THE AMERICAN JOURNAL OF GASTROENTEROLOGY
Vol. 97, No. 7, 2002

ISSN 0002-9270/02/$22.00
Published by Elsevier Science Inc.
PII S0002-9270(02)04197-7

Serotonin Transporter Gene Polymorphism in Irritable Bowel Syndrome

Cengiz Pata, M.D., M. Emin Erdal, M.D., Ebru Derici, R.A., Aziz Yazar, M.D., Arzu Kanık, Ph.D., and Oğuz Ulu, M.D.

Departments of Internal Medicine, Medical Biology and Genetics, and Biostatistics, Faculty of Medicine, University of Mersin, Mersin, Turkey

OBJECTIVES: Serotonin is a key mediator of intestinal peristalsis, and after it is secreted, it is effectively cleansed from the neuronal gap by means of a high affinity substance called serotonin transporter (SERT), which depends on the Na^+ and Cl^- ions localized in the presynaptic neuronal membranes. The aim of this study was to investigate SERT polymorphism in patients with irritable bowel syndrome (IBS).

METHODS: SERT gene polymorphism was assessed by polymerase chain reaction on DNA chains obtained from leukocytes in serum samples from 54 patients diagnosed with IBS and 91 healthy subjects. The polymorphism of two regions (variable number tandem repeats and the SERT gene–linked polymorphic region [5-HTTLPR]) of SERT was assessed.

RESULTS: SERT polymorphisms were found to be similar in healthy subjects and IBS patients ($p > 0.05$). IBS patients were divided into three groups: diarrhea predominant (n = 18), constipation predominant (n = 26), and alternating diarrhea and constipation (n = 10). These groups were compared with respect to gene polymorphism, and it was found that the 5-HTTLPR allele S/S genotype occurred with greater frequency in the constipation predominant group than in the other two subgroups ($p < 0.05$), and L/S genotype frequency in the diarrhea predominant group was higher than those in the constipation and control groups.

CONCLUSIONS: No relationship was found between IBS and SERT gene polymorphism. It is conceivable that the presence of the S/S genotype in IBS patients carries an increased risk of the constipation predominant type of IBS, whereas the presence of the 5-HTTLPR allele L/S genotype carries an increased risk of the diarrhea predominant type. (Am J Gastroenterol 2002;97:1780–1784. © 2002 by Am. Coll. of Gastroenterology)

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic illness manifested by impaired bowel function and abdomen pain and has no explanatory organic diagnosis. It has been reported to have an incidence of 20% in developed countries (1). The etiopathogenesis of IBS is not completely known at present, but visceral hypersensitivity and GI tract hypersensitivity are thought to play an important role in its clinical symptoms (2, 3). Although a number of mediators are believed to be involved in regulating the rhythmic contractions in the intestinal wall, currently serotonin is thought to be the principal molecule responsible for control (4).

Serotonin is an important substance, carrying out functions in various locations such as the brain, mast cells, thrombocytes, and gut. Ninety-five percent of the body's serotonin is synthesized in the bowels, especially enterochromaffin cells in the gut (5). The enterochromaffin cells are sensitive to changes in intraluminal pressure and chemical stimuli, and these cells trigger peristaltic and secretory reflexes via primary afferent neurons by secreting serotonin when the intraluminal pressurre increases (6, 7). The serotonin molecule released from enterochromaffin cells affects the intestinal wall especially by means of the 5-hydroxytryptamine (5-HT) 5-HT_3 and 5-HT_4 receptors, which are found on enteric neurons in the GI tract (8). The cleansing of the serotonin from the neuronal gap is achieved by a substance called serotonin transporter protein (5-HTT, SERT, or SLC6A4) that depends on Na and Cl ions (9, 10). The mucosal transporter, which is expressed in enterocytes, seems to be the same transporter as that expressed in the brain system and enteric serotonergic neurons (11). Activities in two polymorphic sites in the SERT gene have attracted much interest: variable number tandem repeats (VNTRs) of 17–base pair (bp) repeats in intron two, and an insertion/deletion in the 5′-flanking promoter region (SERT gene–linked polymorphic region [5-HTTLPR]) creating a short (S) and a long (L) allele (12). The 5-HTTLPR polymorphism is situated in a GC-rich region composed of 20- to 23-bp repeating units. The S and L alleles have 14 and 16 repeat elements, respectively (12, 13). The SERT gene is located on chromosome 17q11.2, and a functional deletion/insertion polymorphism has been identified in the transcriptional control region (5-HTTLPR) (12).

The aim of this study was to investigate the association of the 5-HTTLPR and VNTR variants with IBS.

MATERIALS AND METHODS

Patients

From November, 2000 to April, 2001, 54 consecutive IBS patients (28 men and 26 women, mean age = 45.81 ± 12.24 yr [range = 18–73]) who were referred to our gastroenterology polyclinics and 91 healthy volunteers (48 men and 43 women, mean age = 41 ± 8.91 yr [range = 19–68]) were included in the study. The IBS patients were required to have had symptoms that fulfilled the Rome I criteria for IBS for >6 months (14). Twenty-six of the IBS patients had a constipation predominant bowel pattern, 18 had a diarrhea predominant bowel pattern, and the remaining 10 had a bowel pattern alternating between diarrhea and constipation. Informed consent was obtained from the subjects involved in the study.

Molecular Analysis

Amplification of DNA and analysis of VNTR polymorphism (with three common alleles: S Tin.2.9, S Tin 2.10, and S tin 2.12) were performed according to the method of Ogilvie *et al.* (15). Insertion/deletion polymorphism (with variants 484 and 528) was analyzed by the polymerase chain reaction (PCR), as described by Heils *et al.* (13).

Genotyping

DNA was extracted from whole blood by standard techniques. Genotyping was accomplished in the Roche Molecular Biochemicals (Mannheim, Germany) system.

PCR primers for intron 2 polymorphism were described by Lesch *et al.* in 1996 (12); the conditions were modified from those described previously (16). Alleles in this system are designed for the following number of repeats: STin 2.9, STin 2.10, and STin 2.12. PCR primers and conditions for the promoter system have been described previously (16). Alleles in this system are designated according to their relative size: "S" (14 repeats), "L" (16 repeats), and "xl" (20 repeats).

A deletion/insertion polymorphism (5-HTTLPR) in the promoter region of the SERT gene was typed by PCR using flanking primers (forward) 5′-GGCGTTGCCGCTCTGAATGC-3′ and (reversed) 5′-GAGGGACTGAGCTGGACAACCAC-3′. PCR was performed with the GC-Rich PCR System (Roche Molecular Biochemicals), a 50-μl reaction containing 20–100 ng of DNA; 100-μm deoxyribonucleoside triphosphate (dNTPs), 20 pmol per primer; and 1.5-mmol/L $MgCl_2$. DNA was denatured at 95.5°C for 3 min and subjected to 35 cycles of 1 min of denaturation at 95.5°C, 1 min of annealing at 60°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% agarose gels and visualized with ethidium bromide staining. Alleles were designated S (484 bp) and L (528 bp) according to Lesch *et al.* (12).

VNTR polymorphism in intron 2 of the SERT gene was typed by PCR using primers 5-HTT F 5′-TGGATTTCCTTCTCTCAGTGATTGG-3′ and 5-HTT R 5′-TCATGTTCCTAGTCTTACGCCAGTG-3′ to amplify a 390 bp (12 copies), 360 bp (10 copies), or 345 bp (nine copies) fragment of the SERT intron 3 variant (17).

PCR was performed at a volume of 50 μl with 20–100 ng of DNA; 100-μm dNTPs, 20 pmol/primer; 1.0-mmol/L $MgCl_2$; 1× PCR buffer with $(NH_4)_2SO_4$ (MBI Fermentas, Vilnius, Lithuania); and 1 U of Taq polymerase (MBI Fermentas). PCR conditions were 2 min of initial denaturation at 94°C, 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 57°C, and 2 min of extension at 72°C, followed by 10 min of final extension at 72°C. Alleles of both polymorphic locations were resolved by 2% agarose gel electrophoresis next to a DNA molecular weight standard.

Statistical Analysis

In all procedures, $p < 0.05$ was considered the level of significance. The findings were compared using the Mann-Whitney U and χ^2 tests. If there was significance in multiple cell χ^2, a one-sided Z approximation test was used to compare two independent proportions in the cells (18).

RESULTS

The study and control groups were similar with regard to age and gender ($p > 0.05$). VNTR and 5-HTTLPR alleles were present in all patients in the IBS and control groups. No significant difference between groups was found in the VNTR alleles 10/10, 10/12, and 12/12 (χ^2 =0.036, $p > 0.05$) (Table 1). The frequencies of 5-HTTLPR allele L/L, L/S, and S/S genotypes were found to be similar (χ^2 = 0.028, $p > 0.05$) (Table 2). The 54 IBS patients were divided into three subgroups—diarrhea predominant (n = 18), constipation predominant (n = 26), and alternating diarrhea and constipation (n = 10)—and gene polymorphism was assessed. The homozygote and heterozygote

Table 1. Polymorphism of the VNTR Region in the SERT Gene

	SERT Gene VNTR Polymorphism	
Genotype	IBS (n = 54)	Control (n = 91)
STin2.10/10	5 (9.3%)	8 (8.8%)
STin2.10/12	19 (35.2%)	31 (34.1%)
STin2.12/12	30 (55.6%)	52 (57.1%)

$\chi^2 = 0.036$, $p > 0.05$.

Table 2. Polymorphism of the 5-HTTLPR Region in the SERT Gene

	SERT Gene 5-HTTLPR Polymorphism	
Genotype	IBS (n = 54)	Control (n = 91)
L/L	8 (14.8%)	22 (23.5%)
L/S	28 (51.9%)	34 (37.0%)
S/S	18 (33.3%)	36 (39.5%)

$\chi^2 = 0.028$, $p > 0.05$.

Table 3. Polymorphism of the VNTR Region in the SERT Gene in Subgroups of Patients With IBS

	SERT Gene VNTR Polymorphism		
Genotype	Constipation Predominant (n = 26)	Diarrhea Predominant (n = 18)	Diarrhea-Constipation (n = 10)
STin2.10/10	3 (11.5%)		1 (10%)
STin2.10/12	9 (34.6%)	7 (38.8%)	4 (40%)
STin2.12/12	14 (53.8%)	11 (61.1%)	5 (50%)

$\chi^2 = 1.18$, $p > 0.05$.

genotypes were compared in consideration of the patients' VNTR alleles, and no significant difference was found ($\chi^2 = 1.118$, $p > 0.05$) (Table 3). Again, these three subgroups were compared with respect to their 5-HTTLPR allele L/L, L/S, and S/S genotypes, reaching a statistical significance ($\chi^2 = 20.94$, $p = 0.002$). Proportions of cells were compared to determine the reason for significance, and the frequency of the S/S genotype in the constipation predominant group was found to be significantly higher than those in the diarrhea predominant and diarrhea-constipation groups ($p = 0.007$ and $p = 0.02$), whereas no significant difference was detected when it was compared with the control group ($p = 0.25$). On the other hand, the frequencies of the S/S genotype in the diarrhea predominant and diarrhea constipation groups were lower than that in the control group ($p = 0.03$ and $p = 0.01$). No difference was found in the frequency of the L/L genotype between each of the subgroups and the control group ($p > 0.05$). The L/S genotype frequency was found at a significantly higher level in the diarrhea predominant group than in the constipation predominant, diarrhea-constipation, and control groups ($p = 0.001$, $p = 0.03$, and $p = 0.001$); however, there were no significant differences among L/S genotype frequencies in the constipation predominant, diarrhea-constipation, and control groups ($p > 0.05$) (Table 4).

DISCUSSION

Ever since the significant role played by the serotonin mediator in the etiopathology of IBS began to become clearer, the number of studies on this subject has been increasing. In the central and peripheral nervous system, 5-HT is inactivated primarily by reuptake into the serotonergic neurons, in which it is secreted (19, 20). This reuptake is accelerated by the SERT gene (21).

The function of VNTR is not well known, but 5-HTTLPR could be proposed as a candidate gene in IBS, as it modulates SERT gene transcription. The S form of this variant is associated with lower basal and induced transcriptional efficiency of the SERT gene promoter, resulting in lower serotonin uptake activity and longer serotonergic activity than with the L form (12, 22). In this case, we would take into consideration the differences between the IBS and control polymorphic sites in the SERT gene, especially 5-HTTLPR variations such as S and L alleles. The functional polymorphism located in the upstream regulatory region in VNTR in intron 2 of the SERT gene was not associated with the incidence of IBS. There were no significant differences with regard to the separation of 5-HTTLPR variations such as S and L alleles between the study and control groups, but the frequency of the allele containing S/S copies of the 5-HTTLPR element was significantly higher in the constipation predominant group in comparison with the other subgroups but not in comparison with the control group, and the frequencies of the S/S genotype in diarrhea predominant and diarrhea-constipation patients were significantly lower than that in control patients. Considering these results, the presence of the S/S allele may be acting as a protecting factor for diarrhea, so one may speculate that serotonin uptake was slower in constipation predominant patients than in diarrhea predominant and diarrhea constipation patients. This reflection appears to be at odds with the bowel motility–increasing effect that serotonin is known to have, but it should not be forgotten that studies

Table 4. Polymorphism of the 5-HTTLPR Region in the SERT Gene in Subgroups of Patients With IBS and the Control Group

	SERT Gene 5-HTTLPR Polymorphism			
Genotype	Constipation Predominant (n = 26)	Diarrhea Predominant (n = 18)	Diarrhea-Constipation (n = 10)	Control (n = 92)
L/L	4 (15.3%)		3 (30%)	22 (23.5%)
L/S	10 (38.4%)	16 (88.1%)*†	6 (60%)	34 (37.0%)
S/S	12 (46.15%)‡§	2 (11.1%)‖	1 (10%)¶	36 (39.5%)

$\chi^2 = 20.94$, $p = 0.002$.
* $p = 0.001$ (diarrhea predominant *vs* control and constipation predominant).
† $p = 0.03$ (diarrhea predominant *vs* diarrhea-constipation).
‡ $p = 0.007$ (constipation predominant *vs* diarrhea predominant).
§ $p = 0.02$ (constipation predominant *vs* diarrhea-constipation).
‖ $p = 0.01$ (diarrhea predominant *vs* control).
¶ $p = 0.03$ (diarrhea-constipation *vs* control).

investigating the relationship between IBS and serotonin have been concerned with 5-HT receptors and postreceptor events (4, 6, 23, 24). There are not enough studies attempting to determine the relationship between the SERT gene's functional capacity and receptor interaction and bowel functioning. In a study on mice (25), Chen *et al*. reported that serotonin uptake in the neuronal gap in the bowels was significantly higher in SERT +/+ subjects than in other groups, and that motility increased in SERT −/− mice at first but later became irregular and constipation attacks ensued. The hypothesis that extended serotonergic activity in the neuronal gap leads to downregulation in the 5-HT receptors over time, decreasing the serotonergic effect, may be an explanation for our results. At the same time, in studies with serotonin agonists it has been shown that as the agonistic effect in the neuronal gap increases, downregulation occurs in the 5-HT_1 and 5-HT_2 receptors, which may be considered to support our hypothesis (26–28). We think that studies establishing the relationship between SERT gene polymorphism and 5-HT_3 or 5-HT_4 receptors, which are effective in bowel contraction, will play an important role in clarifying the matter.

Although the frequency of the L/S genotype in diarrhea predominant patients was higher than those in constipation predominant and control patients, heterozygosity and the unknown metabolic effects of this allele make it hard to come to definite conclusions, but we think that the presence of the L/S allele increases the risk of the diarrhea predominant variant of IBS.

In conclusion, it was found that SERT is not a key factor in determining whether or not an individual will get IBS. If an individual has IBS, and if he or she has the S/S form of SERT, the IBS is more likely to be the constipation predominant variant. On the other hand, the presence of the L/S form of SERT is associated with increased risk of the diarrhea predominant type of IBS. However, it should not be forgotten that a relatively small number of patients may limit the results of this study.

Reprint requests and correspondence: C. Pata, M.D., Department of Internal Medicine, Faculty of Medicine, University of Mersin, Zeytinlibahçe Caddesi Eski Otogar, Yanı 33070, Turkey.
Received Sep. 6, 2001; accepted Jan. 3, 2002.

REFERENCES

1. Olden KW, Schuster MM. Irritable bowel syndrome. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. Gastrointestinal and liver disease. Philadelphia: WB Saunders, 1998:1536–46.
2. Mayer E, Munakata J, Mertz H, et al. Visceral hyperalgesia and irritable bowel syndrome. In: Gebhart GF, ed. Visceral pain. Progress in pain research and management. Seattle: IASP, 1995:429–68.
3. Farthing M. Irritable bowel, irritable body, irritable brain? Br Med J 1995;310:171–85.
4. Kim DY, Camilleri M. Serotonin: A mediator of the brain-gut connection. Am J Gastroenterol 2000;95:2698–708.
5. Tyce GM. Biochemistry of serotonin. In: Vanhoutte PM, ed. Serotonin and gastrointestinal system. New York: Raven, 1985:1–13.
6. Gershon MD. Review article: Roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment Pharmacol Ther 1999;13:15–30.
7. Pan H, Gershon MD. Activation of intrinsic afferent pathways in submucosal ganglia of the guinea pig small intestine. J Neurosci 2000;20:3295–309.
8. Tuludhar BR, Costall B, Naylor RJ. 5-HT3 and 5-HT4 receptor mediated facilitation of THA emptying phase of THA peristaltic reflex in THA marmoset isolated ileum. Br J Pharmacol 1996;117:1679–84.
9. Stahl SM. Essential psychopharmacology. Neuroscientific basis and practical applications. Cambridge, UK: Cambridge University Press, 1996:69–130.
10. Catalano M. Psychiatric genetics'99. The challenges of psychopharmacogenetics. Am J Hum Genet 1999;65:606–10.
11. Chen JX, Pan H, Rothman TP, et al. Guinea pig 5-HT transporter: Cloning expression, distribution, and function in intestinal sensory reception. Am J Physiol 1998;275:433–48.
12. Lesch K-P, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–31.
13. Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66: 2621–4.
14. Thompson W, Creed F, Drossman D. Functional bowel disorders and functional abdominal pain. Gastroenterol Int 1992; 5:75–91.
15. Ogilvie AD, Russel MB, Dhall P, et al. Altered allelic distribution of the serotonin transporter gene in migrain without aura and migrain with aura. Cephalagia 1998;18:23–6.
16. Gelernter J, Kranzler H, Cubells JF. Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African and European American and Japanese populations and in alcohol dependent subjects. Hum Genet 1997;101:243–6.
17. Gutierrez B, Pintor L, Gasto C, et al. Variability in the serotonin transporter gene and increased risk for major depression with melancholia. Hum Genet 1998;103:319–22.
18. Trapp RG. Basic and clinical biostatistics, 4th ed. Philadelphia: Saunders, 1990:147–59.
19. Fuller RW, Wong DT. Serotonin uptake and serotonin uptake inhibition. Ann N Y Acad Sci 1990;600:68–80.
20. Gershon MD. Biochemistry and physiology of serotonergic transmission. In: Brookhart JM, Mouncastle VB, eds. Handbook of physiology: The nervous system. Bethesda, MD: American Physiological Society, 1977:573–623.
21. Blakey RD, Berson HE, Fremeau RT, et al. Cloning and expression of functional serotonin transporter from rat brain. Nature 1991;354:66–70.
22. Collier DA, Stober G, Li T, et al. A novel functional polymorphism within the promotor of the serotonin transporter gene: Possible role in susceptibility to affective disorders. Mol Psychiatry 1996;1:453–60.
23. Wolfe SG, Chey WY, Washington MK. Tolerabilty and safety of alosetron during long-term administration in female and male irritable bowel syndrome patients. Am J Gastroenterol 2001;96:803–11.
24. Graf S, Sarna KS. 5-HT-induced colonic contractions: Enteric locus of action and receptor subtypes. Am J Physiol 1997;273: 68–74.
25. Chen JJ, Li Z, Pan H, et al. Maintence of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity

serotonin transporter: Abnormal intestinal motility and the expression of cation transporters. J Neurosci 2001;21:6348–61.
26. Saucier C, Morris SJ, Albert PR. Endogenous serotin-2A and 2c receptors in Balb/c-3T3 cells revealed in serotonin-free medium: Desensitization and down-regulation by serotonin. Biochem Pharmacol 1998;15:1347–57.
27. Eison AS, Mullins UL. Regulation of central 5-HT2A receptors: A review of in vivo studies. Behav Brain Res 1996;73:177–81.
28. van Huizen F, Bannse MT, Stam NJ. Agonist-induced down-regulation of human 5-HT1A and 5-HT2 receptors in Swiss 3T3 cells. Neuroreport 1993;30:1327–30.