DOI: 10.15171/mejdd.2016.48

Serotonin Transporter Gene (SLC6A4) Polymorphism and Mucosal Serotonin Levels in Southeastern Iranian Patients with Irritable Bowel Syndrome

Mojgan Mohammadi [1,2], Hossein Tahmasebi Abdar [3], Hamid Reza Mollaei [4], Hossein Hajghani [5], Mohammad Reza Baneshi [6], Mohammad Mahdi Hayatbakhsh [7*]

1. Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Gastroenterology, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran
4. Department of Microbiology and Virology, Kerman University of Medical Sciences, Kerman, Iran
5. Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
6. Modeling in Health Research Centre, Institute of Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran
7. Gastroenterology and Hepatology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

*** Corresponding Author:**
Mohammad Mahdi Hayatbakhsh, MD
Department of Gastroenterology, Afzalipour Hospital,Kerman University of Medical Sciences, Kerman, Iran
Tel: + 98 343222270
Fax: + 98 343222270
Email: m24672@yahoo.com

Received: 29 Jul. 2016
Accepted: 28 Nov. 2016

ABSTRACT

BACKGROUND

Irritable bowel syndrome (IBS) is a digestive system disorder with an unknown etiology. Serotonin has a key role in the secretion and motility of the intestine. Polymorphism in serotonin re-uptake transporter (SERT or SLC6A4) gene may have a functional role in the gut of patients with IBS. The aims of the present study were to investigate the association between SLC6A4 gene polymorphism and IBS and to detect the correlation between rectal serotonin levels and IBS sub-types.

METHODS

SLC6A4 gene polymorphism in 131 patients with IBS and 211 healthy controls were analysed using the quantitative polymerase chain reaction high-resolution melting (qPCR-HRM) curve technique. Serotonin was measured in rectal biopsies of patients with IBS using the enzyme-linked immunosorbent assay (ELISA) method.

RESULTS

The patients were categorized into three groups: IBS with diarrhoea (IBS-D): 70 patients, IBS with constipation (IBS-C): 18 patients, and IBS with mixed symptoms (IBS-M): 43 patients. The frequency of SLC6A4 s/s and l/s genotypes was significantly higher in IBS-C than IBS-D, IBS-M, and controls (p=0.036). Serotonin levels were similar in IBS sub-types.

CONCLUSION

SLC6A4 polymorphism is a possible candidate gene associated with the pathogenesis of IBS-C. Although serotonin levels did not differ in rectal biopsies of IBS sub-types, further investigation is recommended.

KEYWORDS:

SLC6A4; SERT; Serotonin, IBS

Please cite this paper as:
Mohammadi M, Tahmasebi Abdar H , Mollaei HR, Hajghani H, Baneshi MR, Hayatbakhsh MM. Serotonin Transporter Gene (SLC6A4) Polymorphism and Mucosal Serotonin Levels in Southeastern Iranian Patients with Irritable Bowel Syndrome. *Middle East J Dig Dis* 2017;9:26-32. DOI: 10.15171/mejdd.2016.48

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic disease, which mainly affects lower gastrointestinal (GI) tract and has a prevalence of approximately 10-15%. The specific characteristic of IBS is altered bowel habits with abdominal pain.[1] Subtypes of IBS according to the Rome III criteria, are as follows; IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C), and IBS with

mixed symptoms (IBS-M).[2] Although the etiology of IBS remains uncertain, some achievements have highlighted its pathophysiology. Psychosocial factors such as depression and anxiety as well as visceral hypersensitivity and dysfunction of gut motility may play major roles in the progress of IBS.[1,3]

Serotonin is a neurotransmitter presenting in the GI tract, the central nervous system (CNS), blood platelets, and the pineal gland. Alternative name of serotonin is 5-hydroxytryptamine (5-HT), which can regulate the motility and mucosal secretion of the GI tract. Results of recent studies imply a link between impaired 5-HT signalling and the pathophysiology of diarrhoea and constipation in IBS.[4,5] Results of previous studies have shown that decreased and increased plasma levels of serotonin are associated with IBS-C and IBS-D, respectively.[4,6]

Enterochromaffin (EC) cells are a type of enteroendocrine and neuroendocrine cells found in the epithelia lining the lumen of the digestive tract. Serotonin is secreted from EC cells; then the serotonin reuptake transporter (SERT) reuptakes serotonin back into the EC cells and reduces the influence of serotonin in the GI tracts. The balance between serotonin production and its reuptake by SERT plays an important role in maintaining gut functions. Studies have shown that a disturbance in the SERT activity impairs this balance and might affect the development of IBS.[7] The gene that encodes the SERT is called solute carrier family 6 member 4 (SLC6A4). Previous studies have shown a polymorphism in the SERT gene SLC6A4 with 14 and 16 repeats namely a short (S) and a long (L) variation, respectively. These variations may affect SERT activity and gut function in IBS.[8-10] Conversely, some studies have shown a negative association between SLC6A4 gene polymorphism and IBS.[11,12] The aims of the present study were to investigate the association between SLC6A4 gene polymorphism and IBS and to detect the correlation between rectal serotonin levels and IBS sub-types.

MATERIALS AND METHODS

Study subjects

All participants were selected from Kerman, southeastern Iran. Patients and healthy controls were unrelated and living (and born) in Kerman. Healthy controls were selected from the Kerman Blood Transfusion Centre. Informed consent was obtained from all the patients. The research was performed between 2012 and 2014 and approved by the Ethics Committee of Kerman University of Medical Sciences. The approval number was K.91.236.

A clinical history was taken by a gastroenterologist from all the patients according to the protocol of Rome III.[2] A rectal biopsy was taken during flexible sigmoidoscopy in order to further measure the serotonin in patients with IBS. Patients with IBS were classified into IBS-D, IBS-C, and IBS-M subtypes using the Rome III criteria. A questionnaire about bowel symptoms was completed by each patient. Symptoms included dyspepsia, bloating, incomplete evacuation, gastroesophageal reflux, passage of mucus, feeling of urgency, frequency of stool, abdominal pain, constipation, and diarrhoea.

SLC6A4 genotyping

DNA was extracted from whole blood using a QiaAmp DNA Mini Kit (Qiagen, Valencia, Ca, USA). The forward and reverse primers for the SLC6A4 gene polymorphism were 5'-CGTTGCCGCTCTGAATGC- 3' and 5'-GAGGGACTGAGCTGGACAACCAC-3', respectively. The real-time PCR HRM-based method was employed to detect SLC6A4 gene polymorphism. The assay was performed using HRM Evagreen 5X Ampliqon master mix (Ampliqon, Hot FIREPol HRM Mix, No ROX) in a Rotor Gene 6000 instrument (Corbett Research). Steps of PCR were as follows; 1: holding at 50°C for 2 minutes, 2: initial denaturation at 95°C for 10 minutes, continued by 40 cycles of the following steps; 3:denaturation at 95°C for 20 seconds, 4: annealing at 65°C for 20 seconds, and 5: extension at 72°C for 20 seconds. Means of Tm for ll, ls, and ss genotypes were 88.2°C, 84.5°C, and 83°C, respectively.

Measurement of serotonin level in rectal biopsy

A rectal biopsy sample was taken by a gastroenterologist during the colonoscopy of each patient with IBS and used to measure serotonin levels. The specimens were archived at -80°C until thawed and homogenized at 4°C in 200 µL PBS (PH 7.2-7.4) by homogenizer. The homogenates were centrifuged for 10 minutes at 10,000 g. The temperature during the centrifugation was 4°C. Serotonin levels were measured employing ELISA kit (IBL International GmbH Hamburg, Germany) according to the manu-

facturer's guidelines.

Statistical analysis

The genotype and allelotype frequency deviations from the Hardy-Weinberg equilibrium were analysed for all the individuals. Statistical analyses such as logistic regression, independent t, Chi-square, and ANOVA tests were performed using the SPSS software version 17.0. Probability (P) values less than 0.05 were assumed as statistically significant.

RESULTS

Study population

Three hundred and forty-two subjects including 211 sex- and age-matched healthy controls (105 women and 106 men, mean age: 36.58±11.85 years) from the Kerman blood centre and 131 patients (63 women and 68 men, mean age: 37.4±12.58 years) with IBS were enrolled in the current study. Of the 131 patients with IBS, 70 (53.4%), 18 (13.7%), and 43 (32.8%) patients had IBS-D, IBS-C, and IBS-M, respectively. The demographic and clinical data of the patients are outlined in table 1.

Association between SLC6A4 polymorphism and IBS

The genotype and allelotype frequencies of SLC6A4 gene polymorphism for all individuals are summarized in table 2. Genotype distributions in the patients and controls, were in Hardy Weinberg equilibrium. In the patients, the genotype frequencies were 29% l/l, 48.1% l/s, and 22.9% s/s whereas in controls, these values were 33.2% l/l, 49.8% l/s, and 17.1% s/s (table 2). Results of logistic regression revealed that healthy controls, compared with the patients with IBS were 22% more likely to have I/I genotype. Corresponding figure for having I/S was 6%. On the other hand, healthy controls were 30% less likely to have S/S genotype. None of these differences were statistically significant.

There were no significant differences in the genotype of the SLC6A4 polymorphism between the patients with IBS and healthy controls. The frequency of s/s and l/s genotypes was significantly higher in IBS-C than in the healthy controls (p=0.032). There was no significant difference in genotype frequencies among the IBS subtypes when compared with each other (table 3).

Table 1: Demographic and clinical characteristics of the patients with irritable bowel syndrome

Parameters	IBS (n= 131)
Age	
(years, mean ± SD)	37.4±12.58
Sex	
Male	68
Female	63
IBS sub-types	
IBS-D n (%)	70 (53.4%)
IBS-C n (%)	18 (13.7%)
IBS-M n (%)	43 (32.8%)
Symptoms	
dyspepsia	4 (3.1%)
Bloating	110 (83.96%)
Feeling of incompletevacuation	53 (40.5%)
Urgency to pass stool	29 (22.1%)
Passage of mucus	34 (26.0%)
Abdominal pain	131 (100%)

IBS: irritable bowel syndrome, HC: healthy controls, IBS-D: IBS with diarrhea, IBS-C: IBS with constipation, IBS-M: IBS with mixed symptoms

However, there was a significant association between allelotype and subtypes of IBS (p=0.036). In particular, the frequency of s allele was higher in IBS-C than either IBS-D or IBS-M (p=0.001, table 4). The frequency of l allele was lower in patients with IBS-C (p<0.001).

Additionally, when the frequencies of SLC6A4 allelotype of the healthy controls and the IBS sub-types were compared, a significant difference, with regard to s allele, was seen between IBS-C and HC groups (63.9% versus 41.9%, p=0.011, table 5).

Relationship between rectal serotonin levels and IBS subtypes

The serotonin levels in the rectal biopsies of patients with IBS-D, IBS-C, and IBS-M was 8.43±1.32, 9.94±2.63, and 8.85±1.6 pg/mL, respectively with no significant difference among IBS sub-types . Moreover, there was no significant relationship between the serotonin levels in rectal biopsies of the patients with IBS and their SLC6A4 genotypes. ANOVA was used for statistical analysis of this data.

Association between serotonin levels and SLC6A4 genotypes and IBS symptoms

Table 2: Distribution of SLC6A4 gene polymorphism in healthy controls and patients with irritable bowel syndrome

SLC6A4 Genotype	Patients with IBS (n=131) # (%)	HC (n=211) # (%)	*p* value	OR (95% CI)
l/l	38 (29)	70 (33.2)	0.473	1.22 (0.760-1.96)
l/s	63 (48.1)	105 (49.8)	0.824	1.063 (0.686-1.64)
s/s	30(22.9)	36(17.1)	0.205	0.694(0.403-1.19)

IBS: irritable bowel syndrome; HC: healthy controls; SLC6A4: solute carrier family 6 member 4; s/s, l/s and l/l: three different types of genotypes as deletion/deletion, insertion/deletion and insertion/insertion respectively. Statistical analysis was performed by logistic regression.

Table 3: Comparison of the frequencies of SLC6A4 genotypes in patients with IBS sub-types and healthy controls

SLC6A4 Genotype	IBS-D n=70	IBS-C n=18	IBS-M n=43	HC n=211
l/l	(35.7%) 25	(5.6%) 1	(27.9%) 12	(33.2%) 70
s/s	(17.1%) 12	(33.3%) 6	(27.9%) 12	(17.1%) 36
l/s	(47.1%) 33	(61.1%) 11	(44.2%) 19	(49.8%) 105
***p* value**	0.917*	0.032**	0.383***	

IBS: irritable bowel syndrome, IBS-D: IBS with diarrhea, IBS-C: IBS with constipation, IBS-M: IBS with mixed symptoms, HC: healthy controls, SLC6A4: solute carrier family 6 member 4, s/s, l/s and l/l: three different types of genotypes as deletion/deletion, insertion/deletion, and insertion/insertion respectively.
Statistical analysis was performed by Chi-square test.
*: comparison between frequency of SLC6A4 genotypes in patients with IBS-D and HC;
**: comparison between frequency of SLC6A4 genotypes in patients with IBS-C and HC;
***: comparison between frequency of SLC6A4 genotypes in patients with IBS-M and HC

Table 4: Comparison of the frequencies of SLC6A4 allelotypes in patients with IBS sub-types

SLC6A4 Allelotype	IBS-D n=70	IBS-C n=18	IBS-M n=43	HC n=211
s allele	(40.7%)57	(63.9%)23	(50%)43	0.036
l allele	(59.3%)83	(36.1%)13	(50%)43	

IBS: irritable bowel syndrome, IBS-D: IBS with diarrhea, IBS-C: IBS with constipation, IBS-M: IBS with mixed symptoms, HC: healthy controls, SLC6A4: solute carrier family 6 member 4, the s and the l are different types of alleles and representatives of the deletion and the insertion, respectively. Statistical analysis was performed by Chi-square test.

Table 5: Comparison of the frequencies of SLC6A4 allelotypes in healthy controls and the patients with IBS sub-types

Allele	IBS-D n=70	IBS-C n=18	IBS-M n=43	HC n=211
s allele	(40.7%) 57	(63.9%) 23	(50%)43	(41.9%) 177
l allele	(59.3%) 83	(36.1%) 13	(50%)43	(58.1%) 245
***p*-value**	0.07*	0.011**	0.17***	

IBS: irritable bowel syndrome, IBS-D: IBS with diarrhea, IBS-C: IBS with constipation, IBS-M: IBS with mixed symptoms, HC: healthy controls, SLC6A4: solute carrier family 6 member 4, the s and the l are different types of alleles and representative of the deletion and the insertion, respectively. Statistical analysis was performed by Chi-square test.
*: comparison between the frequency of SLC6A4 allelotypes in patients with IBS-D and HC;
**: comparison between frequency of SLC6A4 allelotypes in patients with IBS-C and HC;
***: comparison between frequency of SLC6A4 allelotypes in patients with IBS-M and HC

There were no differences in rectal serotonin levels and frequency of SLC6A4 genotypes among patients with IBS reporting dyspepsia, bloating, feelings of urgency, incomplete evacuation, frequency of stool, and abdominal pain (data not shown). ANOVA was used for finding an association between serotonin levels and IBS symptoms. Additionally, Chi-square test was employed for finding an association between SLC6A4 genotypes and IBS symptoms.

DISCUSSION

Serotonin plays an important role in the GI tract. Impairment in SERT may be correlated with IBS symptoms. In the present study, no significant difference was found in rectal serotonin levels among IBS sub-types. Kumar and colleagues,[13] reported higher levels of serotonin in the rectal mucosa of patients with IBS-D. Houghton and co-workers,[6] showed that an exacerbation of postprandial symptoms in patients with IBS-D was correlated with higher plasma levels of serotonin. Faure and colleagues,[14] reported that serotonin in the rectal mucosa was significantly higher in pediatric patients with IBS than in controls. Coates and co-workers,[15] reported a lack of difference in the plasma serotonin levels between patients with IBS and controls and also among IBS sub-types.

According to the results of several studies, functional gene polymorphisms in SERT can be employed as a useful genetic marker for diagnosis of IBS sub-types.[16-18] Controversies regarding the association between SERT gene polymorphism, SLC6A4, and IBS have been reported in different populations. In the current study of a population in southeastern Iran, there were no significant differences in the SLC6A4 genotype frequencies between the patients with IBS and healthy controls. This lack of association between the SLC6A4 gene polymorphism and IBS is in agreement with earlier studies from Korea, Turkey, the USA, England, and a population from southwestern Iran.[19-25] The results of a meta-analysis conducted in 2007, which included 1034 patients with IBS and 1377 healthy controls showed a lack of association between the SLC6A4 gene polymorphism and IBS.[26] Additionally, the results of a recent meta-analysis performed in 2014, which included a total of 25 articles about 3443 patients with IBS and 3359 healthy controls did not conclude that there was a significant association between the SLC6A4 gene polymorphism and IBS.[12]

Several reports have shown an association between the SERT gene polymorphism, SLC6A4, and different IBS sub-types. Some reports have demonstrated an association between the ss genotype of the SERT gene polymorphism and IBS-D, but the results of the current study are inconsistent with those findings. Significant associations were observed between the ss genotype and IBS-D in Indians, Koreans, white Americans, Chinese, and Turks.[9,13,27-29] The results of the present study showed the ss and ls genotypes to be specifically associated with IBS-C compared with healthy controls, which is partially in agreement with a study on northern Indians by Sikander and co-workers,[30] who reported an association between the ss genotype and IBS-C. However, two reports from China, and one recent meta-analysis have demonstrated an association between the ll genotype and an increased risk of IBS-C, which is not in concordance with the current findings.[26,29,30] Moreover, in the present study, an association at the allelic level between the inheritance of s or l alleles and IBS was not found, but a significant association was observed between the s allele and IBS-C as compared with IBS-D and IBS-M. In a population from southwestern Iran, Farjadian and colleagues,[25] reported that the frequency of s allele was higher than l allele in IBS-C, but the difference was not statistically significant. Conversely, Kumar and co-workers,[13] reported a significant association between the s allele and a higher risk of IBS, which is not in agreement with the current findings.

In the present study, no association was found between the rectal serotonin level and the SLC6A4 gene polymorphism in patients with IBS. Furthermore, no difference in rectal serotonin levels among IBS sub-types was detected. Kumar and colleagues,[13] however demonstrated a significant association between the SLC6A4 gene polymorphism and the mucosal serotonin level in the rectal biopsies of IBS patients mainly in IBS-D. Moreover, they demonstrated significant serotonin levels in the rectal mucosal of patients with IBS who had frequent stools and more abdominal pain, which is not similar to the current results.

The inconsistencies between our results and those published by others might be due to the genetic variations among different populations, the influence of other known/unknown polymorphisms on the disease, environ-

mental interactions, the uncertainty in the diagnosis of IBS, differences in sampling from colon versus rectum or plasma, differences in assays employed to measure serotonin in the samples, or even the disparity in the number of samples in different studies.

In conclusion, the ss and the sl genotype and also the s allelotype of the SLC6A4 was associated with IBS-C in our population. The serotonin levels in the rectal biopsies of the patients with IBS were not associated with the SLC6A4 genotype and IBS sub-types. It is suggested that the relationship between other polymorphisms in the SERT gene and IBS be investigated. Moreover, detecting serotonin levels in other samples such as plasma or serum and comparing them with samples from the rectum in patients with IBS is highly recommended for future studies.

ACKNOWLEDGEMENTS

Special thanks to the staff of Kerman Blood Transfusion Centre who assisted us with blood collection from the healthy volunteers. These data have been taken from the thesis of Dr Hossein Tahmasebi Abdar, resident in gastroenterology, Medical School, Kerman University of Medical Sciences, Kerman, Iran. All authors performed the studies, drafted the manuscript and data analysis. All authors read and approved the final draft of the manuscript.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Grundmann O, Yoon SL. Irritable bowel syndrome: Epidemiology, diagnosis and treatment: An update for healthcare practitioners. *J Gastroenterol Hepatol* 2010;**25**:691-9. doi: 10.1111/j.1440-1746.2009.06120.x
2. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006;**130**:1480-91. doi: 10.1053/j.gastro.2005.11.061
3. Lackner JM, Gudleski GD, Thakur ER, Stewart TJ, Iacobucci GJ, Spiegel BM. The impact of physical complaints, social environment, and psychological functioning on IBS patients' health perceptions: looking beyond GI symptom severity. *Am J Gastroenterol* 2014;**109**:224-33. doi:10.1038/ajg.2013.410
4. Atkinson W, Lockhart S, Whorwell PJ, Keevil B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation-and diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2006;**130**:34-43. doi: 10.1053/j.gastro.2005.09.031
5. Dunlop SP, Coleman NS, Blackshaw E, Perkins AC, Singh G, Marsden CA, et al. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005;**3**:349-57.
6. Houghton L, Atkinson W, Whitaker R, Whorwell P, Rimmer M. Increased platelet depleted plasma 5-hydroxytryptamine concentration following meal ingestion in symptomatic female subjects with diarrhoea predominant irritable bowel syndrome. *Gut* 2003;**52**:663-70. doi:10.1136/gut.52.5.663
7. Bertrand PP, Bertrand RL. Serotonin release and uptake in the gastrointestinal tract. *Auton Neurosci* 2010;**153**:47-57. doi: 10.1016/j.autneu.2009.08.002
8. Wang Y, Chang Y, Chang Y, Cheng J, Li J, Wang T,et al. Serotonin transporter gene promoter region polymorphisms and serotonin transporter expression in the colonic mucosa of irritable bowel syndrome patients. *Neurogastroenterol Motil* 2012;**24**:560-5, e254-5. doi: 10.1111/j.1365-2982.2012.01902.x.
9. Park J, Choi M, Park J, Oh JH, Cho YK, Lee IS,et al. Serotonin transporter gene polymorphism and irritable bowel syndrome. *Neurogastroenterol Motil* 2006;**18**:995-1000. doi: 10.1111/j.1365-2982.2006.00829.x
10. Areeshi MY, Haque S, Panda AK, Mandal RK. A serotonin transporter gene (SLC6A4) polymorphism is associated with reduced risk of irritable bowel syndrome in American and Asian population: a meta-analysis. *PloS One* 2013;**8**:e75567. doi: 10.1371/journal.pone.0075567.
11. Kim H, Camilleri M, Carlson P, Cremonini F, Ferber I, Stephens D,et al. Association of distinct α2 adrenoceptor and serotonin transporter polymorphisms with constipation and somatic symptoms in functional gastrointestinal disorders. *Gut* 2004;**53**:829-37. doi:10.1136/gut.2003.030882
12. van Kerkhoven LA, Laheij RJ, Jansen JB. Meta-analysis: a functional polymorphism in the gene encoding for activity of the serotonin transporter protein is not associated with the irritable bowel syndrome. *Aliment Pharmacol Ther* 2007;**26**:979-86. doi: 10.1111/j.1365-2036.2007.03453.x
13. Kumar S, Ranjan P, Mittal B, Ghoshal UC. Serotonin transporter gene (SLC6A4) polymorphism in patients with irritable bowel syndrome and healthy controls. *J Gastrointestin Liver Dis* 2012;**21**:31-8.
14. Faure C, Patey N, Gauthier C, Brooks EM, Mawe GM. Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. *Gastroenterology* 2010;**139**:249-58. doi: 10.1053/j.gastro.2010.03.032.
15. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, et al. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 2004;**126**:1657-64. doi: 10.1053/j.gastro.2004.03.013

16. Colucci R, Blandizzi C, Bellini M, Ghisu N, Tonini M, Del Tacca M. The genetics of the serotonin transporter and irritable bowel syndrome. *Trends Mol Med* 2008;**14**:295-304. doi: 10.1016/j.molmed.2008.05.001

17. Kaiser R, Müller-Oerlinghausen B, Filler D, Tremblay PB, Berghöfer A, Roots I, et al. Correlation between serotonin uptake in human blood platelets with the 44-bp polymorphism and the 17-bp variable number of tandem repeat of the serotonin transporter. *Am J Med Genet* 2002;**114**:323-8. doi: 10.1002/ajmg.10119

18. Mawe G, Coates M, Moses P. Review article: intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther* 2006;**23**:1067-76. doi: 10.1111/j.1365-2036.2006.02858.x

19. Lee DY, Park H, Kim WH, Lee SI, Seo YJ, Choi YC. [Serotonin transporter gene polymorphism in healthy adults and patients with irritable bowel syndrome]. *Korean J Gastroenterol* 2004;**43**:18-22. doi: 10.1111/j.1365-2982.2006.00829.x

20. Pata C, Erdal ME, Derici E, Yazar A, Kanık A, Ulu O. Serotonin transporter gene polymorphism in irritable bowel syndrome. *Am J Gastroenterol* 2002;**97**:1780-4. doi:10.1111/j.1572-0241.2002.05841.x

21. Saito Y, Locke G, Zimmerman J, Holtmann G, Slusser JP, de Andrade M,et al. A genetic association study of 5-HTT LPR and GNβ3 C825T polymorphisms with irritable bowel syndrome. *Neurogastroenterol Motil* 2007;**19**:465-70. doi: 10.1111/j.1365-2982.2007.00905.x

22. Niesler B, Kapeller J, Fell C, Atkinson W, Möller D, Fischer C,et al. 5-HTTLPR and STin2 polymorphisms in the serotonin transporter gene and irritable bowel syndrome: effect of bowel habit and sex. *Eur J Gastroenterol Hepatol* 2010;**22**:856-61. doi: 10.1097/MEG.0b013e32832e9d6b.

23. Kohen R, Jarrett ME, Cain KC, Jun SE, Navaja GP, Symonds S,et al. The serotonin transporter polymorphism rs25531 is associated with irritable bowel syndrome. *Dig Dis Sci* 2009;**54**:2663-70. doi: 10.1007/s10620-008-0666-3.

24. Farjadian S, Fakhraei B, Moeini M, Nasiri M, Fattahi MR. Serotonin transporter gene polymorphisms in Southwestern Iranian patients with irritable bowel syndrome. *Arab J Gastroenterol* 2013;**14**:59-62. doi: 10.1016/j.ajg.2013.03.001

25. Zhang Z-F, Duan Z-J, Wang L-X, Yang D, Zhao G, Zhang L. The serotonin transporter gene polymorphism (5-HTTLPR) and irritable bowel syndrome: a meta-analysis of 25 studies. *BMC Gastroenterol* 2014;**14**:23. doi: 10.1186/1471-230X-14-23

26. Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M, et al. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. *Gut* 2004;**53**:1452-8. doi:10.1136/gut.2003.035451

27. Yuan J, Kang C, Wang M, Wang Q, Li P, Liu H,et al. Association study of serotonin transporter SLC6A4 gene with Chinese Han irritable bowel syndrome. *PloS One* 2014;**9**. doi: 10.1371/journal.pone.0084414

28. Li Y, Nie Y, Xie J, Tang W, Liang P, Sha W,et al. The association of serotonin transporter genetic polymorphisms and irritable bowel syndrome and its influence on tegaserod treatment in Chinese patients. *Dig Dis Sci* 2007;**52**:2942-9. doi: 10.1007/s10620-006-9679-y

29. Wang B, Wang Y, Zhang W, Zhang QY, Liu WT, Jiang K, et al. [Serotonin transporter gene polymorphism in irritable bowel syndrome]. *Zhonghua Nei Ke Za Zhi* 2004;**43**:439-41.

30. Sikander A, Rana SV, Sinha SK, Prasad KK, Arora SK, Sharma SK, et al. Serotonin transporter promoter variant: Analysis in Indian IBS patients and control population. *J Clin Gastroenterol* 2009;**43**:957-61. doi: 10.1097/MCG.0b013e3181b37e8c.