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Rare Variants of the Serotonin Transporter Are Associated With Psychiatric Comorbidity in Irritable Bowel Syndrome

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Abstract

Alterations in serotonin signaling are suspected in the pathophysiology of irritable bowel syndrome (IBS). By modulating the extracellular reuptake of serotonin, the serotonin transporter (SERT) acts as a key regulator of the bioavailability of serotonin. This study is the first to investigate the impact of rare *SERT* variants (i.e., those with a minor allele frequency of < 1%) on the risk for IBS, gastrointestinal (GI) symptom level, response to cognitive–behavioral treatment, and psychiatric comorbidity. We sequenced a 0.19 megabase chromosomal stretch containing the *SERT* gene and surrounding regions in a community sample of 304 IBS patients and 83 controls. We found no significant associations between rare variants in and around the *SERT* gene and IBS risk, GI symptom profile, or response to treatment. We found preliminary evidence, however, that IBS subjects with a history of either depression or anxiety were significantly more likely to carry multiple rare likely functional variant alleles than IBS patients without psychiatric comorbidity.

Keywords

irritable bowel syndrome, serotonin transporter, genetic variants, sequencing, depression, anxiety

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder characterized by recurrent abdominal pain or discomfort associated with altered bowel habits (Canavan, West, & Card, 2014; Drossman, 2006). The pathophysiology of IBS is complex and heterogeneous, involving gut factors, brain–gut interactions, environmental contributions, and genetic factors (Chey, Kurlander, & Eswaran, 2015; Saito et al., 2010; Svedberg, Johansson, Wallander, & Pedersen, 2008; Vehof, Zavos, Lachance, Hammond, & Williams, 2014). A striking feature of IBS is its high degree of psychiatric comorbidity; in particular, the prevalence of depression and anxiety disorders is significantly higher in patients with IBS than in healthy controls (Folks, 2004; Fond et al., 2014; Mykletun et al., 2010; Whitehead, Palsson, & Jones, 2002). More than half of the patients with IBS who seek treatment have a psychiatric comorbidity; at the same time the prevalence of IBS is higher in psychiatric populations (Garakani et al., 2003; Lee et al., 2009; Lydiard, 2001). This frequent comorbidity suggests the possibility of shared etiological factors among IBS, depression, and anxiety.

Serotonin is an important signaling molecule regulating gut motility, secretion, and GI sensation through enterochromaffin cells, serotonergic neurons of the myenteric plexus, and the central nervous system (Gershon, Drakontides, & Ross, 1965; Gershon & Tack, 2007). Hence, authors have suggested impaired serotonergic function as an important etiologic factor in IBS (Crowell, 2004). This idea is supported by the therapeutic success of serotonin-modulating drugs in the treatment of patients with IBS (Barboza, Talley, & Moshiree, 2014). By modulating the extracellular reuptake of serotonin, the serotonin transporter (SERT) acts as a key regulator of the bioavailability of serotonin (Haddley, Bubb, Breen, Parades-Esquivel, & Quinn, 2012). The *SERT* gene is located on chromosome 17q11.1-17q12 and organized into 14 exons spanning approximately 38 kb (Lesch et al., 1994; Ramamoorthy et al., 1993). Prior work has shown associations between decreased expression of SERT and IBS (Coates et al., 2004; Foley et al., 2011; Franke et al., 2010). Mice with congenitally lost or impaired

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SERT function resemble patients with IBS in that they show IBS-like GI symptoms of alternating diarrhea and constipation in combination with behaviors suggesting depression and anxiety (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Chen et al., 2001; Lira et al., 2003; Liu, Rayport, Jiang, Murphy, & Gershon, 2002; Popa, Lena, Alexandre, & Adrien, 2008). Other studies have also shown associations between IBS prevalence, symptom severity, or psychiatric comorbidity in IBS patients on the one hand and common variants in the *SERT* gene on the other (Colucci et al., 2013; Jarrett et al., 2007; Kohen et al., 2009; Zhang et al., 2014). In contrast to prior work investigating common variants in the *SERT* gene, the present study is the first to investigate a possible association between rare *SERT* variants and IBS.

Rare genetic variants (i.e., genetic polymorphisms with a minor allele frequency [MAF] of < 1%) make up the bulk of variation in the human genome (Fu et al., 2013). As purifying selection removes harmful polymorphisms from the genome over time, the chance of a coding genetic variant being harmful rises in inverse proportion to its frequency (Tennessen et al., 2012). In contrast to protein-coding exon variants, however, noncoding mutations are subjected to purifying selection only if they are located in functionally important noncoding regions. The *Encyclopedia of DNA Elements* (*ENCODE*) is a set of publicly available annotations of the human genome that includes information about the location of functional elements in DNA, including protein-coding segments and transcriptional regulatory regions (ENCODE Project Consortium, 2011). Hence we hypothesized that the presence of one or more rare genetic variants, located in ENCODE-annotated functional regions in and around the *SERT* gene, could be associated with IBS, its symptom burden, response to treatment, and/or comorbid psychiatric conditions.

Material and Method

Participants

For this study we used DNA samples and survey data from four case–control studies of IBS carried out in western Washington State. Extensive description of the study samples can be found in previous reports (Heitkemper et al., 2012; Jarrett et al., 2007, 2009; Motzer, Jarrett, Heitkemper, & Tsuji, 2002). Briefly, we recruited patients with IBS with a prior diagnosis who currently met the Rome III criteria and healthy controls without history of functional GI disorders through community advertisement (Drossman, 2006). We excluded IBS patients or healthy control subjects if they (a) had a history of coexisting GI pathology (e.g., inflammatory bowel disease) or surgery, renal or reproductive pathology (e.g., endometriosis), severe fibromyalgia, or severe cardiovascular disease or (b) were currently taking any of the following medications on more than 3 days a week: antibiotics, anticholinergics, cholestyramine, narcotics, colchicines, docusate, an enema preparation, iron supplements, or laxatives. The University of Washington's institutional review board approved the protocols for this study and the four parent studies, and all participants gave written informed consent. Inclusion and exclusion criteria were dictated by the parent studies from which our samples were drawn. For this study, we restricted our analysis sample to individuals who identified themselves as White to avoid population stratification bias, as prior studies have identified marked ethnic differences in *SERT* sequence (Gelernter, Kranzler, & Cubells, 1997; Nakamura, Ueno, Sano, & Tanabe, 2000). Our final analysis sample consisted of 304 IBS participants and 83 controls; Table 1 shows participant characteristics. Depression and anxiety were significantly more common among participants with IBS than among controls.

Table 1. Participant Characteristics.

Characteristic	IBS ($n = 304$), n (%)	Controls ($n = 83$), n (%)	p Value
Gender, female	264 (87)	76 (92)	.243[a]
Age[b]	40.1 ± 14.3	37.5 ± 13.3	.056[b]
Predominant bowel pattern			
Normal	19 (6)	0	
Constipation	69 (23)	0	
Diarrhea	150 (49)	0	
Alternating	8 (3)	0	
Mixed	35 (12)	0	
Not assessed	23 (8)	83 (100)	
Psychiatric comorbidity			
Depression	127 (44)	21 (27)	.005[a,c]
Anxiety	149 (52)	24 (31)	.001[a,c]
Both depression and anxiety	90 (31)	12 (15)	.006[a,c]
Neither depression nor anxiety	102 (35)	45 (58)	<.000[a,c]

Note. IBS = irritable bowel syndrome.
[a]p Value is based on χ^2 test.
[b]p Value is based on t-test.[c]Psychiatric comorbidity data are missing in some cases and controls: a history of depression was assessed in 286 IBS cases and 78 controls; a history of anxiety was assessed in 288 IBS cases and 78 controls; percentages are based on these numbers.

SERT Sequencing

Genomic DNA was extracted from fresh whole blood or frozen isolated white blood cells by buffy coat preparation (Miller, Dykes, & Polesky, 1988) using Puregene DNA Purification kits (Gentra Systems Inc., Minneapolis, MN) or Qiagen DNeasy Blood & Tissue kits (Qiagen, Valencia, CA). Haloplex Target Enrichment kits (Agilent Technologies, Santa Clara, CA) were customized to prepare sequencing libraries from a 0.19 megabase (MB) chromosomal region centered around the *SERT* gene (coordinates: chr17:28,435,000–28,625,000). Multiplexed bar-coded libraries (96 per lane) were sequenced with 76 bp paired-end reads on an Illumina HiSeq 2000 instrument (Illumina, San Diego, CA). Sequenced reads were mapped to the hg19 reference genome, and genetic variants were identified with the GATK HaplotypeCaller, Version 3.3, using default settings. Variants called in less than 90% of subjects or with a genotype quality of less than 50 or with deviation from Hardy–Weinberg equilibrium at $p < .01$ were

excluded from further analysis. Subjects with <30× sequencing depth were excluded as well.

Additional Measures

Lifetime history of mental health disorders. We assessed the lifetime history of mental health disorders using the World Health Organization Composite International Diagnostic Interview (CIDI) for the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Andrews & Peters, 1998). Specifically, we used data about depression and anxiety from the CIDI mood-disorders module (depression as single and recurrent episodes; subtypes mild, moderate, and severe and dysthymia) and anxiety-disorders module (panic disorder with or without agoraphobia, agoraphobia, generalized anxiety disorder, obsessive-compulsive disorder, phobias including social phobia, and post-traumatic stress disorder). We coded results as 1 if a participant met the criteria for a present or past diagnosis of a particular depressive or anxiety disorder or 0 if she or he did not meet the criteria.

IBS-related GI symptom score (IBS-GI). We assessed IBS symptom severity in participants with IBS using a daily diary filled out over 28 days. In the diary, participants rated 26 symptoms on a scale of 0 (*not present*), 1 (*mild*), 2 (*moderate*), 3 (*severe*), or 4 (*very severe*). Of these symptoms, six were GI symptoms related to IBS: abdominal pain or discomfort, bloating, constipation, diarrhea, intestinal gas, and urgency. We computed an IBS symptom score by first determining the severity of the worst IBS symptom on each day to get an IBS severity for that day then collapsing across the 28 diary days for each participant to determine the percentage of days with moderate to very severe GI symptoms (Jarrett et al., 2009).

IBS-related quality of life (IBS-QOL). We assessed IBS-QOL in participants with IBS using a 42-item questionnaire with nine scales: sleep, emotional, mental health beliefs, energy, physical functioning, diet, social role, physical role, and sexual relations (Hahn, Kirchdoerfer, Fullerton, & Mayer, 1997). Examples of questions are, ''How often did your IBS make you feel fed up or frustrated'' (1 = *always*, 2 = *often*, 3 = *sometimes*, 4 = *seldom*, or 5 = *never*) or ''My IBS affected my ability to succeed at work/main activity'' (1 = *strongly agree* to 5 = *strongly disagree*). We transformed the scales to a standard 0–100 scale and computed a total score by averaging all but two of the scales (eating/diet and sexual relations), as described in our previous work (Jarrett et al., 2009).

Treatment outcomes. We analyzed treatment outcomes in relation to functional rare *SERT* variant burden in a subgroup of 116 subjects with IBS who had participated in a treatment trial and who had been randomized to the active cognitive–behavioral intervention, Comprehensive Self-Management (CSM; Jarrett et al., 2009). We assessed outcomes as change in IBS-GI or IBS-QOL from baseline to 3 months after study start (shortly after the end of the CSM intervention), change from baseline to 6 months, and change over 12 months.

Statistical Analysis

Hardy–Weinberg equilibrium was tested using χ^2 tests. Rare variants were single nucleotide polymorphisms (SNPs) or small insertion–deletion variants with an MAF of less than 1% that occurred in our sample of White subjects. Variants were classified as likely to be functionally important (LF) if ENCODE identified them as lying within one or more of the following three regions: (a) a coding region, (b) a DNAseI hypersensitive site, and/or (c) a transcription factor binding site within or surrounding the *SERT* gene. LF variants were not distinguished by the number of ENCODE annotations they carried (i.e., they were grouped together regardless of whether they carried one, two, or three ENCODE annotations). Variants with none of these three ENCODE annotations were considered likely nonfunctional (NF). Associations of LF or NF variant tallies across the entire sequenced region with participant characteristics were tested with a nonweighted collapsing method using χ^2 tests for dichotomized measures (e.g., IBS subject vs. control, history of depression or not) and analysis of variance for continuous measures (e.g., IBS-GI, or IBS-QOL; Dering, Hemmelmann, Pugh, & Ziegler, 2011).

Results

Across the entire 0.19 MB sequenced region we identified 628 variants in our 387 participants, including 578 SNPs and 50 small insertion–deletion polymorphisms up to 12 bp in length. Our sequencing methodology was not able to detect larger insertion–deletion variants like the 43 bp 5-HTTLPR length polymorphism but was able to identify SNPs (e.g., rs25531) lying within this length polymorphic region. Of these 628 variants, 418 had an MAF < 1%, while the remaining 210 variants were common (MAF ≥ 1%). Of the 418 rare variants, 102 fell into an ENCODE-annotated region and were therefore classified as LF, with the remaining 316 being NF. The majority of ENCODE annotations referred to DNAseI hypersensitive sites (85 of 102 rare LF variants) followed by transcription factor binding sites (38 of 102 rare LF variants), and coding variants located either within *SERT* (3 of 102 rare LF variants) or in either of two immediately flanking genes within the sequenced region (nuclear speckle splicing regulatory protein 1 [*NSRP1*] and bleomycin hydrolase [*BLMH*], 4 of 102 rare LF variants). We observed the three rare *SERT* coding variants once each in our sample. Two of these polymorphisms were synonymous substitutions (rs145183821 and rs114814153) and the third nonsynonymous (Lys605Asn, rs6352). Many variants carried dual annotations, most commonly for being located in both a DNAseI hypersensitive site and a transcription factor binding site.

No Association of Rare LF Variants With GI Symptoms or Response to Treatment

IBS patients (n = 304) and controls (n = 83) did not differ significantly in the number of rare LF variant alleles each

Table 2. Rare Likely Functional (LF) Variant Burden in Irritable Bowel Syndrome (IBS) Patients and Controls With Comorbid Depression or Anxiety Versus Neither Condition.

	IBS ($n = 288$)		Controls ($n = 78$)	
Number of Rare Alleles per Subject	Depression or Anxiety, *n* (%)	No Depression or Anxiety, *n* (%)	Depression or Anxiety, *n* (%)	No Depression or Anxiety, *n* (%)
0	120 (65)	65 (64)	19 (58)	24 (53)
1	47 (25)	35 (34)	10 (30)	15 (33)
2	15 (8)	***1 (1)***	3 (9)	6 (13)
3	4 (2)	***1 (1)***	1 (3)	0 (0)

Note. For each of four groups (IBS with comorbidity, $n = 186$; IBS without comorbidity, $n = 102$; controls with comorbidity, $n = 33$; and controls without comorbidity, $n = 45$), the number (% of group) of subjects carrying 0, 1, 2, or 3 rare minor alleles is shown, which is tallied across all rare LF variants observed in our sample. As highlighted in bold italic font, IBS patients without psychiatric comorbidity were less likely to carry multiple rare LF variants compared to those with comorbid depression and/or anxiety ($p = .039$).

subject carried. In IBS patients, the number of rare LF variant alleles per subject was not associated with symptom severity, as measured by IBS-GI and IBS-QOL scores. Moreover, in IBS patients who had undergone active treatment with CSM ($n = 116$), rare LF variant tally was not associated with treatment outcomes as measured by changes in IBS-GI and IBS-QOL from baseline to follow-up at 3, 6, or 12 months after the initial assessment (data not shown).

Association of Rare LF Variants With Comorbid Depression and Anxiety in IBS Patients

IBS patients with a history of either depression or anxiety were significantly more likely to carry multiple rare LF variant alleles than IBS patients without psychiatric comorbidity (χ^2 $p = .039$; Table 2). By contrast, control subjects with and without depression/anxiety comorbidity did not differ significantly in their rare LF variant tally (Table 2). The tally of NF rare variants was not associated with psychiatric comorbidity in either cases or controls (not shown).

Discussion

The present study is the first to examine possible associations of rare *SERT* variants with IBS. While we saw no associations with IBS, GI symptoms, or response to treatment, we did find evidence of higher LF variant burden among IBS patients with comorbid depression or anxiety.

Our study of rare variants was motivated by the observation that the bulk of genetic variation between humans is due to rare genetic polymorphisms, yet these variants have been understudied (Nelson et al., 2012; Sadee et al., 2014). Genetic variants outside of protein-coding regions exert their influence by modulating the expression levels of their target genes. Such functional noncoding variants can be located at a considerable distance from the genes whose expression they control, raising the question of how broad a region around the *SERT* gene cover in the search for variants influencing its level of expression (Albert & Kruglyak, 2015). CCCTC-binding factor (CTCF) is a ubiquitously expressed protein that helps establish a boundary between different DNA stretches and thus separates the regulation of a gene like *SERT* from that of its neighbors (Atkinson & Halfon, 2014; Phillips & Corces, 2009). Hence we chose our sequenced region to extend beyond the *SERT* gene on both sides up to and including its closest flanking CTCF binding sites, capturing most, if not all, regulatory elements that might play a role in *SERT* expression. We then further subdivided genetic variants within that region by whether they co-localized with ENCODE-annotated regions. The majority of ENCODE annotations refer to DNAseI, the enzyme most frequently used to identify regulatory elements by the degree to which the corresponding stretches of DNA are open and accessible to regulatory proteins (Marsman & Horsfield, 2012). However, since regulation of gene expression is tissue dependent, it cannot be assumed that all ENCODE annotations refer to regulatory elements that are active in the colonic mucosa, where they might contribute to the phenotype of IBS. Hence, the distinction between ENCODE-annotated LF variants and nonannotated NF variants in our study only denotes differing degrees of likelihood that a given variant may have functional effects in the GI tract, resulting in the classifications of variants as LF, or likely NF. Our investigation was further based on the hypothesis that rare LF alleles at polymorphic loci are more likely to be damaging than the common alleles, since the latter are more likely to represent the result of evolutionary pressures toward a healthy phenotype (Tennessen et al., 2012). Moreover, by interfering in the binding of regulatory proteins to DNA, an LF variant would be more likely to interfere with *SERT* expression than to raise its expression level, according to the general principle that loss-of-function mutations are far more common than gain-of-function mutations. Hence a higher cumulative burden of *SERT* LF variants could be expected to lead to reduced expression of the *SERT* protein.

We did not find any significant association between LF variants in and around *SERT* and IBS risk, GI symptom profile, or response to treatment. These findings speak against the hypothesis that subtle underexpression of *SERT* contributes to IBS, mirroring the results of prior, largely negative studies that showed no association of 5-HTTLPR with the risk for IBS. Intriguingly, however, the one rare nonsynonymous coding variant (Lys605Asn, rs6352) we observed in a subject with IBS is a gain-of-function mutation that results in reduced regulation

and overall enhancement of *SERT* expression (Prasad et al., 2005; Prasad, Steiner, Sutcliffe, & Blakely, 2009).

We found evidence of a possible association of LF variant burden (i.e., carrying multiple rare LF variants) with comorbid depression or anxiety in IBS patients. This finding is in keeping with previous observations that the lower expressing short *SERT* 5-HTTLPR genotype is associated with comorbid depression in IBS (Jarrett et al., 2007) as well as in patients with stroke and other medical conditions (Karg, Burmeister, Shedden, & Sen, 2011; Kohen et al., 2008). A possible explanation for this association lies in observations that the carriers of lower expressing *SERT* variants may have higher sensitivity to emotional stimuli, resulting in both enhanced detrimental effects of negative environmental conditions and a better response to psychotherapy (Jonassen & Landro, 2014; Kohen et al., 2011). Interestingly, in our study the association between LF variants and comorbid psychiatric illness appears to be driven by a lower than expected burden of multiple LF variants in IBS patients (Table 2). While IBS patients with a history of depression or anxiety carried one or multiple minor LF alleles in a similar frequency as control subjects with or without psychiatric comorbidity, the percentage of multiple rare alleles was less in IBS patients without comorbidity. Hence it is possible that not carrying a high LF variant allele burden confers resistance against anxiety and depression induced by a chronically disabling medical condition. If confirmed in other studies, this observation would provide an important insight into the etiology of psychiatric comorbidity in IBS. Often the question is raised whether the GI distress in IBS patients is caused, or exacerbated, by a "fragile psyche." The fact that the genetic architecture of *SERT* in depressed or anxious IBS patients resembles that of GI-healthy controls, however, argues that psychiatric comorbidity is a consequence rather than a cause of IBS.

In keeping with prior reports on the interaction between *SERT* genotypes influencing the gene's expression level and response to psychotherapy, one would expect carriers of multiple rare LF alleles to respond better to treatment with CSM. The size of our sample of subjects who received CSM, either in person or by telephone, may have been too small to detect a subtle effect. Our analyses are also likely to be affected by the fact that probably only a subset of LF variants are actually of functional importance in the GI tract or brain. Future generations of ENCODE annotations are likely to contain much more detailed, tissue-specific information about the performance of different regulatory DNA elements in humans.

In conclusion, our study is the first to investigate rare *SERT* variants in IBS. We found evidence of a possible association of rare variant burden with a history of comorbid depression or anxiety. If confirmed in a larger sample, our findings could indicate that absence of rare variants may be a resilience factor against the development of comorbid psychiatric illness in patients with IBS. Our results must be interpreted with caution, however, as our sample size was small for a genetic study, and *p* values were not corrected for multiple testing. At the same time, our study shows the feasibility of gene-targeted population resequencing studies combined with the use of ENCODE annotations to identify potentially functionally important regulatory variants in common diseases.

Author Contributions

RK contributed to conception and design, acquisition, analysis, and interpretation and design and implementation of the research, data analysis, and writing of the paper; drafted and critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. JHT contributed to acquisition and laboratory work, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. EH contributed to analysis and data analysis, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. KCC contributed to analysis and interpretation and data analysis, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. MEJ contributed to acquisition and implementation of the study, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. MMH contributed to conception, design, acquisition, and interpretation and implementation of the study; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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