divided the IBD patients into two groups according to a previous history of surgery, we found no significant effect of the CB2 Q63R polymorphism (data not shown).

## DISCUSSION

Genetic predisposition is a well-documented risk factor for the development of IBD (27). In the present study, we performed a case-control association analysis to test whether the risk for IBD might be influenced by the CB2 Q63R polymorphism in an ethnically homogeneous Turkish sample. The rationale for studying the CB2 Q63R polymorphism in the present study was based on (i) the linkage between the 1p36 locus, where CNR2 maps (21), and IBD (22); ii) the altered expression of CB2 in the inflamed gut mucosa (19); and (iii) the potential role of CB2 as a therapeutic target in IBD (28). However, the CB2 Q63R polymorphism was associated with neither susceptibility to IBD nor with its disease phenotype. Based on the observed prevalence of the minor Q allele, our study had an 80% power to detect a relative risk of 1.84 with a significance level (alpha) of 0.05 (two-tailed) between IBD cases and controls.

Previous studies have shown that the Q63R polymorphism of the CB2 gene is associated with alcoholism and depression in Japanese individuals (29,30). Moreover, this polymorphism may predispose to the development of childhood immune thrombocytopenic purpura in independent populations from Italy (31) and Egypt (32). In the field of gastroenterology, the CB2 Q63R variant has been suggested to modulate hepatic inflammation and the risk of liver damage in obese children (25). Moreover, the Q63R polymorphism seems to increase more than six-fold the risk for developing celiac disease (33).

As is the case for any inflammatory disorder, other factors may...
be involved in the pathogenesis of IBD besides genetic predisposition, including environmental and host factors (8,9). Moreover, a single polymorphism may have no clinical significance if evaluated independently, but specific combinations of common polymorphisms and the combination of related polymorphisms might play a relevant role in IBD.

One of the major limitations of this work was its small sample size, which may limit the generalizability of its conclusions. Moreover, the limited sample size could affect the association analyses performed in subgroups, e.g., in relation to the extent of the disease, because subgroup analysis reduces sample sizes and may introduce some bias in the statistical calculations. Finally, because we did not have information on environmental factors, such as smoking habits and alcohol consumption, we were unable to assess the interactions between those environmental factors and the CB2 Q63R polymorphism. These caveats notwithstanding, our study provided preliminary evidence that the CB2 Q63R polymorphism is not associated with IBD or its clinical phenotypes in the Turkish population.

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Conflict of Interest: No conflict of interest was declared by the authors.

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