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# Association of Fatigue With TPH2 Genetic Polymorphisms in Women With Irritable Bowel Syndrome

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**Abstract**

Fatigue is the most common extraintestinal symptom in women with irritable bowel syndrome (IBS). Genetic polymorphisms of monoamines are associated with fatigue in many chronic diseases. In this pilot exploratory study, the primary aim was to determine whether genetic polymorphisms of tryptophan hydroxylase (*TPH1*/*TPH2*), serotonin reuptake transporter (*SERT*), or catechol-O-methyltransferase (*COMT*) are associated with fatigue in women with IBS. Additionally, analysis explored whether these genetic associations with fatigue would be present when controlling for abdominal pain, psychological distress, feeling stressed, and sleepiness during the day. Secondary analysis of two randomized controlled trial baseline data sets in Caucasian women with IBS ($N = 185$) was conducted. Participants kept a daily diary with one dimension (i.e., severity) for each of the 26 symptoms, including fatigue, for 28 days prior to randomization. DNA samples were tested for single-nucleotide polymorphisms (SNPs) of *TPH1* (four SNPs)/*TPH2* (one SNP), *SERT* (one SNP), and *COMT* (one SNP). Analysis of covariance was used to examine associations of percentage of diary days with *moderate* to *very severe* symptoms with genetic polymorphisms. Only one SNP, *TPH2* rs4570625, was significantly associated with fatigue ($p = .005$). T-allele (low functional) carriers of *TPH2* (i.e., G/T or T/T genotypes) reported a greater percentage of days with *moderate* to *very severe* fatigue than G/G homozygotes ($p = .001$). Reduced synthesis of tryptophan in the central nervous system may contribute to reports of fatigue in women with IBS. Understanding genetic risk factors for fatigue may elucidate preemptive strategies to reduce fatigue in individuals with IBS.

**Keywords**

fatigue, irritable bowel syndrome, genetic polymorphisms, tryptophan hydroxylase, serotonin reuptake transporter, catechol-O-methyltransferase

Approximately 10–20% of all adults in the United States meet the diagnostic criteria for irritable bowel syndrome (IBS), and women are more frequently diagnosed with IBS than men (Drossman, 2016). IBS is a common functional gastrointestinal (GI) disorder characterized by abdominal pain or discomfort accompanied by a disturbance in defecation. The etiology and pathophysiology of IBS are complex and likely multifactorial and include genetics. According to the Rome IV criteria, IBS is subtyped by bowel habits (i.e., constipation-dominant [IBS-C], diarrhea-dominant [IBS-D], a mix of constipation and diarrhea, and IBS-unsubtyped). Individuals with IBS often report other GI (e.g., bloating, urgency) and non-GI symptoms, such as fatigue and psychological distress (Lackner, Gudleski, DiMuro, Keefer, & Brenner, 2013).

Fatigue is one of the most common and disturbing extraintestinal symptoms reported by patients with IBS (Frändemark, Jakobsson, Törnblom, Simrén, & Jakobsson, 2017; Lackner et al., 2013). In a systematic review and meta-analysis of fatigue in IBS (24 studies), the pooled prevalence of fatigue in IBS was 54.2% (Han & Yang, 2016). Fatigue in IBS is associated with reduced health-related quality of life and higher health-care costs (Lackner et al., 2013). It is positively correlated with severity of abdominal pain (Heitkemper et al., 2011), psychological distress (i.e., depression, and anxiety; Han & Yang, 2016; Lackner et al., 2013), poor sleep and stress (Lackner et al., 2013).

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The underlying mechanisms of fatigue (including genetics) in IBS are unknown. However, authors have described associations between fatigue and genetic variation in monoamine neurotransmitter systems in reviews of the genetics of fatigue in other patient groups, such as patients with chronic fatigue syndrome (CFS), hepatitis, and breast cancer (Landmark-Høyvik et al., 2010; Wang, Yin, Miller, & Xiao, 2017). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin. TPH1 is mostly expressed in the periphery, and TPH2 is exclusively expressed in neurons in the central nervous system (CNS; Smith, White, Aslakson, Vollmer-Conna, & Rajeevan, 2006). Although less is known about the associations of the *TPH1* gene with fatigue in humans, one animal study showed that *TPH1*-knockout mice exhibited behaviors reflective of fatigue such as decreased physical activity (Côté et al., 2003). Low expression of the *TPH2* gene in the brain is positively associated with subjective fatigue in patients with CFS (Goertzel et al., 2006; Smith et al., 2006) and in patients with a diagnosis of depressive disorder (Utge et al., 2010). Low *TPH2* gene expression in the brain was also associated with negative affective traits (e.g., anxiety, depression, fatigue, and aggression) in 420 German patients with personality disorders (Gutknecht et al., 2007), in 334 healthy Japanese adults (Latsko et al., 2016), and in 85 healthy Caucasian adults (Strobel et al., 2007). Therefore, there is some support for the link between *TPH1/TPH2* genes and fatigue. Such findings suggest there would be value in examining the association of *TPH1/TPH2* genes with fatigue along with psychological distress in IBS.

The serotonin transporter (*SERT*) gene regulates the reuptake of serotonin in the synaptic cleft. In one study, the *l* allele frequency was more prevalent in 78 Japanese patients with CFS as compared to 50 healthy volunteers (Narita et al., 2003). In a cross-sectional study in healthy Russian men and women reporting fatigue ($N = 143$), men with the *ll* genotype had lower levels of serum serotonin and more reports of fatigue than those with the *s* genotype (Maluchenko et al., 2009). In contrast, women with the *s* genotype experienced more severe fatigue. In a systematic review of the role of antidepressants, including serotonin-selective reuptake inhibitors (SSRIs), in patients with CFS, the authors stated that the findings regarding the use of SSRIs for fatigue are inconsistent, and the efficacy of these medications in these patients needs further exploration (Pae et al., 2009). Catechol-O-methyltransferase (COMT) is one of the several enzymes that degrade catecholamines (Sommerfeldt, Portilla, Jacobsen, Gjerstad, & Wyller, 2011). The methionine (Met) allele of *COMT* Val158Met polymorphism results in a 3- to 4-fold decrease in the activity of the COMT enzyme, compared to the valine (Val) allele. Sommerfeldt et al. found that patients with CFS ($N = 53$) with the *COMT* Val158-Met single-nucleotide polymorphism (SNP) rs4680 had a higher frequency of the Met/Met genotype and a lower frequency of the Val allele. Whether the polymorphisms of these serotonin and catecholamine-related genes are associated with fatigue in IBS remains unexplored.

Because of the established roles of these monoamine neurotransmitters in colonic motility, visceral pain sensitivity, and emotional regulation, the fatigue-related genetic polymorphisms of *TPH1/TPH2*, *SERT*, and *COMT* may also be important in IBS. Minor alleles of *TPH1* and *TPH2* were positively associated with self-report of diarrhea and loose stools in Caucasian women with IBS (Jun, Kohen, Cain, Jarrett, & Heitkemper, 2011). Other researchers found associations between the *s/s* (less functional) genotype of *SERT* polymorphisms and abdominal pain (Colucci et al., 2013) and depression and anxiety (Jarrett et al., 2007) in Caucasian men and women with IBS. Han et al. (2017) found that IBS patients with at least one Val allele of *COMT* Val158Met benefited (i.e., reduced psychological distress and abdominal pain) more from comprehensive self-management than those who were homozygous for the Met allele.

Taken together, the roles of genetic polymorphisms of *TPH, SERT*, and *COMT* in fatigue in other chronic diseases and in symptoms of IBS suggest that these polymorphisms may be potential risk factors for fatigue in patients with IBS. The primary aim of this pilot study was to explore possible associations of genetic polymorphisms of *TPH1/TPH2, SERT*, and *COMT* genes with fatigue in women with IBS. Given the positive associations of fatigue with frequent cooccurring symptoms (Han & Yang, 2016; Heitkemper et al., 2011; Lackner et al., 2013), as an exploratory aim we also sought to determine whether the relationships of these genetic polymorphisms with fatigue were influenced by abdominal pain, psychological distress (anxiety and depression), sleepiness, or feeling stressed.

## Method

### Design, Settings, and Participants

Women and men with IBS, 18–70 years of age, were recruited from two prior randomized clinical trials on the effectiveness of comprehensive self-management, conducted at the University of Washington in the Pacific Northwest (Study 1, Jarrett et al., 2009; Study 2, Jarrett et al., 2016). Studies 1 and 2 used similar eligibility criteria and research procedures (i.e., inclusion/exclusion criteria, diagnostic criteria, recruitment), which are described in previous publications (Jarret et al., 2009; Jarret et al., 2016). For the current study, we used only data from the baseline periods limited the sample to Caucasian women because men and non-Caucasian women were not sufficiently represented. A total of 185 Caucasian women were included in the present study ($n = 123$ from Study 1, $n = 62$ from Study 2).

### Ethical Considerations

The two parent studies received institutional review board (IRB) approval from the University of Washington Human Subject Division prior to recruitment. The IRB approval was renewed annually until each study was closed. Investigators obtained written consent, including for deoxyribonucleic acid (DNA) genetic testing, prior to collecting baseline data.

## Procedures

In both parent studies, eligibility screening of participants occurred over the phone. A research nurse met with participants who met study criteria in person and obtained written informed consent. The nurse then conducted a health interview and completed baseline questionnaires that included demographic data and clinical/symptom characteristics. After the in-person visit, the participant completed a daily diary each evening for 28 days at home. Following the initial visit, a research nurse collected 10 ml of ethylenediaminetetraacetic acid–anticoagulated blood by venipuncture and sent it to the university laboratory. Blood samples were centrifuged and used for genotyping. In this secondary data analysis, we used demographic data, clinical/symptom characteristics, daily diary data, and DNA samples collected as the baseline assessments before randomization into study groups occurred.

## Measures

*Demographic data and clinical/symptom characteristics.* Participants provided information regarding their age, race and ethnicity, marital status, education, occupation, and annual personal income levels using a demographic questionnaire. General history and IBS-specific history were assessed using a Health History Questionnaire (mean Cronbach's $\alpha = .93$). IBS bowel-pattern subtypes (e.g., IBS-D, IBS-C) were assessed in Study 1 with the Rome II Diagnostic Questionnaire (mean Cronbach's $\alpha = .95$; Drossman, Corazziari, Talley, Thompson, & Whitehead, 2000) and in Study 2 with the Rome III Diagnostic Questionnaire (mean Cronbach's $\alpha = .98$; Thompson, Drossman, Talley, Walker, & Whitehead, 2006).

*Daily symptom diary.* Participants completed a daily symptom diary over 28 consecutive days during the baseline assessment period prior to randomization. Every day, women recorded symptom severity over the past 24 hr for each of 26 symptoms including fatigue, abdominal pain, psychological distress (i.e., depression or anxiety), feeling stressed, and sleepiness during the day. Each symptom item assessed one dimension of a symptom. Participants rated each symptom on a Likert-type scale of 0 (*not present*), 1 (*mild*), 2 (*moderate*), 3 (*severe*), and 4 (*very severe*) for 28 days. For each woman, we collapsed the daily symptom assessments over the 28 days into the percentage of days with *moderate* to *very severe* symptoms (e.g., [numbers of days with *moderate, severe*, or *very severe* fatigue/28 days] $\times$ 100%). For present study, fatigue was the primary focus. We also included abdominal pain, psychological distress, feeling stressed, and sleepiness during the day as covariates associated with fatigue.

In our prior study, the daily diary demonstrated good reliability. The internal consistency had a mean Cronbach's $\alpha = .74$ in 61 women with dysmenorrhea (Jarrett, Heitkemper, & Shaver, 1995). In the current study, the internal consistency had a mean Cronbach's $\alpha = .93$. For the test–retest reliability of the current study, we measured the intraclass correlation coefficient with baseline diary data between mean symptom severity for first 2 weeks and mean symptom severity for last 2 weeks. The daily diary had good stability (test–retest reliability for fatigue: $r = .77$, $p < .001$; test–retest reliability of the six symptoms used in this analysis ranged from $r = .76$ to .77). We evaluated the construct validity using a Pearson correlation, that is, the correlation between the mean fatigue severity for the 28 diary days and the mean energy-subscale score of IBS-quality-of-life (QOL) Questionnaire. The energy-subscale item is, "During the past 4 weeks, how much of the time did you feel emotionally or physically worn out and tired because of your IBS?" which respondents rated on a Likert-type scale of 1 (*every day*) to 5 (*none*). A higher score denotes better QOL. The mean fatigue severity of daily diary was negatively correlated with the mean energy subscale of IBS-QOL ($r = -.34$, $p < .001$), indicating good construct validity because the two instruments measure opposite concepts (i.e., fatigue vs. energy QOL).

## Genotyping

DNA was isolated from whole blood using buffy coat preparations and Puregene DNA purification kits (Gentra Systems, Inc., Minneapolis, MN, for *TPH* and *SERT* genes; Qiagen Sciences LLC, Louisville, KY, for *COMT* gene) according to the manufacturer's instructions. TaqMan protocol with a polymerase chain reaction (PCR) was used for SNP genotyping. In the parent studies (Jarrett et al., 2016; Jarrett et al., 2009), tagging SNPs were required to be common (defined as having a minor-allele frequency $\geq 0.05$) in public databases (e.g., HapMap). The quality-control filtering of all SNPs was performed in the parent studies to exclude SNPs with call rates of <95% or Hardy–Weinberg $p$ values of <.001. Measures of linkage disequilibrium (LD) were computed in the parent studies from the participants' genotypes with Haploview 4.2 (Jarrett et al., 2009, 2016). Based on the literature (Landmark-Høyvik et al., 2010; Wang et al., 2017), we used preselected genes and SNPs of serotonin and catecholamines from the existing data set collected by the parent studies (Jarrett et al., 2009, 2016), selecting *TPH1/TPH*2, *SERT* (short/long allele), and *COMT* Val158Met genes were selected for the present study. The seven SNPs from the existing data set were rs4537731 (promoter), rs684302 (intron 2), rs211105 (intron 3), and rs1800532 (intron 7) of *TPH1*; rs4570625 (promoter) of *TPH*2; rs25531 (promoter) of *SERT*; and rs4680 (exon 4) of *COMT*.

## Statistical Analyses

*Power analysis.* The sample size of 185 for the current analysis was determined by the parent studies (Jarrett et al., 2009, 2016), which were powered for the primary aims of those studies. With this sample size ($N = 185$), a three-group analysis of covariance (ANCOVA) has 80% power with an

**Table 1.** Demographic and Clinical/Symptom Characteristics of Women With Irritable Bowel Syndrome (IBS).

| Characteristic | Study 1 $n = 123$ | Study 2 $n = 62$ | Merged $N = 185$ | $p$[a] |
|---|---|---|---|---|
| Age in years, mean (*SD*) | 44.9 (14.1) | 41.0 (14.7) | 43.4 (14.5) | .187 |
| Married/partnered, *n* (%) | 65 (52.8) | 32 (51.5) | 97 (52.4) | .532 |
| >Bachelor's degree, *n* (%) | 77 (62.6) | 39 (62.9) | 116 (62.7) | .488 |
| Income >$ 60,000/year, *n* (%) | 22 (17.9) | 16 (25.0) | 38 (20.5) | .123 |
| Bowel-pattern subtypes, *n* (%) | | | | .121 |
| Constipation (IBS-C) | 28 (22.8) | 22 (35.3) | 50 (26.9) | |
| Diarrhea (IBS-D) | 61 (49.6) | 30 (48.5) | 91 (49.2) | |
| Mixed (IBS-M) | 19 (15.4) | 9 (14.7) | 28 (15.1) | |
| Unknown (IBS-U) | 15 (12.2) | 1 (1.5) | 16 (8.6) | |
| % of days with *moderate to very severe* symptoms,[b] mean (*SD*) | | | | |
| Fatigue | 36.2 (22.4) | 36.6 (27.2) | 36.4 (21.3) | .267 |
| Abdominal pain | 37.3 (26.2) | 40.1 (27.2) | 38.1 (26.5) | .487 |
| Depression | 10.6 (16.5) | 10.3 (20.9) | 10.5 (17.8) | .917 |
| Anxiety | 19.8 (22.3) | 21.6 (26.4) | 20.3 (23.5) | .596 |
| Feeling stressed | 25.8 (24.5) | 32.3 (30.1) | 27.5 (26.2) | .097 |
| Sleepiness during the day | 25.6 (25.4) | 31.9 (27.8) | 27.4 (26.2) | .110 |
| Duration (years) of IBS symptomology, mean *SD* | 9.9 (0.9) | 10.1 (1.1) | 10.0 (0.7) | .952 |

*Note.* *SD* = standard deviation.
[a]Testing differences in demographic data and clinical/symptom characteristics between Study 1 and Study 2. [b]From a daily diary for 28 days.

$\alpha$ value of .05 for detecting an effect size of $f = 0.30$ (moderate-to-large effect size).

*Data analysis.* Descriptive statistics were used to calculate the mean and standard deviation (*SD*) or the total number and percentage of outcome variables. Group differences between Study 1 and Study 2 in demographic data, clinical/symptom characteristics, and the frequency of genetic polymorphisms were tested for using Student's $t$ test and $\chi^2$ test. For our primary aim, ANCOVA was used to explore associations of genetic polymorphisms with percentage of days with *moderate* to *very severe* fatigue. Age was adjusted as a covariate because it was associated with fatigue ($r = .12, p = .023$). In preliminary analyses of these baseline data in our study ($N = 185$), the percentage of days with *moderate* to *very severe* fatigue was significantly ($p < .001$) and positively correlated with the percentage of days with *moderate* to *very severe* abdominal pain ($r = .72$), anxiety ($r = .66$), depression ($r = .69$), feeling stressed ($r = .79$), and sleepiness during the day ($r = .54$). Therefore, for the exploratory aim the ANCOVA was repeated, controlling for these fatigue-related symptoms as covariates. A $p$ value < .05 was considered statistically significant, and all tests were two-tailed. To adjust for multiple comparisons and correct for Type I error, we used a Bonferroni adjustment. We tested one hypothesis (i.e., association of percentage of days with *moderate* to *very severe fatigue*) and seven SNPs; hence, we considered a $p$ value < .05/7 = .007 to be significant. Unadjusted $p$ values were presented and compared to the threshold of <.007. The analyses were performed with IBM SPSS Statistics for Windows, version 22.0 (SPSS, Inc., Armonk, NY: IBM Corp).

## Results

### Demographic Data and Clinical/Symptom Characteristics

There were no statistically significant differences in demographic data or clinical/symptom characteristics (i.e., bowel-pattern subtypes, mean daily symptoms, and duration of illness) between Study 1 and Study 2 participants (Table 1) or in the frequency of genetic polymorphisms of *TPH*, *SERT*, and *COMT* between the two studies (data not shown). In the merged data set, the majority of participants had a college or graduate-level degree (63%), and IBS-D was the most common IBS subtype (49%). Women reported on average 36% of days with *moderate* to *very severe* fatigue (Table 1). Medications they used included tricyclic antidepressants (3%), SSRIs (22%), oral contraceptives (68%), and Vitamin C as antioxidant (20%; data not shown).

### Genetic Associations With Fatigue

There were significant mean differences in percentage of days with *moderate* to *very severe* fatigue (unadjusted $p = .005$) by polymorphisms of *TPH*2 SNP rs4570625 (Table 2). Fatigue severity was higher in women with G/T and T/T genotypes compared to the G/G homozygotes. However, there were no statistically significant differences in fatigue between the G/T and T/T genotypes (Table 2). We found no significant differences in fatigue by the other polymorphisms of *TPH1* SNPs, *SERT*, or *COMT* genes. *TPH1* rs211105 was associated with fatigue with a trend toward significance (unadjusted $p = .051$), but this became nonsignificant with a Bonferroni correction (Table 2). Based on these results, we performed a post hoc analysis, in which we combined G/T and T/T genotype carriers into one group as T-allele carriers

**Table 2.** Differences in Fatigue by Polymorphisms of *TPH1, TPH2, SERT,* and *COMT* Genes in Women with Irritable Bowel Syndrome.

| Genotype | Fatigue[a] | $p$[b] | $p$[c] |
|---|---|---|---|
| *TPH1* (rs4537731) | | .857 | .806 |
| AA ($n = 72$) | 37.8 (28.0) | | |
| AG ($n = 91$) | 35.5 (23.8) | | |
| GG ($n = 22$) | 36.1 (28.3) | | |
| *TPH1* (rs684302) | | .538 | .833 |
| CC ($n = 59$) | 34.6 (26.1) | | |
| CT ($n = 94$) | 36.1 (25.3) | | |
| TT ($n = 32$) | 40.9 (27.7) | | |
| *TPH1* (rs211105) | | .051 | .060 |
| TT ($n = 109$) | 40.2 (27.3) | | |
| GT ($n$ =70) | 30.6 (22.8) | | |
| GG ($n = 6$) | 39.5 (27.1) | | |
| *TPH1* (rs1800532) | | .525 | .923 |
| CC ($n = 63$) | 34.9 (26.0) | | |
| AC ($n = 91$) | 35.9 (25.2) | | |
| AA ($n = 31$) | 41.2 (28.1) | | |
| *TPH2* (rs4570625)[d] | | .005* | <.001* |
| GG ($n = 115$) | 31.7 (24.8) | | |
| GT ($n = 61$) | 43.6 (27.4) | | |
| TT ($n = 9$) | 48.9 (14.8) | | |
| *SERT* (rs25531)[e] | | .341 | .422 |
| s/s ($n = 36$) | 38.7 (28.3) | | |
| s/l ($n = 84$) | 36.9 (25.3) | | |
| l/l ($n = 64$) | 30.9 (22.6) | | |
| *COMT* (rs4680) | | .434 | .286 |
| Val/Val ($n = 43$) | 33.6 (25.8) | | |
| Val/Met ($n = 93$) | 35.7 (26.9) | | |
| Met/Met ($n = 49$) | 40.4 (24.3) | | |

*Note.* $N = 185$. Data are presented as mean (*SD*). All comparisons controlled for age. *COMT* = catechol-O-methyltransferase; *SERT* = serotonin transporter; *TPH* = tryptophan hydroxylase.[a]Percentage of days with *moderate* to *very severe* fatigue with a daily diary for 28 days. [b]Testing differences in fatigue by genotype. [c]Testing differences in fatigue by genotype, controlling for percentage of days with *moderate* to *very severe* abdominal pain, depression, anxiety, feeling stressed, and sleepiness during the day. [d]Pairwise group comparisons among genotypes: GG ≠ GT $p = .004$, GG ≠ TT $p = .002$, GT = TT $p = .604$. [e]$N = 184$ due to missing genetic data.

($n = 70$) and compared them to G/G homozygotes ($n = 115$). The T-allele carriers showed a higher percentage of days with *moderate* to *very severe* fatigue (mean ± *SD*: 31.7 ± 24.5 in G/G genotype vs. 44.3 ± 26.0 in T-allele carriers; unadjusted $p = .001$, data not shown). The associations of *TPH2* rs4570625 (either "G/G, G/T, T/T genotypes," or "G/G and T-allele carriers") with fatigue were statistically significant, even after controlling for multiple comparisons. When we controlled for abdominal pain, psychological distress, feeling stressed, and sleepiness during the day, the results did not change substantially (Table 2). That is, the association of polymorphisms *TPH2* SNP rs4570625 and fatigue remained statistically significant, when we controlled for these covariates ($p < .001$).

## Discussion

In this exploratory pilot study focusing on fatigue in women with IBS, we found a significant association between polymorphisms in the *TPH2* SNP rs4570625 and the percentage of days with *moderate* to *very severe* fatigue in our sample. The significant association of *TPH2* rs4570625's low-expressing T allele with fatigue suggests that there may be dysregulation of serotonin synthesis in the brain in a subset of women with IBS.

Our *TPH2* finding is consistent with those of other investigators who found significant associations of fatigue with minor alleles of different *TPH2* SNPs in other populations. Studies reported positive associations of fatigue with other *TPH2* SNPs in Caucasian women with depression (rs1229394 in Utge et al., 2010), in Caucasian women and men with CFS (rs1843075 in Goertzel et al., 2006; rs 2171363, rs4760816, and rs4760750 in Smith et al., 2006), and in Korean men and women following stroke (rs10879355 in Choi-Kwon, Ko, Choi, & Kim, 2014). Our findings also add to the list of symptoms in IBS for which there may be a genetic vulnerability that involves the production of serotonin. The T allele of *TPH2* rs4570625 has been associated with altered *TPH2* messenger RNA (mRNA) expression (i.e., low functioning) in animal models (Chen, Vallender, & Miller, 2008; Scheuch et al., 2007) and humans (Chen et al., 2008; Gutknecht et al., 2007; Latsko et al., 2016; Markett et al., 2017). In addition, the T allele of *TPH2* rs4570625 is positively associated with the risk of emotional dysregulation in anxiety disorders (Gutknecht et al., 2007), major depressive disorder (Gao et al., 2012), and poor impulse and executive control (Strobel et al., 2007). Given that *TPH2* is primarily expressed by neurons in the CNS, T alleles that reduce its expression may ultimately impact the amount of serotonin available for neurotransmission in CNS areas associated with emotion regulation, perception, and sleep (Russo, Kema, Bosker, Haavik, & Korf, 2009). Although the mechanism of fatigue in IBS is unknown, we conjecture that the reductions in brain serotonin levels, such as may occur in T-allele carriers, contribute either directly or indirectly (in concert with other genetic and epigenetic factors) to fatigue.

When we adjusted for fatigue-related symptoms as covariates (i.e., abdominal pain, psychological distress, feeling stressed, and sleepiness during the day), the association of *TPH2* rs4570625 with fatigue did not change substantially and remained significant. This finding indicates that the link between *TPH2* polymorphisms and fatigue is not likely due to effects of symptoms comorbid with fatigue in IBS. We previously reported that *TPH2* rs4570625 is not associated with daily abdominal pain and bowel-pattern subtypes or overall psychological distress in IBS (Jun et al., 2011). Thus, the relationship of this SNP to fatigue may be unique or distinct from its relationship to other symptoms reported by women with IBS. Despite reports in the literature of possible associations of *TPH1*, *SERT*, and *COMT* with fatigue in other patient groups (Landmark-Høyvik et al., 2010; Wang et al., 2017), we did not find significant associations between polymorphisms of these genes and fatigue in our IBS population. This inconsistency could be due to the heterogeneities in participant characteristics across studies and in fatigue measures or to the possibility that

Gene × Environment or Gene × Drug interactions moderate genetic associations with fatigue.

*Limitations.* This study had several important limitations. Overall, genetic association studies require large sample sizes, which were beyond the potential of this exploratory study. Although SNP rs4570625 may be important in terms of affecting the expression of *TPH2* through modification of transcription factor binding sites (Latsko et al., 2016; Markett et al., 2017), the functional elements of the *TPH2* gene remain to be clarified. The majority of participants were middle-aged and Caucasian women who volunteered for a self-management intervention; thus, they may not accurately represent all patients with IBS. Furthermore, we tested only a limited number of SNPs for the seven candidate genes and did not cover all of the different SNPs or haplotypes of the other possible candidate genes (e.g., cytokines) in relation to fatigue. Lastly, we used a one-dimension daily diary item for each symptom. A major advantage of a one-dimension measure is the reduction in participant burden. However, one-dimension measures are limited in their capture of the full range of symptom characteristics (Diamantopoulos, Sarstedt, Fuchs, Wilczynski, & Kaiser, 2012). In the current study, it is unknown whether the fatigue item reflects physical or mental fatigue.

*Research implications.* Because fatigue is multidimensional (Lackner et al., 2013), we suggest replication studies be conducted that use validated multidimensional fatigue measures such as the Fatigue Impact Scale and Patient-Reported Outcomes Measurement Information System–Fatigue including physical, cognitive, and social fatigue. Measures of regular physical activity levels, antioxidant use, and objective sleep quality may provide greater insight into factors that negatively or positively contribute to fatigue levels. We also suggest the use of mRNA or proteomics analyses to better describe how alterations in serotonergic pathways relate to fatigue in IBS.

*Clinical implications.* Determination of unique genetic markers would allow for identification of patients with IBS at higher risk of fatigue. Knowledge of genetic risk factors could assist clinicians in providing early preventive measures and more targeted clinical or supportive-care interventions beyond bowel-pattern regulation for those patients identified as being vulnerable to fatigue. For example, physical activity has been shown to increase central tryptophan and serotonin levels (Young, 2007). However, for the present findings to be used for meaningful clinical decision-making, additional large-scale studies are warranted.

## Conclusions

This pilot study was the first to examine the associations of genetic polymorphisms of *TPH1/TPH2*, *SERT*, and *COMT* with fatigue in women with IBS. We found a significant association between the *TPH2* gene and fatigue. Women with IBS who carry the T allele (less functional) in *TPH2* rs4570625 may be at greater risk of experiencing fatigue. Increased understanding of the genetic mechanisms of fatigue may lead to better prediction of risk for fatigue and personalized symptom management in IBS.

### Author Contributions

C. Han contributed to conception and design contributed to acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; agrees to be accountable for all aspects of work ensuring integrity and accuracy. M. Jarrett contributed to conception, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. K. Cain contributed to analysis and interpretation, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. S. Jun contributed to acquisition, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. M. Heitkemper contributed to conception, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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