

IRRITABLE BOWEL SYNDROME: A DYSFUNCTION OF THE ENDOCANNABINOID SYSTEM?

Park JM, Choi MG, Cho YK. Cannabinoid receptor 1 gene polymorphism and irritable bowel syndrome in the Korean population: a hypothesis-generating study. *J Clin Gastroenterol* 2011;45:45–49.

Up to 20% of adults have symptoms consistent with a diagnosis of irritable bowel syndrome (IBS), a functional gastrointestinal (GI) disorder characterized by the Rome III criteria, which include abdominal discomfort, bloating, and altered bowel habits in absence of organic abnormalities. The pathophysiology of IBS remains unclear, incorporating biological as well as psychosocial factors. The current understanding suggests that multiple factors contribute to the development of IBS in individual patients. There is principal agreement that, among other infections, altered gut motility, disturbed gut–brain signaling, visceral hypersensitivity, and bacterial overgrowth as well as psychosocial, environmental, and genetic factors may contribute to the pathophysiology of IBS.

The recent publication by Park et al investigated AAT triplet repeat polymorphisms in the Cannabinoid Receptor 1 Gene (CNR1) in a group of patients with IBS. The overarching hypothesis was that the CNR1 polymorphism may be associated with IBS. This hypothesis was based on information available from basic and clinical research. Animal studies show that cannabinoid receptors 1 and 2 are involved in antinociceptive pathways when visceral rectal pain is tested, and other animal studies provide evidence that the cannabinoid 1 receptor is crucially involved in reducing intestinal secretion and diarrhea in various animal models (*Pharmacol Ther* 2010;126:21–38). These studies suggest that the cannabinoid 1 receptor may play a role in the pathophysiology of visceral hypersensitivity and diarrhea, both cardinal symptoms of IBS. Additionally, human studies show that external cannabinoids can reduce colonic compliance during a colonic barostat examination and can reduce colonic motility in healthy volunteers, observations that strengthen the overarching hypothesis (*Am J Physiol Gastrointest Liver Physiol* 2007;293:G137–G145).

The study was performed in a tertiary referring center in Seoul, Korea, where 162 patients with IBS and 423 healthy individuals without GI or GI symptoms, who served as controls, were included. The recruitment took place from 2004 until 2007; thus, ROME II criteria were used to identify people with IBS. Before inclusion, colonoscopy was normal and patients were excluded from entering the study if history comprised any abdominal surgery (except appendectomy), if they were pregnant or breastfeeding, if they had a mental disorder or any systemic disease, or if they were receiving drug treatment for GI diseases. Another criteria for nonin-

clusion was drug treatment for IBS in the 3 months before study inclusion.

Patients were classified into constipation-predominant (IBS-C; n = 42) diarrhea-predominant (IBS-D; n = 85), or alternating symptoms (IBS-M; n = 35) groups. The collection of demographic information included age and gender. Abdominal pain or discomfort was assessed using a 7-point response scale (0 [no symptoms] to 6 [extreme symptoms]). Patients were additionally asked to quantify symptoms in the 30 days before inclusion in the study. In addition to the symptom quantification, a blood draw for genetic testing was taken and after extraction of genomic DNA, the occurrence of AAT-triplet polymorphisms within the CNR1 gene was quantified.

Demographic comparisons were made using the *t*-test and the chi-square test where applicable. The chi-square test was furthermore used to compare the frequency of the AAT triplet polymorphisms between healthy individuals and patients with IBS. Associations between CNR1 genotypes and abdominal symptom score were tested using the Kruskal–Wallis test. Age and gender distribution were not different when comparing the group of patients with IBS with control subjects. The allelic distribution of the AAT triplets in the CNR1 gene differed significantly when distribution in patients with IBS was compared with healthy controls; whereas the (AAT)₉ and (AAT)₁₀ alleles were most frequent in healthy controls, the (AAT)₁₃, (AAT)₁₀, and (AAT)₁₄ alleles were the most frequent in the group of patients with IBS. For further analysis, the 13 possible alleles were divided into 2 groups, with group 1 comprising the shorter alleles (≤ 10 repeats) and group 2 comprising longer alleles (> 10). In consequence, 3 CNR1 genotypes (homozygote for ≤ 10 ; heterozygote; homozygote for > 10) were analyzed. The homozygote genotype $> 10 / > 10$ was significantly more common among patients with IBS and this finding was significant for the total IBS patient cohort, as well as the male or female patient cohorts. There was no difference in allele distribution between male and female or between different age groups. Interestingly, when comparing the CNR1 genotype for the IBS subgroups, the IBS-C, the IBS-D and the IBS-M cohorts had a significant higher frequency of the $> 10 / > 10$ genotype. There was no significant difference of genotype distribution between the 3 IBS subtypes. Interestingly, the $> 10 / > 10$ genotype was significantly associated with higher abdominal pain and discomfort scores, whereas no association was found for the CNR1 genotype and symptom frequency.

In summary, the study by Park et al. suggests that polymorphisms in the CNR1 receptor are more common among patients with IBS and that the AAT $> 10 / > 10$ allele genotype is associated with a higher symptom score but not higher symptom frequency. No differences were found between IBS subgroups, age, or gender.

Comment. A genetic component in the pathophysiology of IBS was suggested by twin studies and epidemiologic studies in the past. More recently, genetic association studies investigating the IBS phenotype and the possible link of phenotypic pattern to specific genes or gene polymorphisms provided stronger evidence on such genetic components. Although the evidence of a genetic link to IBS is unexplained if not equivocal, multiple genes or gene polymorphisms were suggested to be associated with IBS (for review, see *Dig Dis Sci* 2009;54:2318–2324).

The strongest evidence for such genetic associations presently exists for genes within the serotonin (5-HT) system. Different polymorphisms in the genes encoding for the serotonin transporter (SERT) like the *5-HTTLPR*, the *STin2 VNTR*, the *SERT-P*, and the *rs25531* polymorphisms in the *SLC6A4* gene, or 5-HT receptor encoding genes like polymorphisms in the *5-HT2A* and the *5-HT3* receptor gene were reported to be associated with IBS or specific IBS phenotypes, but most of the studies are limited by a small number of included patients, especially when IBS subtypes are investigated. Furthermore, evidence was published that polymorphisms in the $\alpha 2C$ -adrenoreceptor gene, the interleukin (IL)-10 gene, the *GN β 3* G-protein-coupled receptor gene, the catechol-*o*-methyltransferase (COMT) gene, and the *SCN5A* gene (encoding a Na⁺ channel on interstitial cells of Cajal), are associated with different IBS phenotypes. Additional preliminary evidence was published that polymorphisms in the genes of the cholecystokinin-1 (CCK-1) receptor, IL-6, IL-4, IL-10, and tumor necrosis factor- α may be associated with IBS. With more studies being published investigating genotype-phenotype associations, the numbers of studies questioning previously reported associations of the *GN β 3*, IL-10, and tumor necrosis factor- α , and other genes with IBS is increasing.

This *cacophony* leads to a number of general criticisms on polymorphism-phenotype association studies. Polymorphism studies are generally hampered by the fact that the functional consequences of the genetic alterations are uncertain and speculative, and most of the studies do not address or investigate a possible relevant functional change arising in consequence of the polymorphism. Moreover, the hypotheses on possible factors linking the genotype with a respective phenotype are frequently unclear or weak at best. Especially in the context of IBS, where multiple factors contribute to a variable phenotype, these genetic associations have to be considered carefully as a multifactorial pathophysiology that is unlikely to be explained by a single genetic variation. In addition, our phenotypic classification of patients with IBS is based on the largely subjective ROME III criteria. As suggested recently, this classification may be helpful for the direction of symptomatic treatment, but may be misleading in studies investigating the pathophysiology of IBS. Additionally, owing to small patient numbers and the lack of direct gender-

and/or age-matching protocols in these association studies, the results are likely to be misleading. Larger sample sizes and multicenter approaches are ideally required to result in meaningful information. Such large, multicenter approaches are nowadays state of the art in genetic studies of IBD and this should be adapted to genetic studies in IBS and other functional GI disorders.

Other studies tested the presence of a genotype in the context of a testable functional parameter rather than only the phenotypic classification as IBS. For example, a gene polymorphism in the 5-hydroxytryptamine transporter long polymorphic region (*5-HTTLPR*) within the promoter for the serotonin transporter protein, is associated with increased pain sensation and increased rectal compliance, as tested with rectal barostat measurements (*Am J Physiol Gastrointest Liver Physiol* 2008;295:G219–G225). This functional relevance was shown for patients hetero- or homozygote for this polymorphism (LS/SS genotype) compared with the heterozygote “normal” LL genotype. This study demonstrated that, in patients with the LS/SS genotype, pain sensation ratings were increased, which emphasizes a possible functional relevance of this polymorphism. A smaller study in 28 healthy volunteers who are homozygote holders of the S allele of the *5-HTTLPR* gene noted central responses to rectal balloon sensation (blood flow measured by positron emission tomography) was increased in emotion-regulating brain regions (*Neuroimage* 2009;47:946–951). This suggests that a homozygote phenotype in this SERT gene polymorphism may play a role in visceral hypersensitivity. Unfortunately, this thought was never tested in patients with IBS.

At a genetic level, the study by Park et al suggested an association of the presence of the *CNR1* gene (which encodes the CB₁ receptor) polymorphism to the presence of IBS and to higher symptom scores in these IBS patients. Though multiple limitations, this study is the first study linking the *CNR1* receptor to IBS and IBS symptom severity.

One important limitation is that the AAT-repeat polymorphism in the *CNR1* gene is only 1 known polymorphism type in the *CNR1* gene that was studied. Other polymorphisms in the *CNR1* gene like the more frequently studied rs1049353 (G1359A) polymorphism or another 11+ published polymorphisms were not investigated. The functional relevance of all of these polymorphisms, which impact receptor expression, receptor metabolism, receptor-binding characteristics, or receptor functioning, are not clear. Therefore, choosing an “ideal” polymorphism to study remains a challenge and studying all known polymorphisms for this gene may be superior over selecting just 1 or a limited number of polymorphisms. In this context, it seems important that none of the AAT repeat polymorphisms seem to be in the receptor encoding region, and thus the relevance of these polymorphisms remains debatable. In this small study population, it may have been worthwhile to test additional rectal sensitivity in a

barostat examination, as such data may have resulted in stronger and more convincing results.

It remains somewhat unclear why medication use for IBS during the last 3 months was deemed to be an exclusion criterion. This may have led to a significant bias in patient selection as patients with more severe forms of the disease were likely to be excluded. Whether this bias may have resulted in positive, negative, or no bias to the results remains unknown. Additional minor limitations of the study are the single-center nature of the small study population and the Korean population, because allelic distribution may vary in different populations.

Previously, evidence for a genetic association of IBS and the endocannabinoid system was presented for a single-nucleotide polymorphism (C385A) was shown in the fatty-acid-amide-hydrolase (*FAAH*) gene, an enzyme important in the degradation of endogenous cannabinoids (*Am J Physiol Gastrointest Liver Physiol* 2008; 294:G13–G19). In this study, the *FAAH* gene polymorphism was associated with functional changes and colonic transit was accelerated in presence of the polymorphism. This acceleration was significant in patients with diarrhea-predominant IBS (IBS-D) and mixed-form IBS (IBS-M), stressing the putative role of the endocannabinoid system and possible role in the genetics of the endocannabinoid system in patients with IBS. Data investigating the *FAAH* or the *CNR1* polymorphism in association with tests assessing rectal sensation or rectal pain are not available, despite being of great interest as evidence of an endocannabinoid role in visceral sensation in animal research.

A concept of “clinical endocannabinoid deficiency” being associated with IBS was suggested in the past (*Neuroendocrinol Lett* 2004;25:31–39), and the study by Park et al extends this hypothesis to the point where endocannabinoid deficiency rather than changes at the receptor level may be of importance in the pathophysiology of IBS. Whether this role is relevant in the control of motility, sensation, mucosal integrity, maintaining intestinal microbiota, cross-talk with intestinal microbiota, or even inflammation associated with IBS remains to be speculative but basic research is highly supportive of such interactions. Interestingly, studying *CB₁^{-/-}* receptor knockout mice showed that *CB₁^{-/-}* mice have an altered response to adverse memory extinction, broadly suggesting that an anxious phenotype would be expected if *CNR1* is dysfunctional. To speculate on how that knowledge would translate into a possible involvement in IBS pathophysiology remains academically appealing.

Additional support for a potential role of *CNR1* comes from recent studies where antagonists at the

CNR1 receptor like rimonabant and taranabant were tested in short- and long-term clinical trials. The majority of these trials indicated that patients or volunteers taking these drugs have a high and, throughout the different studies, consistent likelihood of developing diarrhea as a side effect (*J Clin Pharmacol* 2008;48: 734–744; *N Engl J Med* 2005;353:2121–2134; *JAMA* 2008;299:1547–1560), which may serve as an indirect indicator of the putative role of the *CNR1* in maintaining motility and *CNR1* blockade or dysfunction resulting in diarrhea. This notion is emphasized by numerous animal studies using cannabinoid receptor-1 (*CB₁*) antagonists or *CB₁^{-/-}* receptor knockout mice. Human studies treating patients with IBS using a *CNR1* antagonist are presently not available, but may help to understand the exact involvement of *CNR1* in IBS pathophysiology. On the other hand, there are studies investigating GI motility or visceral sensation of GI origin. Despite supporting the concept of a cannabinoid role in the generation or treatment of GI symptoms that may be linked to IBS, interpretation of these studies is limited owing to the central side effects of the used compounds.

Finally, the endocannabinoid system, including their receptors and metabolic pathways, may be involved in the pathophysiology of IBS and it is now multifold evidence from genetic studies, clinical trials, and basic science that supports this notion. Park et al add another puzzle stone, by showing that a polymorphism in the *CNR1* gene is associated with IBS and greater IBS symptoms, a finding that is supported by numerous publications suggesting such a role of *CNR1*. The exact role of endocannabinoid control within the pathophysiology of IBS or the development of some of the associated symptoms has yet to be determined. With multiple potential drugs targeting the endocannabinoid system at different sites on the horizon, it seems appealing to learn more about the role of the endocannabinoid system in the context of IBS or other functional GI disorders, because this bears the potential of discovering potential future treatments for IBS or some of its symptoms.

MARTIN STORR

II Medical Department
Klinikum Groshadern
Ludwig-Maximilians University Munich

HANS-DIETER ALLESCHER

Zentrum Innere Medizin
Klinikum Garmisch-Partenkirchen,
Academic Teaching Hospital of the LMU Munich
Munich, Germany