Serotonin Transporter Gene (*SLC6A4*) Polymorphism in Patients with Irritable Bowel Syndrome and Healthy Controls

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Abstract

Background: Polymorphisms in serotonin re-uptake transporter (SERT or *SLC6A4*) gene may play role in disturbance in gut function in irritable bowel syndrome (IBS). The aim of this study was to evaluate the association between *SLC6A4* polymorphism of SERT-P and serotonin (5-hydroxytryptamine, 5-HT) concentration in IBS as compared with controls. **Methods**: 150 patients with IBS (Rome-III criteria) and 252 controls were subjected to *SLC6A4* genotyping. 5-HT was measured in the rectal biopsy of patients only. **Results**: Patients and controls were age and gender-matched. Patients were classified into D-IBS: 79 (52%), C-IBS: 52 (35%) and A-IBS: 19 (13%). *SLC6A4* polymorphism differed in IBS and controls [genotypes s/s, 89 (59%), l/s, 44 (29%), and l/l, 17 (12%) vs. s/s, 92 (37%), l/s, 114 (45%), and l/l, 46 (18%), p<0.001]. *SLC6A4* s/s genotype was commoner in D-IBS than C-IBS, A-IBS and controls (p<0.001). 5-HT level was higher in D-IBS than A-IBS and C-IBS (154.7±37.1 vs. 112.4±24.6 vs. 104.3±23.7-pmol/mL, p<0.001) and in s/s than l/s and l/l genotypes (151.1±37.3 vs. 105.0±20.9 vs. 100.9±28.0-pmol/mL, p<0.001). IBS with s/s genotype more often had abdominal pain than l/s and l/l [78/89 (87.6%) vs. 19/44 (43%) vs. 5/17 (29%), p<0.001]. 5-HT level was higher among IBS patients with abdominal pain and diarrhea than without (142.9±39.4 vs. 108.4±28.9-pmol/mL, p<0.001) and (140.2±41.3-pmol/mL vs. 121.3±35.0-pmol/mL, p=0.003). **Conclusion**: The frequency of *SLC6A4*-polymorphism and higher levels of 5-HT were significantly associated with IBS, particularly in patients with diarrhea and abdominal pain, suggesting that *SLC6A4* is a potential candidate gene involved in the pathogenesis of IBS.

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Key words

Functional gastrointestinal disorders – genetic polymorphism – gastrointestinal infection – gastroenteritis – post-infectious IBS – serotonin receptor.

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter in the enteric nervous system promoting gut motility, visceral sensation and secretion [1, 2]. 5-HT is thought to play a key role in the pathophysiology of irritable bowel syndrome (IBS) [1, 2]. The role of 5-HT in the pathophysiology of IBS is supported by its higher levels in patients with IBS than healthy controls [3]. Plasma 5-HT concentration was higher in females with diarrhoea-predominant IBS (D-IBS) following ingestion of a meal [3]. Numerous 5-HT receptors including 5-HT1P, 5-HT3, 5-HT4 are expressed in the intestinal wall and a variety of drugs for treatment of IBS have been recently developed, which target these receptors [4-6]. Drugs antagonising 5-HT3 receptor such as alosetron, cilansetron, ramosetron, granisetron and ondansetron have been used for treatment of D-IBS by reducing abdominal pain, discomfort and diarrhea [7]. Once 5-HT is secreted, it is transported through a protein called serotonin reuptake transporter (SERT) [8, 9]. Reduced function of the SERT is therefore expected to result in excess of 5-HT with consequent hypercontractility of gut muscles, increased secretion, and sensation resulting in diarrhea and abdominal pain [10]. A polymorphism consists of two repeat variations: a short (s) variation of 14 repeats of a sequence and a long (l) variation of 16 repeats; both these variations have been shown to promote expression of the SERT gene *SLC6A4* [solute carrier family 6 (neurotransmitter transporter, serotonin), member 4] differentially: 's' carriers show a decreased expression of the gene, which may lead to increased serotonin level in patients with IBS [11].

In a Turkish study, patients with D-IBS more often had *SLC6A4* insertion/deletion genotype, as compared to healthy subjects [12]. In another study from the USA, female patients with D-IBS more often had *SLC6A4* deletion/deletion

genotype [13]. However, in another US study, the distribution of *SLC6A4* polymorphisms was comparable among patients with functional lower gastrointestinal disorders including IBS and controls or among patients with D-IBS and controls [14]. In another study from Korea, a strong association was observed between *SLC6A4* deletion/deletion genotype and D-IBS [15]. The discrepancies in the results in different studies might be related to ethnic differences.

There is no study demonstrating the relationship between the mucosal 5-HT level and *SLC6A4* polymorphism of SERT-P in IBS. We therefore aimed to study the association between *SLC6A4* polymorphism of SERT-P in patients with IBS as compared with healthy controls and to compare the level of 5-HT among various IBS subtypes.

Methods

Patients and control populations

One hundred and fifty of 240 patients (62.5%) with functional gastrointestinal disorders fulfilling Rome III criteria [16] for IBS and showing normal results on hematology, blood biochemistry and flexible sigmoidoscopy and 252 healthy controls (HC) were included. Rectal biopsy was obtained during flexible sigmoidoscopy for measurement of 5-HT in patients with IBS. Both patients and controls were unrelated natives from northern India. The study was approved by the Institutional Ethics Committee. Informed consent was obtained from each subject.

Sub-typing of IBS

Patients with IBS were classified into diarrhoea-predominant (D-IBS), constipation predominant (C-IBS), and alternating diarrhoea and constipation (A-IBS) subtypes using the Rome III criteria [16]. In addition to this, history of previous acute gastroenteritis was obtained. Post-infectious IBS (PI-IBS) was diagnosed using standard criteria [17].

Clinical evaluation

A bowel symptom questionnaire was administered to each patient. Symptoms included bloating, excess gas, diarrhea, constipation, abdominal pain, passage of mucus, feeling of incomplete evacuation, straining, and urgency. The patients were asked how they felt about their bowel movements (diarrhea or constipation). Stool frequency and form (using Bristol stool form chart) were also noted.

SLC6A4 genotyping

DNA was extracted from venous blood stored at -70ºC by alkaline lysis method using QIAamp DNA Blood Midi Kit (Qiagen Inc., Valencia, CA, USA). Polymerase chain reaction was carried out in a total volume of 25 µL, containing genomic DNA (100-150 ng), 20 pmol of each primer, 5 µL of 10 X Taq polymerase buffer and 1.5 units of Taq DNA polymerase (Bangalore Genei, Bangalore, India). The primers for the *SLC6A4* (SERT-P repeats) were forward: 5'-GGC GTT GCC GCT CTG AAT GC- 3 and (reversed) 5'-GAG GGA CTG AGC TGG ACA ACC AC-3' (Lesch et al 1996)[18]. PCR amplification was performed under the following conditions: denaturation at 95.5°C for 3 min, 35 cycles of 95.5°C for 1 min, 1 min of annealing at 76°C, 1 min of extension at 72°C, and 7 min of final extension at 72°C. Amplification products were resolved by electrophoresis on 2.5% agarose gels and visualized with ethidium bromide staining. Alleles were designated 's' (484 bp) and 'l' (528 bp) according to Lesch et al [18].

Measurement of 5-HT level in rectal biopsy

Rectal biopsy specimens, obtained during colonoscopy, were kept at -70°C until used for the measurement of 5-HT level. These biopsies were thawed and then homogenized at 4°C in 200 µL PBS (pH 7.4) using homogenizer. The homogenates were centrifuged at 10,000g for 10 minutes at 4°C for measurement of 5-HT level. The total 5-HT estimation was done using a commercially available ELISA kit (IBL International GmbH Hamburg, Germany). The mucosal level of 5-HT was expressed in pmol/mL.

Statistical analysis

Allele and genotype frequency in patients with IBS and controls were compared using 2 x 2 contingency table using $\chi 2$ test with Yates' correction, as applicable. The direct gene counting method was used to determine the frequency of genotypes and alleles. To ensure that the controls included were representative of the general population and to exclude the possibility of genotyping error, deviation of the observed genotype frequencies of *SLC6A4* (SERT-P repeats) polymorphisms in the controls was compared with that expected under Hardy–Weinberg equilibrium using the goodness-of-fit $\chi 2$ test. Genotype risk was expressed as an age- and gender-adjusted OR (odds ratio) with 95% confidence interval (CI) estimated using binary logistic regression statistics. Bonferroni's correction (a multiple-comparison correction) was applied to significant associations. Continuous data were analysed using Student's *t* test. The one way analysis of variance (ANOVA) was used to analyse continuous data between three groups. P values less than 0.05 were considered significant. Data were analyzed using the statistical software SPSS, ver. 15.0 (SPSS, Inc., Chicago, IL, USA).

Results

Demographic and clinical parameters

Patients with IBS were comparable in age and gender with HC (Table I). According to the patient's perception, of 150 patients with IBS, 99 (66%), and 39 (26%) were constipation, and diarrhea predominant subtypes respectively, while 12 (8%) were of alternating type. Patients with IBS were classified into D-IBS 79 (52%), C-IBS 52 (35%) and A-IBS 19 (13%) using Rome III criteria.

Association between *SLC6A4* polymorphism and IBS

Genotype and allele frequency were observed to be in Hardy-Weinberg equilibrium in the control population ($p = 0.59$). The genotype distribution and allele frequency in patients with IBS and controls are shown in Table II. Patients with IBS more often had s/s genotype [89 (59%)

Table I. Demographic and clinical parameters of patients with IBS and healthy controls

Parameters	IBS (n = 150)	HC (n = 252)	P - value
Age (years, mean ± SD)	36.7 ± 11.8	37.2 ± 11.5	0.70
Gender (Male, no. and %)	114 (76%)	197 (78%)	0.61
Hemoglobin (g/dl)	13.2 ± 1.9	-	-
ESR (mm 1st hr)	19.3 ± 12.9	-	-
TSH (IU/L)	2.6 ± 2.8	-	-
D-IBS (n, %)	79 (52%)	-	-
C-IBS (n, %)	52 (35%)	-	-
A-IBS (n, %)	19 (13%)	-	-
Symptoms			
Abdominal pain	102 (68%)	5 (2%)	< 0.0001
Abdominal discomfort	109 (73%)	5 (2%)	< 0.0001
Abdominal distension /bloating	142 (95%)	13 (5%)	< 0.0001
Passage of mucus	105 (70%)	0	< 0.0001
Feeling of incomplete evacuation	140 (93%)	17 (6.7%)	< 0.0001
Abdominal pain/discomfort relieved after defecation	147 (98%)	5 (2%)	< 0.0001
Urgency to pass stool	59 (39%)	0	< 0.0001
Loose stool at onset of pain	108 (72%)	3 (1.2%)	< 0.0001
Straining during defecation	82 (55%)	0	< 0.0001
Number of stool/week (median and range)	21 (2 – 60)	7 (7 – 14)	< 0.0001
Relief of pain with bowel movement	141 (94%)	5 (2%)	< 0.0001
More frequent stools at onset of pain	103 (69%)	5 (2%)	< 0.0001

IBS: irritable bowel syndrome; HC: healthy controls; ESR: erythrocyte sedimentation rate; TSH: thyroid stimulating hormone; D-IBS: diarrhea predominant-IBS; C-IBS: constipation predominant-IBS; A-IBS: diarrhea and constipation alternating type-IBS.

vs. 92 (37%), p < 0.001], l/s genotype [44 (29%) vs. 114 (45%), p < 0.001] and l/l genotype [17 (12%) vs. 46 (18%), p = ns] compared to HC. s/s (deletion/deletion) genotype was associated with higher risk of IBS as compared to l/l genotype (p = 0.003, OR = 2.6, 95% Cl = 1.4 – 4.9). At allelic level, presence of "s" allele was associated with a higher risk of IBS as compared to the presence of "l" allele (p < 0.001, OR = 1.9, 95% Cl = 1.4 – 2.7) (Table II). When the l/s and l/l genotypes were combined into one group, the frequency of the s/s genotype tended to be higher than non-s/s genotypes among patients with IBS than controls (P < 0.001).

The frequency of s/s genotype was significantly higher among D-IBS as compared to C-IBS and A-IBS. However, frequency of l/s genotype was higher in C-IBS as compared to D-IBS and A-IBS. The genotypic distribution and allele frequency in subgroups of patients with IBS are shown in Table III.

Table II. Distribution of the SERT (*SLC6A4*) gene polymorphism in healty controls and patients with IBS.

SERT-P Genotype	Patients with IBS n[a] (%)	HC n[a] (%)	P-value	OR (95% Cl)
l/l	17 (12%)	46 (18%)	-	1 (Reference)
s/s	89 (59%)	92 (37%)	0.003	2.6 (1.4 – 4.9)
l/s	44 (29%)	114 (45%)	0.92	1.0 (0.5 – 2.0)

n[a] = total number of patients (150) and healthy controls (252); Statistical test: Binary logistic regression model was used to calculate age and gender adjusted odds ratio (OR) and 95% confidence interval (95% Cl); IBS: irritable bowel syndrome, HC: healthy controls, SERT: serotonin re-uptake transporter gene promoter, SLC6A4: solute carrier family 6 (neurotransmitter transporter, serotonin), member 4.

Relationship between level of 5-HT and *SLC6A4* genotypes in patients with IBS

The level of 5-HT in rectal biopsy was higher among patients with D-IBS as compared to A-IBS and C-IBS (154.7 ± 37.1 pmol/mL vs. 112.4 ± 24. 6 pmol/mL vs. 104.3 ± 23.7 pmol/mL, p < 0.001, respectively) (Fig.1A). Level of 5-HT in rectal biopsy was higher in patients with IBS with s/s genotype than those with l/s and l/l genotypes (151.1 ± 37.3 vs. 105.0 pmol/mL ± 20.9 vs. 100.9 ± 28.0 pmol/mL, p < 0.001, respectively) (Fig.1B). The level of 5-HT was significantly higher in patients with IBS with s/s genotype compared to non-s/s genotype (151.06 ± 37.3 pmol/mL vs. 103.8 ± 22.9 pmol/mL, p < 0.0001). There was no difference in the level of 5-HT among female and male patients with IBS.

Table III. Distribution of the SERT-P (SLC6A4) gene polymorphisms between each of the IBS subtypes.

Genotype	D-IBS (n = 79)*†	C-IBS (n = 52)	A-IBS (n = 19)	HC (n = 252)	p-value
l/l	6 (8%)	7 (13%)	4 (21%)	46 (18%)	0.84
s/s	66 (83%)	15 (29%)	8 (42%)	92 (37%)	0.001
l/s	7 (9%)	30 (58%)	7 (37%)	114 (45%)	0.001
Allele (no. and %)					
"s" allele	139 (88%)	60 (58%)	23 (61%)	298 (59%)	0.001
"l" allele	19 (12%)	44 (42%)	15 (39%)	206 (41%)	-

IBS: irritable bowel syndrome; D-IBS: diarrhea predominant-IBS; C-IBS: constipation predominant-IBS; A-IBS: diarrhea and constipation alternating type-IBS; HC: healthy controls; SERT-P: serotonin re-uptake transporter gene promoter; SLC6A4: solute carrier family 6 (neurotransmitter transporter, serotonin); member 4; s/s: deletion/deletion; l/s: insertion/deletion; l/l: insertion/insertion types of genotypes; "s": deletion; "l": insertion types of alleles. *P < 0.001 for s/s vs. non-s/s genotype. † Odds ratio (95% confidence interval) for s/s vs. non-s/s genotypes: 8.83 (4.44 – 17.83).

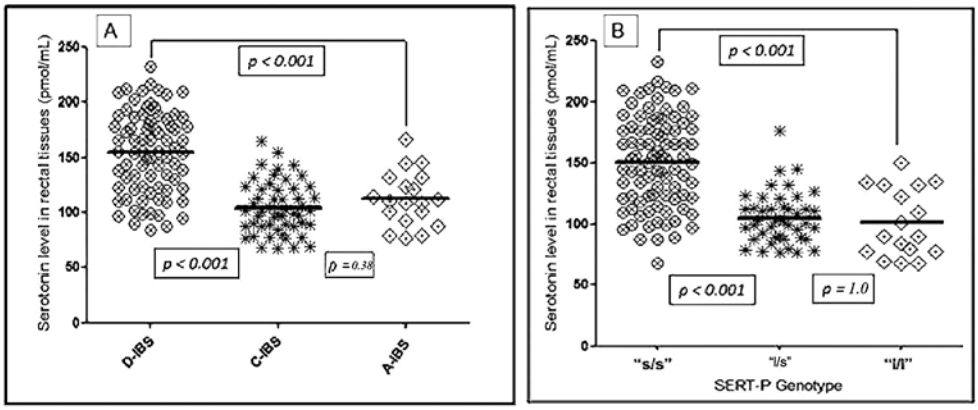

Fig 1. Comparison of level of 5-HT among (A) subgroups of irritable bowel syndrome patients and (B) SERT-P (SLC6A4) genotypes. IBS: irritable bowel syndrome; D-IBS: diarrhea predominant-IBS; C-IBS: constipation predominant-IBS; A-IBS: diarrhea and constipation alternating type-IBS; SERT-P: serotonin re-uptake transporter gene promoter; SLC6A4: solute carrier family 6 (neurotransmitter transporter, serotonin); member 4; s/s: deletion/deletion; l/s: insertion/deletion; l/l: insertion/insertion types of genotypes; NS: not significant.

Association between *SLC6A4* genotypes and IBS symptoms

Patients with IBS with s/s genotype more often had abdominal pain as compared to l/s and l/l genotypes [78/89 (87.6%) vs. 19/44 (43%) vs. 5/17 (29%), p < 0.001]. However, there was no difference in the frequency of bloating, urgency, feeling of incomplete evacuation, straining and passage of mucus per rectum between patients with these genotypes. s/s carriers more often reported abdominal pain and frequent stools (> 3 per day) than non-s/s carrier [78/89 (87.6%) vs. 24/61 (39.3%), p < 0.0001] and [56/89 (62.9%) vs. 28/61 (45.9%), p = 0.046], respectively.

Association between the level of 5-HT and symptoms of IBS

The level of 5-HT in rectal mucosa was higher among patients with IBS reporting abdominal pain and frequent stools (more than 3 per day) as compared to those without these symptoms (142.9 ± 39.4 pmol/mL vs. 108.4 ± 28.9 pmol/mL, p < 0.0001) and (140.2 ± 41.3 pmol/mL vs. 121.3 ± 35.0 pmol/mL, p = 0.003), respectively. However, there was no difference in the level of 5-HT among patients with IBS reporting bloating, urgency, loose stools at onset of pain, feeling of incomplete evacuation and passage of mucus per rectum as compared to those without these symptoms.

***SLC6A4* polymorphisms according to gender**

The frequency of s/s, l/s and l/l genotypes were 36%, 44% and 20%, in male and 38%, 49% and 13% in female patients with IBS, respectively. The frequency of s/s, l/s and l/l genotypes were 59%, 30% and 11% in male and 61%, 28% and 11% in female patients with IBS, respectively. *SLC6A4* genotype frequency was comparable between males and females among patients with IBS and controls. The frequency of *SLC6A4* genotypes was comparable among either gender in patients with C-IBS, D-IBS and A-IBS.

Clinical profile of patients with PI-IBS

Of 150 patients with IBS, 11 (7.3%) had PI-IBS, all of whom had D-IBS. None of the 68 (45.3%) other D-IBS and of the 71 non-diarrheal IBS patients reported a history

Table IV. Clinical and demographic parameters of PI-IBS, D-IBS without prior history of gastroenteritis and non-diarrheal IBS

Parameters	PI-IBS (N = 11)	D-IBS without prior history of gastroenteritis (N = 68)	Non-diarrheal IBS (N = 71)	P-value
Age (y) mean ± SD	33.8 ± 10.5y	36 ± 11.7 y	37.8 ±12.03y	0.46
Gender, male (%)	8 (73)	53 (78)	53 (75)	0.87
Hemoglobin (g/dl)	13.1 ± 2.8	13.3 ± 1.7	13.1 ± 1.9	0.78
ESR (mm 1st hr)	16.9 ±14.9	19.9 ± 12.7	19.2 ± 13.1	0.79
TSH (IU/L)	2.31 ± 1.8	2.13 ± 1.7	3.1 ± 1.8	0.71
Number of stool/ Week	28 (10 – 56)	28 (8 – 56)	18 (2 – 30)	0.01

Abbreviations: PI-IBS; post-infectious irritable bowel syndrome, D-IBS; diarrhea predominant-IBS, ESR: erythrocyte sedimentation rate; TSH: thyroid stimulation hormone, SD: standard deviation. Categorical data were analyzed using Chi-square test; continuous data between three groups were analyzed using one-way ANOVA with Bonferroni correction, p-value less than 0.05 were considered significance.

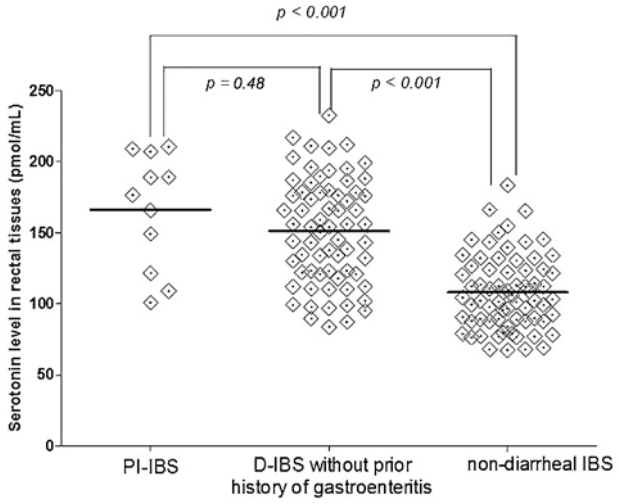

Fig 2. Comparison of level of 5-HT between PI-IBS, D-IBS without prior history of gastroenteritis and non-diarrheal IBS. IBS: irritable bowel syndrome, PI-IBS: post-infectious irritable bowel syndrome, D-IBS: diarrhea predominant-IBS.

of gastroenteritis before onset of IBS. The clinical and demographic parameters of PI-IBS, D-IBS without prior history of gastroenteritis and non-diarrheal IBS are given in Table IV.

Relationship between 5-HT level and PI-IBS

Patients with PI-IBS had higher mucosal 5-HT level compared to non-diarrheal IBS (166.1 ± 40.6 pmol/mL vs. 108.02 ± 26.1 pmol/mL, $p < 0.001$). Also, the mucosal 5-HT level was significantly higher in patients with D-IBS without prior history of gastroenteritis compared to non-diarrheal IBS (151.23 ± 36.99 pmol/mL vs. 108.02 ± 26.1 pmol/mL, $p < 0.001$) (Fig 2).

Association between *SLC6A4* genotypes and PI-IBS

Patients with PI-IBS more often had s/s genotypes compared to D-IBS without prior history of gastroenteritis and non-diarrheal IBS [9/11 (82% vs. 55/68 (81%) vs. 25/71 (35%), $p < 0.001$]. However, the frequency of l/s genotype was higher in non-diarrheal IBS compared to D-IBS without prior history of gastroenteritis and PI-IBS [36/71 (51%) vs. 7/68 (10%) vs. 1/11 (9%), $p < 0.001$]. Furthermore, the frequency of l/l genotype was similar in all the three groups.

Discussion

The present study showed that deletion/deletion genotype of *SLC6A4* polymorphism and higher level of 5-HT in rectal mucosa were associated with IBS, particularly D-IBS.

Although the complex multidimensional nature of IBS and ethnic differences will affect the results for *SLC6A4* polymorphism, our findings are consistent with the original hypothesis [1]. The SERT protein is responsible for reuptake of 5-HT in serotonergic nerves and mucosa of bowel, and is a factor that determines 5-HT activity [1]. In a lymphoblast cell line, s/s genotype at promoter polymorphic site of SERT gene, which encodes this protein, was associated with lower transcriptional efficiency, resulting in lower SERT expression and therefore lower cellular uptake of 5-HT [18]. In animal models, *SLC6A4* knockout mice had diarrhoea, which was associated with faster colonic motility that resulted in increased excretion of water in stool [19]. These observations could explain our results showing *SLC6A4* s/s genotype to be associated with D-IBS. The association of D-IBS with the *SLC6A4* s/s genotype is also supported by the studies demonstrating changes in postprandial 5-HT levels in platelet-depleted plasma [3, 4]. These studies showed that 5-HT concentration was higher in D-IBS patients after meals than healthy controls [4]. These findings suggested that patients with IBS had a disorder primarily involving the metabolism and reuptake of 5-HT rather than its synthesis and/or release [4]. As SERT is the main molecule responsible for 5-HT reuptake, and *SLC6A4* polymorphism determines SERT activity, our results offer another plausible explanation in pathogenesis of D-IBS.

The result of the present study showing s/s genotype to be a risk factor for IBS is similar to the results of US and Korean studies [13, 20]. Moreover, the results showing SERT genotype to be particularly associated with D-IBS are in accordance with studies from Korea [20], China [21-23], US [13] and Turkey [12]. In contrast, a northern Indian study by Sikander et al, reported that s/s genotype

was associated with C-IBS [24]. A Chinese study showed that l/l genotype was associated with C-IBS [21]. The latter studies, however, had limitations due to inclusion of small number of patients and controls, raising the possibility of statistical error [12]. Moreover, IBS may be heterogeneous in different populations as it is a syndrome diagnosed using symptom-based criteria, its prevalence being variable in different countries [25-27] with different ethnicity, and in addition, the female predominance of this syndrome is not universal [28]. Such heterogeneity in phenotypes may also partly explain the result of genetic studies. Variation has also been identified in *SLC6A4* polymorphism studies among various ethnic groups [29]. Frequency of l/l genotype was low in the Korean control population [20]. Low frequency of l/l genotype was also reported among patients with IBS (5.3% and 3.0%) from Korea [20, 30, 31]. A Japanese study on migraine also showed a low frequency of l/l genotype in the control population (5.8%) [32]. In contrast, Turkish [12] and US [13], studies showed frequency of l/l genotype as high as 23.5% and 31.4% among healthy controls population, respectively. Therefore, it seems that the *SLC6A4* l/l genotype may be less common in Eastern Asia than in Western countries. Such variation in the frequency of l/l genotype may influence the statistical power to detect a genotype-related association. Thus, ethnic differences should be considered when evaluating the results of a study.

In contrast to the US study [13] in which only female patients were enrolled, our study included almost equal proportions of male and female patients. The distribution of *SLC6A4* genotypes in male subjects was comparable between IBS and controls, regardless of IBS subtype. However, the frequency of s/s genotype in female patients with D-IBS tended to be higher than that of non-s/s genotype, though this finding did not reach statistical significance. This result is consistent with the results of one US study [13]. Recently, Saito et al suggested that *SLC6A4* polymorphism had no association with IBS though alternating diarrhea and constipation subtype of IBS (A-IBS) was associated with this polymorphism [33]. Niesler et al showed that among 196 Caucasian patients with IBS, *SLC6A4* s/s genotype was less frequent among patients with IBS in general and even among D-IBS (14.6%) and C-IBS (14.3%) in particular compared with 94 HC (25.5%) [34]. The variation in the result of that study might be related to difference in gender of patients compared to the other studies.

Our result showing higher levels of 5-HT in rectal mucosa among patients with abdominal pain and diarrhea may have clinical implications as antagonists of this biomolecule may help alleviating these troublesome symptoms in patients with IBS. Our results are in agreement with those of Houghton et al [3], who showed that postprandial symptom exacerbation in female patients with IBS-D was associated with increased levels of plasma 5-HT [3]. Endogenous level of platelet-depleted plasma 5-HT correlated with sigmoid-colonic motility in fasting and fed state, both in patients with IBS and controls [35]. In a recent study, spontaneous release of 5-HT was found to be 10 times higher among patients with IBS (either with diarrhea or constipation) than controls [36]. 5-HT release, however, was comparable between IBS-D and IBS-C [36]. In another study on pediatric patients with IBS, 5-HT content of rectal mucosa was higher than in control subjects [37]. In contrast to these studies, a study by Coates et al showed that mucosal 5-HT release was comparable among patients with IBS-D or IBS-C and controls [38]. Such contradictory results might be explained by methodological differences between the studies, including patient selection, site of mucosal biopsy sampling (for example, rectum vs. colon), and methods used to detect mucosal 5-HT release.

Mucosal level of 5-HT was higher among patients with diarrheal IBS, particularly those with PI-IBS, than non-diarrheal IBS. However, 5-HT level was comparable among patients with PI-IBS and D-IBS without prior history of acute gastroenteritis. Also, s/s genotype was more frequent among patients with PI-IBS than non-diarrheal IBS but was comparable to D-IBS without prior history of acute gastroenteritis. The results of the present study are in accordance with earlier studies on PI-IBS. Increased number of enterochromaffin (EC) cells has been reported in PI-IBS: EC cells, CD3 lymphocytes and intraepithelial lymphocytes (IEL) were increased in PI-IBS patients one year after campylobacter enteritis. In another study, Dunlop et al [39] reported that 5-HT containing EC cells and T lymphocytes were increased in rectal lamina propria of patients with PI-IBS as compared to non PI-IBS 3-month after campylobacter enteritis. 5-HT and its receptors are the target molecules for treatment of functional gastrointestinal disorders [40]. Most 5-HT is found in enterochromaffin cells, which are the best characterized subset of entero-endocrine cells [39, 40]. 5-HT activates gastrointestinal motility through 5-HT3 or 5-HT4 receptors and induces gastric relaxation through 5-HT7 receptors [41]. As shown in the present study, 5-HT was found to be increased in PI-IBS. It has been shown that 5-HT containing EC cells were found to be increased in D-IBS, particularly among patients with PI-IBS [40]. Because 5-HT is involved in gut motility and secretion, its mucosal level and various receptor functions might be related to development of IBS symptoms [40].

Previous studies evaluated fasting and/or fed-state levels of 5-HT in platelet-depleted plasma [36]. The results suggested that patients with IBS-D or PI-IBS had reduced 5-HT reuptake and/or metabolism, whereas impaired release might be associated with IBS-C [4, 36, 42]. Infiltrating mast cells might release 5-HT, as evidenced by a positive correlation between the number of these cells in mucosa and degree of 5-HT release [36]. A recent study in pediatric patients with IBS demonstrated that children showing a mild increase in rectal mucosal immune cell counts had a higher mucosal availability of 5-HT and lower SERT mRNA [43]. Moreover, conditions characterized by overt mucosal inflammation, such as celiac disease [44] or Crohn's disease [45], are known to be associated with increased mucosal 5-HT content and its release. In animal models, colonic inflammatory conditions, such as tri-nitrobenzene sulfonic acid colitis [46] and ileitis were associated with

increased EC cell numbers, increased 5-HT release, and evidence of impaired SERT activity, leading to increased 5-HT availability [47]. Enterochromaffin cell hyperplasia and reduced SERT expression may persist as a consequence of short term inflammatory insult in the gut, as shown in a Trichinella spiralis mouse model of post-infectious bowel dysfunction [47].

In conclusion, the frequency of *SLC6A4*-polymorphism and higher levels of 5-HT were associated with IBS, particularly those with diarrhea and abdominal pain, suggesting that *SLC6A4* is a potential candidate gene involved in the pathogenesis of IBS.

Conflicts of interest

There is no conflict of interest to disclose.

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