

Introduction: To assess the potential association between serotonin transporter gene insertion/deletion polymorphism and the cancer-related constipation phenotype.

Material and methods: A total of 120 patients diagnosed with malignant solid tumors were subjected to genotyping. For the two groups – patients with constipation and constipation-free patients with non-gastrointestinal cancer, 60 cases in each group – we collected the peripheral venous blood. We extracted genomic DNA, and used polymerase chain reaction (PCR) to analyze the serotonin transporter (5-HT) link polymorphic region (5-HTTLPR) polymorphism of the serotonin transporter gene.

Results: The frequency of S/S genotype in cancer patients with constipation was 66.67% (40/60), and the frequency of the S allele was 79.17% (95/120); the frequency of S/S genotype in cancer patients without constipation was 48.33% (29/60), and the frequency of the S allele was 65.83% (79/120). There was a significant difference between the two groups ($p < 0.05$).

Conclusions: The presence of 5-HTTLPR S/S genotype and the S allele in patients with cancers probably carry an increased risk of constipation. However, its role as a cause of cancer-related constipation needs to be further investigated.

Key words: cancer, constipation, serotonin transporter, genetic polymorphism.

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Relationship between serotonin transporter gene polymorphism and constipation in cancer patients

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Introduction

Constipation is highly prevalent in patients with cancer and is a source of tremendous suffering [1]. Cancer-related constipation has a significant influence on patient quality of life. There are many possible causes but the mechanism is not clear. Information is lacking regarding available therapies for cancer-related constipation among current medications approved by the US Food and Drug Administration (FDA) [2]. Some scholars believe that constipation is correlated with visceral anomalies, genetic susceptibility, the neural immune mechanism, psychological factors and so on. The role of genetic susceptibility during the occurrence of constipation has drawn more and more attention recently. Hence the importance of serotonin in regulation of intestinal function has been a hot research subject.

Serotonin is an important gastrointestinal neurotransmitter and paracrine molecule, which regulates peristalsis in the gastrointestinal tract. It is secreted by an abundant amount of enterochromaffin cells and serves as a critical messenger for gastrointestinal fluid secretion and gut motility [3]. To prevent excessive 5-HT signaling and 5-HT receptor desensitization, the activities of enteric 5-HT are terminated by reuptake through the serotonin transporter protein (SERT) [4, 5]. Human SERT is encoded by a single gene on chromosome 17q11 and is composed of 14 exons that encode 630 amino acids [6].

SERT-P polymorphism results from a 44 base-pair insertion/deletion, approximately 1 kb upstream of the serotonin transporter gene. The polymorphism results in an insertion (L) and a deletion (S) allele [7]. In functional studies using a transfected cell line, homozygous deletion (S/S) and heterozygous (L/S) SERT genotypes were associated with lower transcriptional activity compared with that of the homozygous insertion (L/L) genotype, leading to a reduction in 5-HT reuptake [8].

Several studies have investigated the association between SERT gene polymorphism and irritable bowel syndrome (IBS), with contradictory results [9–11]. Two twin studies support a genetic and environmental basis for pathophysiology [12, 13]. In a study of 54 patients with IBS and 91 healthy subjects, it was found that the presence of the S/S genotype in IBS patients carries an increased risk of the constipation-predominant type of IBS (C-IBS),

whereas the presence of the 5-HTTLPR allele L/S genotype carries an increased risk of the diarrhea-predominant type (D-IBS) [14]. SERT gene polymorphism in patients with cancer may also participate in the occurrence of constipation. However, pathogenesis studies about cancer-related constipation are rarely reported. Therefore, the aim of this study was to assess the potential association between the SERT gene polymorphism and cancer-related constipation in a Chinese population.

Material and methods

Patient and control populations

A total of 120 patients with diagnosed malignant solid tumors participated in the study. The patients were recruited randomly from the Department of Oncology of the First Affiliated Hospital of Xinxiang Medical University from May 2009 to December 2009. Among the 120 patients, 60 of them had had symptoms that fulfilled the Rome III criteria for constipation for more than 6 months. The study also included 60 other patients who were constipation-free as a control group. Patients were excluded if they had a history of gastrointestinal cancer or other organic diseases, such as diabetes, hyperthyroidism, connective tissue disease, systemic neuromuscular disease, major organ diseases, or mental disorders. Informed consent was obtained from the subjects involved in the study.

Study design and molecular analysis

A peripheral venous blood sample from each individual was collected in ethylene diamine tetraacetic acid (EDTA) containing tubes. For analysis of 5-HTTLPR polymorphism of the serotonin transporter gene, genomic DNA of blood samples was extracted using phenol-chloroform [15]. The insertion/deletion polymorphism of the SERT gene was amplified [16] using an Eppendorf thermal cycler (Germany). The forward primer used was 5'-ATG CCA GCA CCT AAC CCC TAA TGT-3' and the reverse primer was 5'-GG ACC GCA AGG TGG GCG GGA-3' (Sangon). The expected product sizes for the insertion (L) and deletion (S) alleles were 419 bp and 375 bp, respectively. Amplification was performed in a 50 μ l reaction volume (Sangon), containing 2 \times PCR Master reaction buffer 25 μ l (containing MgCl₂ 3.75 μ l, dNTP 2.5 μ l, Tap enzyme 1.25 μ l and 2 \times PCR Master 17.5 μ l), upstream primer 1 μ l, downstream primer 1 μ l, template DNA 4 μ l, and sterile double-distilled water 19 μ l. Polymerase chain reaction cycle conditions were 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 2 min, and final extension at 72°C for 10 min. The amplified products were separated by electrophoresis on 2.0% agarose (Sangon) and visualized with ethidium bromide staining (Sangon). The gel was visualized under UV radiation using a gel electrophoresis visualizing system, photographed and analyzed with image analysis software.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS Inc, Chicago, IL). Values were expressed as mean \pm SD. Genotype and allele frequencies

were expressed as percentages. Differences in allele and genotype frequencies between patients and controls were analyzed using the χ^2 test. Demographic comparisons made in the study used the *t* test and χ^2 test for continuous and categorical variables respectively. Deletion/insertion and insertion/insertion genotype frequencies were collapsed into one group to form 2 \times 2 tables of deletion/deletion and non-deletion/deletion individual against disease status. Odds ratios and confidence intervals were also estimated. In all procedures, *p* < 0.05 was considered the level of significance.

Results

Characterization of experimental and control groups

In this study, 60 patients with constipation and 60 patients without constipation were enrolled. The demographic data of experimental and control groups are shown in Table 1. Control and experimental groups were similar in characteristics with respect to race, age, sex, marital status, education level, tumor type, and stage (*p* > 0.05 for all factors).

Gene polymorphism

Amplified products of the 5-HTTLPR gene separated by agarose electrophoresis are shown in Fig. 1. The product sizes of the insertion (L) and deletion (S) alleles were 419 bp and 375 bp, respectively.

The genotype distribution of the controls and patients with constipation were checked for deviation from the Hardy-Weinberg equilibrium using the χ^2 test. Genotype distribution and allele frequencies in the patients and control groups are given in Table 2. The results showed that frequency of S/S genotype in patients with constipation was 66.67%, and that of the controls was 48.33%. Comparing the two groups, the difference was significant ($\chi^2 = 4.13$, *p* < 0.05). No significant difference was found in the L/L and L/S genotype between the patients with constipation and the control group. The frequency of the S allele was significantly higher than that of L allele between the patients with constipation and the control group ($\chi^2 = 5.35$, *p* < 0.05).

Discussion

The pathogenesis of cancer-related constipation has not been reported in the literature; however, many scholars believe the pathogenesis of constipation may be related to intestinal motility disorders, visceral anomalies, intestinal infection, brain-gut interaction, genetic and environment factors, dietary factors, psychological factors and control disorders of the neuroendocrine network in the intestine, which was proposed in recent years. Concerning the role of genetic factors, recent studies have shown that the occurrence of constipation may be greatly controlled by genes [17]. There is a phenomenon of family aggregation and intergenerational transmission in functional constipation, and twins have higher incidence of gastrointestinal symptoms than the control subjects.

Table 1. Demographic data of constipation patients and controls

	Control n = 60 (%)	Constipation n = 60 (%)
Age (mean \pmSD), years	56.2 \pm 12.3	55.7 \pm 11.0
Male, n (%)	30 (50.0)	30 (50.0)
Ethnic group		
Chinese population	60 (100.0)	60 (100.0)
Marital status, n (%)		
Married	52 (86.7)	49 (81.7)
Single	8 (13.3)	11 (18.3)
Education level		
University and above	12 (20.0)	9 (15.0)
Secondary and below	48 (80.0)	51 (85.0)
Tumor type		
Esophageal cancer	15 (25.0)	13 (21.7)
Lung cancer	16 (26.7)	15 (25.0)
Breast cancer	10 (16.6)	11 (18.3)
Liver cancer	7 (11.6)	8 (13.3)
Lymphoma	4 (6.7)	4 (6.7)
Nasopharyngeal carcinoma	4 (6.7)	3 (5.0)
Others	4 (6.7)	6 (10.0)
Stage		
II	16 (26.7)	15 (25.0)
III	26 (43.3)	25 (41.7)
IV	18 (30.0)	20 (33.3)

Serotonin is an important gastrointestinal neurotransmitter and paracrine molecule [3], which regulates peristalsis in the gastrointestinal tract. It has become a hot research topic in the pathogenesis of constipation, and 5-HTTLPR has been studied more. The study about the relationship between SERT gene polymorphism and gastrointestinal functional diseases is focused on irritable bowel syndrome (IBS). This study indicated that the SERT gene polymorphism was associated with cancer-related constipation in the Chinese population.

Single-nucleotide polymorphism (SNP) refers to the variation between a single nucleotide base occurring on DNA sequences, in the population, the frequency of this mutation being at least 1%, otherwise it is considered to be a point mutation. Single-nucleotide polymorphisms are the most frequent type of variation in the human genome, and they provide powerful tools for a variety of medical genetic studies. This study showed that the frequency of S/S genotype was higher in patients with constipation than in the controls, and the difference was significant. Meanwhile, the frequency of the S allele was significantly higher than that of the L allele between the patients with constipation and the control group. *In vivo* experiments showed that the S allele of the SERT gene led to lower basal and induced transcriptional efficiency, reducing serotonin transporter expression and serotonin reuptake [18], and should theoretically result in increasing colonic motility and diarrhea. However, our results showed a different trend. Our results were consistent with the results of Pata [14], who reported no association between SERT-P polymorphisms and the presence of IBS, but did suggest the S/S homozygous polymorphism as a risk factor for the constipation-predominant form of

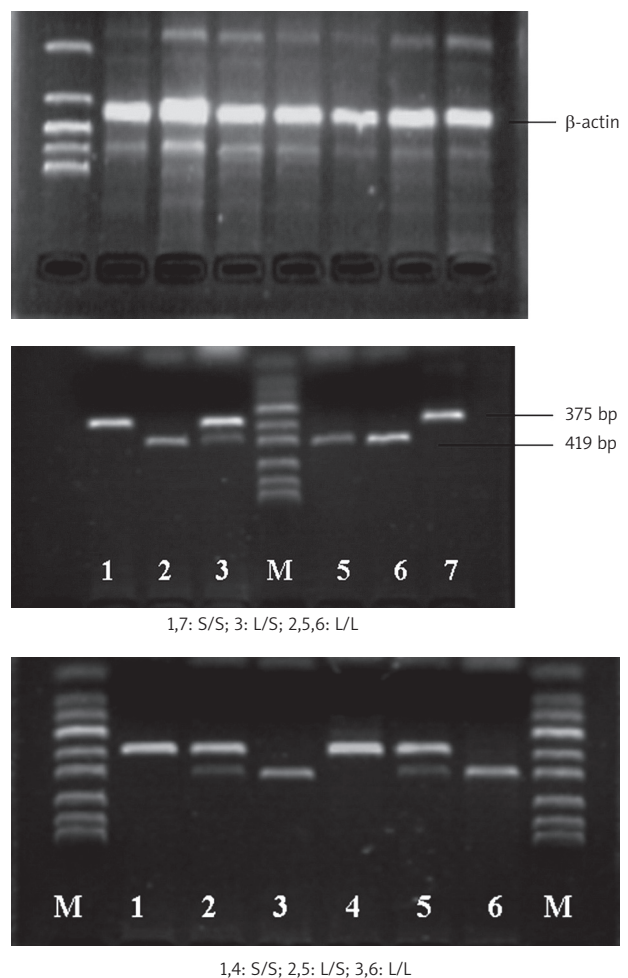
**Fig. 1.** Amplified products of the 5-HTTLPR gene separated by agarose electrophoresis

Table 2. Distribution of genotype and allele frequency of SERT-P polymorphism in the constipation patients and controls

Genotypic distribution (frequency)	Control n = 60 (%)	Constipation n = 60 (%)
wild type L/L	10 (16.67)	5 (8.33)
heterozygous L/S	21 (35.00)	15 (26.67)
homozygous polymorphism S/S	29 (48.33)	40 (66.67)*
allele frequency		
S allele	79 (65.83)	95 (79.17)**
L allele	41 (34.17)	25 (20.83)

* $\chi^2 = 4.13$, $p < 0.05$ (Control vs. Constipation)

** $\chi^2 = 5.35$, $p < 0.05$ (Control vs. Constipation)

the disease and the L/S heterozygous polymorphism similarly for D-IBS. Sikander *et al.* [19] also reported that the SERT-P allele was associated with C-IBS in the Indian population. The frequency of the SERT-P S/S genotype was higher in C-IBS than in healthy patients. This could be due to extended serotonergic activity in the neuronal gap because the polymorphism in the serotonin transporter leads to down-regulation in the 5-HT receptors over time, decreasing the serotonergic effect, which leads to constipation.

SERT-P polymorphism is known to show frequency variability between ethnic groups [20, 21]. The frequency of the S/S genotype in eastern patients was higher than in western patients [14, 22–24]. In our study, the frequency of the S/S genotype was 66.67% in cancer patients with constipation, and it was reported as 52% in Indian C-IBS patients [19]. On the other hand, when we evaluated the L allele and S allele as two groups, the frequency of the S allele (65.83%) and L allele (34.17) in the control group was consistent with the frequency reported in other studies: 39% long and 61% short alleles in one study with 174 controls [25]. In another study with 85 controls, the proportion of alleles was 25.3% long and 74.7% short [26].

In conclusion, our findings suggest that the serotonin transporter gene is a potential candidate gene for involvement in cancer patients with constipation in the Chinese population. In particular, the S/S genotype of SERT-P may cause a higher risk for constipation in cancer patients. However, the cause of constipation in patients with cancer and pathophysiology is very complex, and no single factor can explain it. It may be related to various factors including cell biology, neuroimmunology, behavioral medicine and psychology and other fields. Thus, further studies are needed to elucidate the physiologic importance of 5-HT receptor gene polymorphisms and to find new alternatives for the treatment of cancer-related constipation.

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