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Associations of tryptophan hydroxylase gene polymorphisms with irritable bowel syndrome

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Abstract

Background *Alterations in serotonin (5-HT) are suspected in the pathophysiology of irritable bowel syndrome (IBS). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of serotonin and has two isoforms: TPH1 and TPH2. Genetic variants in both genes have been studied in various disorders related to serotonin dysregulation. The aim of this study was to examine whether TPH gene variants were associated with IBS and IBS-related gastrointestinal (GI) symptoms.* ***Methods*** *Five single nucleotide polymorphisms (SNPs) from the TPH1 and one SNP from the TPH2 were genotyped in 199 IBS patients and 79 healthy controls. All subjects were Caucasian women of European origin. Irritable bowel syndrome patients filled in a daily diary with five GI symptoms and stool characteristics for 28 days.* ***Key Results*** *The TPH1 SNPs showed no association with the diagnosis of IBS. However, among IBS patients, all five TPH1 SNPs showed some association with diarrhea and loose type of stool consistency, with P-values rating from 0.01 to 0.20. The TPH2 SNP showed a trend towards a reduced risk of IBS and possible associations with stool characteristics, both hard and loose stools. However, no P-values were less than the conservative multiple-comparison-adjusted threshold of 0.001 and hence these results must be interpreted cautiously.* ***Conclusions & Inferences*** *This study is the first to assess associations of TPH gene variants with IBS-related GI symptoms and stool characteristics. The possible association of TPH gene variants with diarrhea needs to be verified in an independent sample.*

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder characterized by recurrent abdominal pain or discomfort associated with altered bowel habits.[1]Although the underlying pathophysiology of IBS is still not clearly understood, serotonin [5-hydroxy tryptophan (5-HT)] is known to be an important signaling molecule in the regulation of GI motility, sensation and secretion.[2] Approximately 95% of 5-HT in the body is found in the GI tract with 90% in enterochromaffin (EC) cells and 10% in serotonergic neurons of the myenteric plexus.[3] The therapeutic success observed with serotonin-modulating agents in patients with IBS supports the pivotal role of 5-HT in IBS pathophysiology.[4–6]

Tryptophan hydroxylase (TPH) is the first and rate-limiting enzyme of 5-HT biosynthesis, catalyzing the oxygenation of tryptophan.[7] There are two isoforms of TPH: TPH1 and TPH2, with overall 71% identity in amino acid sequence in humans.[8,9] The gene encoding TPH1 is located on chromosome 11p15.3-p14 with a size of 29 kilobases (kb) and composed of 11 exons.[10] TPH2 is encoded by a 93.6 kb gene on chromosome 12q21.1 which is also composed of 11 exons.[11] *TPH1* and *TPH2* have different expression patterns: *TPH1* is expressed in the pineal gland, pituitary gland and peripheral organs, mostly EC cells in the gut, while *TPH2* is mainly expressed in the CNS and peripheral serotonergic neurons.[8,9,12]

Polymorphisms in the *TPH* genes have been studied intensively in psychiatric or behavioral disorders whose underlying pathophysiology is related to 5-HT. However, there is as yet no published data related to *TPH* gene polymorphisms and GI symptoms or IBS. Therefore, the purpose of this study was to examine a

possible association of *TPH* gene polymorphisms with IBS in women and their relationship to severity of GI symptoms.

MATERIALS AND METHODS

Subjects

This study used DNA samples and survey data from three case–control studies of IBS carried out in western Washington State. Extensive description of the study samples can be found in previous reports.[13–15] Irritable bowel syndrome patients with a prior diagnosis who currently met the Rome III criteria[1] and healthy controls without history of functional GI disorders were recruited through community advertisement. Irritable bowel syndrome patients or healthy control subjects were excluded if they (i) had a history of co-existing GI pathology (e.g., inflammatory bowel disease) or surgery, renal or reproductive pathology (e.g., endometriosis), severe fibromyalgia, severe cardiovascular disease; or (ii) were currently taking the following medications more than 3 days a week: antibiotics, anticholinergics, cholestyramine, narcotics, colchicines, docusate, an enema preparation, iron supplements, or laxatives. The protocols for this study and the three parent studies were approved by the University of Washington's institutional review board and all the patients gave written informed consent. For this study, we restricted the sample to women who identified themselves as Caucasian to avoid population stratification bias. This resulted in an analysis set of 199 IBS subjects and 79 controls. Of the women with IBS, 20% (n = 41) met criteria for constipation-predominant, 44% (n = 88) met criteria for diarrhea-predominant and 26% (n = 52) met criteria for mixed IBS (Table 4).

Single nucleotide polymorphism selection and genotyping

Based on previously reported association studies we selected five single nucleotide polymorphisms (SNPs) in the *TPH1* gene and one SNP in the *TPH2* gene for genotyping. *TPH1* SNPs are spanning the *TPH1* gene from the upstream region (−6.5 kilo-basepairs (kbp); rs4537731, also known as −6526A/G), through intron 2 (rs684302) and intron 3 (rs211105) to intron 7 (rs1800532, also known as 218A/C and rs1799913, also known as 779A/C). The *TPH2* SNP is located in the promoter region (−703 kbp; rs4570625, also known as −709G/T). For convenience, five *TPH1* SNPs were numbered by their location in the gene and the *TPH2* SNP was marked as SNP6 (Table 1).

Genomic DNA was extracted from fresh whole blood or frozen isolated white blood cells by buffy coat preparation[16] using Puregene DNA Purification kits (Gentra Systems Inc., Minneapolis, MN, USA). Genotyping was done using TaqMan custom genotyping assays and an ABI 7300 Real-time PCR System (Applied Biosystems Inc., Foster City, CA, USA). PCR reactions containing 50 ng genomic DNA, 1× Genotyping Master Mix, 900 nmol L^{-1} of each primer and

Table 1 Characteristics of *TPH* polymorphisms and their association with irritable bowel syndrome in women

			Polymorphism		Frequency, n (%)			Odds ratio*		
Gene	SNP no.	SNP ID	Location	MAF	Genotype	IBS (n = 199)	Control (n = 79)	OR	95% CI	P-value†
TPH1	1	rs4537731	Promoter region	0.38	AA	76 (38)	29 (37)	1		0.84
					AG	96 (48)	41 (52)	0.86	0.49,1.5	
					GG	27 (14)	9 (11)	1.03	0.43,2.5	
	2	rs684302	Intron 2	0.44	CC	63 (32)	27 (34)	1		0.84
					CT	97 (48)	37 (47)	1.18	0.65,2.1	
					TT	39 (20)	15 (19)	1.21	0.57,2.6	
	3	rs211105	Intron 3	0.21	TT	124 (62)	48 (60)	1		0.82
					GT	66 (33)	28 (35)	0.86	0.49,1.5	
					GG	9 (5)	3 (4)	1.20	0.31,4.7	
	4	rs1800532	Intron 7	0.42	CC	72 (36)	28 (35)	1		0.97
					AC	89 (45)	3 (46)	0.96	0.53,1.7	
					AA	38 (19)	15 (19)	1.04	0.49,2.2	
TPH2	6	rs4570625	Promoter region	0.21	GG	131 (66)	46 (58)	1		0.05
					GT	62 (31)	25 (32)	0.92	0.51,1.6	
					TT	6 (3)	8 (10)	0.25	0.08,0.8	

Values in parentheses are expressed as percentages.
SNP, single nucleotide polymorphism; MAF, minor allele frequency; IBS, irritable bowel syndrome; OR, odds ratio; CI, confidence interval; TPH, tryptophan hydroxylase.
*Logistic regression adjusted for age.
†Overall P-value.

200 nmol L^{-1} of each probe were performed in 96-well plates using the standard protocol for TaqMan MGB probes in a total volume of 25 μL. After the cycling, end-point fluorescence was measured and genotype calling was carried out by the allelic discrimination analysis module. To assure quality control, 5% of genotyping was performed in duplicate which showed 100% reproducibility of our results.

Measures

IBS-related GI symptom score. IBS subjects filled in a daily diary for 28 days, including five items related to GI symptoms: abdominal pain or discomfort, bloating, constipation, diarrhea and intestinal gas. These were rated on a scale of 0 (not present), 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe). The severity of each symptom was summarized as percentage of days with moderate to very severe symptoms.[13] The consistency of each stool was also recorded in the diary with a scale of 1 (very hard), 2 (hard), 3 (formed), 4 (loose) and 5 (watery). Stool consistency over 28 days was summarized as percentage of days with either 'very hard' or 'watery' stool.

Statistical analysis

Hardy–Weinberg equilibrium was tested using chi-square tests. To test genotypic association between *TPH* SNPs and IBS, odds ratios (OR) and 95% confidence intervals were estimated using logistic regression, controlling for age. The homozygous genotype of the more common allele of each SNP served as the reference category. Associations with GI symptom scores of individuals SNPs were analyzed by the analysis of covariance (ANCOVA) with age as a covariate. Associations of SNPs with IBS predominant bowel pattern subgroups (Rome III criteria) were tested with chi-square tests. To adjust for multiple comparisons with correct type I error, we used a very conservative method, the Bonferroni adjustment. We tested nine hypotheses and five SNPs, hence we considered a *P*-value of less than 0.05/45 = 0.001 to be significant. Unadjusted *P*-values are presented and compared to the threshold of 0.001. Results are also discussed in terms of the overall pattern of results.

As a further exploration of the primary analyses described above, the HAPLOVIEW 4.0 program (http://www.broad.mit.edu.offcampus.lib.washington.edu/mpg/haploview) was used to explore the haplotype structure of the *TPH1* gene and determine linkage disequilibrium (D′ and R^2) between SNP pairs.[17] Haplotype analysis was performed with HAPSTAT (http://www.bios.unc.edu/~lin/hapstat/) using a sliding window approach with three consecutive markers throughout the gene.

RESULTS

One hundred and ninety-nine women with IBS and 79 healthy women completed the study. The control group was younger than the IBS group (IBS: 40 ± 14; controls: 36 ± 13 years of age, $P = 0.03$). To reduce ethnic variation and stratification effects, only unrelated Caucasian women were included in the analysis.

Genotype distributions

Genotype distributions for all SNPs were in Hardy–Weinberg equilibrium in both control and patient groups (data not shown). Genotypes for SNP4 and SNP5 in the *TPH1* gene were identical in all cases and controls, indicating complete LD between SNPs (D′ = 1, $r^2 = 1.0$). Therefore, we excluded SNP5 from further analysis.

Association of *TPH* gene variants with IBS diagnosis

Table 1 shows the logistic regression analysis of the association between *TPH* gene variants and IBS. There was no significant association between *TPH1* gene SNPs and IBS. Although homozygosity for the rare allele (T) of *TPH2* gene SNP6 was less common in IBS patients (3%) than in healthy controls (10%), the difference is based on only a few individuals and the unadjusted *P*-value was 0.05, not less than the threshold of 0.001.

The association between *TPH1* variants and IBS was also examined at the level of haplotypes. The LD pattern of *TPH1* variants was similar between our sample and the HapMap CEU data set with one block of strong LD and limited haplotype diversity with only five common haplotypes (Fig. 1, Table 2). These five haplotypes accounted for 99% of the segregating haplotypes in this study population. No evidence was found for an association between any of the *TPH1* haplotypes and IBS.

Association of *TPH* gene variants with the severity of GI symptoms in women with IBS

We also investigated possible associations of *TPH* SNPs and IBS-related GI symptoms (Table 3). None of these associations was significant at the multiple-comparison-adjusted threshold of $P < 0.001$. However,

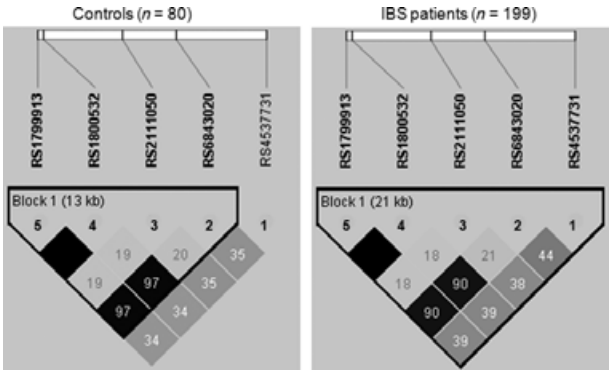

Figure 1 LD plot of the *TPH1* single nucleotide polymorphisms genotyped in this study. Each diamond in the LD plot represents the strength of pairwise LD, with dark color indicating strong LD. The pairwise R^2 values are written in the boxes. Haplotype blocks are indicated with a surrounding black line using the method of Gabriel *et al.*[39]

an interesting pattern does appear. Two SNPs in the *TPH1* gene showed associations with diarrhea symptoms. Irritable bowel syndrome patients homozygous for the minor allele (G) of *TPH1* SNP1 reported higher severity of diarrhea symptoms than the other two genotype groups (AA and AG genotypes, $P = 0.02$), and also showed a trend towards more days with watery stools ($P = 0.08$). For *TPH1* SNP3, patients carrying the minor allele (GT and GG genotypes) reported higher severity of diarrhea symptoms ($P = 0.02$) and more days with watery stools than patients homozygous for the major allele (TT genotype, $P = 0.01$) (Table 3). Minor allele (G) carriers at SNP1 also reported more severe bloating symptoms ($P = 0.02$).

Patients who were homozygous for the minor allele (T) of *TPH2* SNP6 reported more days with both very hard and watery stools compared to other genotype groups (GG and GT genotypes). However, this result was due to two IBS patients, homozygous for the minor allele, who are very high on both hard and watery stool types.

Irritable bowel syndrome patients in the diarrhea-predominant subgroup showed a trend towards increased frequency of the SNP3 minor allele (GT and GG genotypes), but overall the genotype distribution of SNP3 did not significantly differ between subgroups (Table 4). None of the other GI symptoms such as abdominal pain or constipation was associated with any of the *TPH* gene variants.

DISCUSSION

Our study is the first to show possible associations of *TPH* gene variants with IBS and IBS-related GI symptoms in unrelated Caucasian women. While SNPs in the *TPH1* gene were not associated with a diagnosis of IBS, they showed some association with daily reporting of GI symptoms including diarrhea, bloating and loose stools in women with IBS. A SNP in the *TPH2* gene promoter region showed a trend of association with a reduced risk of IBS and stool consistency with both hard and loose stools in IBS patients. While these results were not statistically significant after accounting for multiple comparisons, they are intriguing and an attempt at replication with a larger independent data set would be warranted.

Alterations in 5-HT synthesis and metabolism have been considered as a possible underlying mechanism of IBS, due to the importance of 5-HT in the regulation of gut motility and secretion. Although findings have been inconsistent, various studies have been reported changes in 5-HT content, *TPH1* expression, serotonin reuptake transporter (*SERT*) expression, and postprandial plasma serotonin levels in patients with IBS.[18,19] *TPH1* is predominantly expressed in EC cells in the gut.[9] Several studies have reported altered mucosal *TPH1* transcript levels of RNA in patients with functional GI disorders. One study reported significantly reduced *TPH1* mRNA levels in the colonic mucosa in both diarrhea-predominant and constipation-predominant IBS patients compared to healthy controls.[18] Another study showed higher mucosal *TPH1* mRNA levels in patients with chronic constipation compared to healthy controls.[20] Several studies

Table 2 *TPH1* haplotype frequencies in women with irritable bowel syndrome and controls

	Haplotype					Frequency			
	SNP1	SNP2	SNP3	SNP4	SNP5	IBS	Control	Chi-square	*P*-value
a	A	T	T	A	A	0.408	0.402	0.009	0.926
b	G	C	G	C	C	0.202	0.199	0.001	0.971
c	A	C	T	C	C	0.180	0.203	0.329	0.566
d	G	C	T	C	C	0.169	0.158	0.074	0.786
e	A	T	T	C	C	0.025	0.006	2.061	0.151
f	A	C	G	C	C	0.009	0.016	0.310	0.578

SNP, single nucleotide polymorphism; TPH, tryptophan hydroxylase; IBS, irritable bowel syndrome.

Table 3 Association between *TPH* polymorphisms and the severity of gastrointestinal symptoms in women with irritable bowel syndrome

	Symptom Score*, mean (SD)			
SNP1 (rs4537731)	AA (*n* = 70)	AG (*n* = 93)	GG (*n* = 25)	*P*-value†
GI symptom				
Abdominal pain	27.9 (23.5)	35.6 (25.2)	34.8 (25.7)	0.15
Bloating	21.5 (22.5)	33.9 (30.9)	36.4 (32.7)	0.02
Intestinal gas	32.7 (24.4)	38.1 (30.4)	40.2 (34.0)	0.61
Constipation	16.9 (21.6)	18.9 (21.1)	24.7 (24.4)	0.33
Diarrhea	9.8 (15.4)	12.3 (15.9)	20.5 (30.3)	0.05
Stool consistency				
Very hard stool	13.1 (19.2)	11.1 (14.7)	12.7 (17.3)	0.73
Watery stool	7.2 (9.1)	10.3 (16.5)	14.4 (20.3)	0.08
SNP2 (rs684302)	CC (*n* = 59)	CT (*n* = 93)	TT (*n* = 36)	
GI symptom				
Abdominal pain	32.8 (21.8)	34.6 (27.2)	27.6 (22.6)	0.38
Bloating	34.9 (30.1)	28.3 (29.1)	24.2 (25.3)	0.22
Intestinal gas	37.7 (30.1)	36.2 (30.9)	34.5 (26.9)	0.96
Constipation	19.4 (20.8)	18.4 (23.1)	19.7 (20.2)	0.93
Diarrhea	15.2 (23.7)	12.7 (16.0)	7.2 (13.2)	0.13
Stool consistency				
Very hard stool	10.4 (15.3)	11.8 (15.5)	15.5 (21.7)	0.36
Watery stool	11.0 (15.5)	10.7 (16.7)	5.1 (6.0)	0.11
SNP3 (rs211105)	TT (*n* = 116)	GT (*n* = 63)	GG (*n* = 9)	
GI symptom				
Abdominal pain	31.1 (24.6)	36.8 (25.6)	23.2 (17.1)	0.20
Bloating	27.0 (26.9)	35.0 (31.7)	26.0 (29.5)	0.27
Intestinal gas	36.2 (27.8)	38.6 (33.3)	24.0 (28.9)	0.54
Constipation	19.0 (22.3)	19.1 (21.9)	17.4 (12.2)	0.98
Diarrhea	9.5 (14.2)	17.5 (24.0)	15.0 (16.1)	0.02
Stool consistency				
Very hard stool	13.8 (18.3)	9.5 (14.2)	7.3 (10.1)	0.18
Watery stool	7.2 (10.0)	14.0 (20.5)	12.1 (16.5)	0.01
SNP4 (rs1800532)	CC (*n* = 68)	AC (*n* = 85)	AA (*n* = 35)	
GI symptom				
Abdominal pain	35.3 (23.7)	32.4 (26.4)	28.3 (22.5)	0.40
Bloating	34.7 (30.5)	27.7 (28.5)	24.5 (25.6)	0.14
Intestinal gas	38.0 (30.2)	35.4 (31.0)	35.5 (26.6)	0.73
Constipation	19.3 (21.9)	18.2 (22.4)	20.0 (20.5)	0.88
Diarrhea	14.3 (22.7)	13.0 (16.2)	7.4 (13.3)	0.20
Stool consistency				
Very hard stool	11.3 (16.9)	11.1 (14.0)	15.8 (21.9)	0.35
Watery stool	10.9 (15.3)	10.5 (16.9)	5.2 (6.0)	0.13
SNP6 (rs4570625)	GG (*n* = 126)	GT (*n* = 57)	TT (*n* = 5)	
GI symptom				
Abdominal pain	32.0 (24.1)	33.8 (25.2)	35.0 (40.4)	0.84
Bloating	30.8 (29.3)	27.1 (28.2)	26.9 (29.9)	0.83
Intestinal gas	37.9 (30.0)	33.3 (29.6)	32.8 (31.8)	0.76
Constipation	19.2 (21.8)	17.0 (18.7)	34.3 (44.9)	0.25
Diarrhea	11.8 (17.8)	12.1 (16.1)	30.7 (45.1)	0.08
Stool consistency				
Very hard stool	12.2 (18.0)	9.9 (11.6)	32.7 (23.9)	0.01
Watery stool	8.3 (12.5)	11.5 (17.7)	22.5 (29.4)	0.07

TPH, tryptophan hydroxylase; GI, gastrointestinal; SNP, single nucleotide polymorphism.
*Each symptom score was calculated by the percentage of days with moderate-to-very-severe symptoms.
†Analysis of covariate controlling for age.

Table 4 *TPH* polymorphisms and irritable bowel syndrome subgroups by Rome III criteria

			IBS subgroups			
Gene SNP no.	SNP ID	Genotype	IBS-C (*n* = 41)	IBS-D (*n* = 88)	IBS-M (*n* = 52)	IBS-U (*n* = 17)
TPH1	rs4537731	AA	15 (37)	32 (36)	20 (38)	8 (47)
1		AG	20 (49)	42 (48)	27 (52)	7 (41)
		GG	6 (15)	14 (16)	5 (10)	2 (12)
2	rs684302	CC	14 (34)	31 (35)	15 (29)	3 (18)
		CT	18 (44)	46 (52)	23 (44)	10 (59)
		TT	9 (22)	11 (13)	14 (27)	4 (24)
3	rs211105	TT	28 (68)	51 (58)	31 (60)	13 (77)
		GT	12 (29)	31 (35)	19 (37)	4 (24)
		GG	1 (2)	6 (7)	2 (4)	0
4	rs1800532	CC	16 (39)	35 (40)	17 (33)	4 (24)
		AC	16 (39)	42 (48)	21 (40)	10 (59)
		AA	9 (22)	11 (13)	14 (27)	3 (18)
TPH2	rs4570625	GG	27 (66)	57 (65)	34 (65)	12 (71)
6		GT	12 (29)	30 (34)	15 (29)	5 (30)
		TT	2 (5)	1 (1)	3 (6)	0

Values in parentheses are expressed as percentages.
TPH, tryptophan hydroxylase; IBS, irritable bowel syndrome; SNP, single nucleotide polymorphism; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, mixed IBS; IBS-U, unsubtyped IBS.

have shown increased postprandial plasma 5-HT levels in diarrhea-predominant IBS,[21–24] and decreased plasma 5-HT levels in constipation-predominant IBS.[22,25]

Although we found no association between individual SNPs or haplotypes of the *TPH1* gene and a diagnosis of IBS, SNP1 and SNP3 showed associations with daily diarrhea symptoms and loose stools in women with IBS, though these were non-significant after multiple comparison adjustment. TPH is the rate-limiting enzyme of 5-HT synthesis, therefore *TPH* gene variants could be candidates to influence concentrations of 5-hydroxyindoleacetic acid (5-HIAA, the major metabolite of 5-HT). One study reported that the AA genotype of *TPH1* SNP1 was not associated with 5-HIAA concentrations in cerebrospinal fluid (CSF) although this genotype was associated with suicide attempts among patients with major depressive disorder.[26] However, a recent study showed that the major (A) allele of *TPH1* SNP1 was associated with decreased CSF 5-HIAA concentrations ($P = 0.0028$) in healthy Caucasians.[27] This discrepancy may be due to different ethnicities of the study populations or different health conditions (major depressive patients *vs* healthy controls).

The *TPH1* gene variants we investigated here have been shown to be associated with psychiatric disorders such as major depression, schizophrenia, and suicidal behavior.[26,28] These findings provide indirect evidence that they are either functionally important in

themselves or in LD with other functional genetic variants. Therefore, additional studies with larger sample sizes are needed to verify that the *TPH1* gene represents a diarrhea susceptibility factor in IBS. In addition functional studies are needed to explain the biological mechanisms where by these SNPs contribute to IBS phenotype.

The SNP6 of *TPH2* gene (also known as −703 G/T) is located in the promoter region and has been reported to modify serotonin availability by influencing gene expression.[29,30] In addition, several genetic studies reported that *TPH2* polymorphisms which are high LD with SNP6 are associated with CSF 5-HIAA levels.[31,32] The TT genotype of SNP6 has been reported to be associated with higher amygdala response to emotional stimuli.[33,34] Several genetic association studies demonstrated an association of the T allele with increased startle response,[35] higher risk for personality disorder[36] and attention-deficit/hyperactivity disorder (ADHD).[37]

Our result showed that being homozygous for the minor allele (T) of the *TPH2* SNP6 was marginally associated with a reduced risk of IBS, without correction for multiple testing. This finding may be supported by the observation that the frequency of the minor allele of SNP6 in our controls was similar to the frequencies in the HapMap CEU data (21%) and healthy controls of German European origin (20%) in other genetic studies.[35,38] However, we could not exclude the possibility that this is only by chance. Therefore, additional studies with larger sample sizes are needed to confirm this association as well.

Regarding GI symptoms, women with IBS who were homozygous for the minor allele (T) of SNP6 were more likely to have stools that were characterized as both 'hard' and 'loose'. Among the five IBS patients with the TT genotype of SNP6, two subjects reported very high percentages of days with hard stool and also very high percentages of days with watery stools. These two subjects also reported high abdominal pain, intestinal gas and fatigue. At this time, we cannot determine whether these subjects were more likely to experience abdominal discomfort as a result of their genotype or whether they had a tendency to overendorse GI symptoms as part of a more generalized tendency to report heightened levels of distress. Given the contradictory results of SNP6 in relation to presence of IBS *vs* the severity of symptoms, the fact that they are based on a very small number of patients with the TT genotype, and the concern about multiple comparisons, these results for SNP6 should not be interpreted too strongly.

A strength of this study lies in its use of a well-characterized cohort of women with IBS, who logged their GI symptoms by daily diary for 28 days. However, there are several limitations to this study. Firstly, our study samples originated from three different studies which might have introduced sampling bias. However, patients and controls of all three studies were recruited in the same way, from the same geographic region, and with the same inclusion and exclusion criteria. Secondly, the relatively small sample size and the exploration of a number of SNPs and outcome measures means the power of this study was low after accounting for multiple comparisons. The suggestive findings need to be verified in a larger independent sample.

In conclusion, we reported a preliminary finding of possible associations of *TPH1* gene variants with IBS-related GI symptoms including diarrhea, bloating and loose stool in Caucasian women with IBS. Our findings also suggest a possible association of a *TPH2* SNP with IBS diagnosis and stool characteristics.

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DISCLOSURES

The authors of this manuscript declare that they have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

SJ contributed to the design and implementation of the research, data analysis and writing of the paper; RK contributed to the implementation of the study and writing of the paper; KCC contributed to data analysis and writing of the paper; MJ and MH contributed to the design of the research and writing of the paper.

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