Int J Clin Exp Med 2015;8(3):4581-4585
www.ijcem.com /ISSN:1940-5901/IJCEM0004792

Original Article

Association of genetic polymorphisms in HTR3A and HTR3E with diarrhea predominant irritable bowel syndrome

Qiao-Yan Gu[1,2], Jun Zhang[1], Yi-Chao Feng[2], Guang-Rong Dai[2], Wei-Ping Du[2]

[1]Department of Gastroenterology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, Shaanxi Province, P. R. China; [2]Department of Gastroenterology, Affiliated Hospital of Yan'an University, Yan'an 716000, Shaanxi Province, P. R. China

Received December 13, 2014; Accepted January 30, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: Objective: The present study aims to investigate the relationship between genetic polymorphisms in HTR3A and HTR3E and diarrhea predominant irritable bowel syndrome (D-IBS) in a Chinese population. Methods: We enrolled 500 D-IBS patients and 500 age- and sex-matched healthy control subjects to detect the genotypes in HTR3A and HTR3B gene by using of PCR-RFLP method. Results: There were significant difference between the D-IBS patients and the health control subjects in the distribution of genotype and allele of rs1062613 in HTR3A gene. As regarding rs62625044 in HTR3E gene, we found there was a significant different between the case and the control group in the distribution of GA genotype and A allele in female but not in male. Conclusion: The present study suggested that there are associations of D-IBS risk with genetic polymorphisms in HTR3A and HTR3E.

Keywords: Diarrhea predominant irritable bowel syndrome, gene, polymorphism, HTR3A, HTR3E

Introduction

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a functional bowel disorder which is very common worldwide, especially in women [1-3]. Serotonin (5-HT), a brain - gut axis contact key neurotransmitter, plays an important role in the pathogenesis of irritable bowel syndrome (IBS) [4, 5]. Among all the 5-HT receptors, the serotonin receptor 3 (5-HT3) is an important medium. It has been shown 5-HT3 plays a key role in the motor-sensory function of the gut [5, 6]. HTR3A, one of the 5-HT3 subunits (HTR3A, HTR3B, HTR3C, HTR3D and HTR3E), plays a critical role in the formation of 5-HT3 receptor function [7, 8]. However, HTR3E only expressed in the gastrointestinal tissues, suggesting HTR3E receptor subtype may play a special role in the human gastrointestinal tract function [8].

Genetic variations that alter gene expression cause phenotypic diversity and play an important role in disease susceptibility especially with regard to complex conditions [9]. Previous studies indicated that HTR3A genetic polymorphisms may be associated with IBS susceptibility [10, 11], but the conclusions are still controversial. Therefore, in the present study, we aimed to further investigate the relationship between D-IBS susceptibility and the genetics polymorphisms in HTR3E and HTR3A gene.

Subjects and methods

Subjects

D-IBS patients were collected from January 2011 to May 2014 in the Second Affiliated Hospital of Xi'an Jiaotong University and the Affiliated Hospital of Yan'an University. A total of 500 D-IBS patients (314 cases of female, 186 cases of male) aged 20 to 76 years old were enrolled the present study. All the patients were examined the blood routine test, blood biochemistry, HBsAg, anti -HCV, and anti -HIV and were found to be normal. Stool routine test and flora analysis and intestinal endoscopy examination showed no abnormalities. In addition, no subject had a history of major psychiatric disor-

Table 1. The sequences of primers for the PCR

SNPs	Gene	Sequence of primers	Endonuclease	Products
rs1062613	HTR3A	Sense: 5'CAGCTGTCCCCTCCCCTTTCCT3'	Hpy188III	348 bp
		Antisense: 5'AGAGCGGGCCTGGTGGTGTTT3'		
rs62625044	HTR3E	Sense: 5'CGTCATATGCCTCTGGAACA3'	Hpy188III	397 bp
		Antisense: 5'ATAGGCGTGAACCACTGCAC3'		

der or history of alcohol or substance abuse. A total of 500 healthy controls (321 cases of female, 179 cases of male) were selected from the same hospitals as the D-IBS patients. All the control subjects must meet to the following criteria: 1) no gastrointestinal symptoms and gastrointestinal diseases; 2) normal bowel habits; 3) no history of abdominal surgery; 4) non-immune diseases, infection and a history of recent drug taking history; 5) colonoscopy examination showed no abnormal in intestinal mucosa; 6) the age, sex and other general information were matched with D-IBS group. There are not significant difference between case and control groups on gender, age, and occupation (all $P > 0.05$).

Diagnostic criteria

All the D-IBS diagnostic criteria have been reported previously [12]. Briefly, two or more recurrent abdominal pain or discomfort in the last three months and at least 3 d with the above symptoms each month, and also must meet to at least 2 criteria of the following: 1) symptoms disappear after bowels; 2) onset with changes in stool frequency (> 3 times daily); 3) onset with stool appearance changed.

Methods

Sample collection and DNA extraction: All patients and controls were taken 2 ml of whole blood (within 1 ml of 2% $EDTANa_2$) on the day to enroll. Genomic DNA was prepared from blood samples using standard protocols.

Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP): PCRs were performed in 25 ml volumes containing 50 ng of genomic DNA as template, 10 pmol of each primer, 200 Mm dNTPs (MBI Fermentas), 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 0.1% Triton X-100 and 1.25 U of Taq DNA Polymerase (NEB). Thermal cycling was performed in a Mastercycler gradient thermal cycler (Eppendorf). Annealing temperatures (TA), sequences of the HTR3A and HTR3E primers, and endonuclease are shown in (**Table 1**). Cycling conditions were: Initial denaturation at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, TA for 30 s and 72°C for 30 s. The final extension step was at 72°C for 5 min. The products after digestion of Hpy188III (NEB) were analyzed on a 2.0% agarose gel.

Statistics: Comparison of genotype frequencies, association analyses and test for deviation from the HWE were performed using Chi-square test. For the association analyses, the frequencies of genotypes were compared in a minor allele dominant model using a 2 × 2 contingency table. We calculated *P*-values using the Chi- square test. In those cases where the expected value of at least one cell of the contingency table was below 5, we used the Fisher's exact test. Corrections for multiple testing were performed as indicated in the results.

Results

Hardy-Weinberg equilibrium test

The genotype distributions of rs1062613 and rs62625044 were agreement with H-WE both in case and control groups (both $P > 0.05$, data not shown).

Distributions of genotypes and alleles in case and control groups

As shown in (**Table 2**), there were significant differences between patients D-IBS and controls in rs1062613 polymorphism distribution both in men and women. The T allele frequency was significantly higher in the D-IBS patients than that in the control group. As regarding rs62625044, not only the GA genotype frequency but also the allele frequency was higher in female patients than that in the female controls. We did not found significant difference between male patients and male controls in either GA genotype or A allele.

Table 2. Distribution of genotypes and alleles between each group

Groups	Genotypes, n									Allele, n					
	Total			Male			Female			Total		Male		Female	
rs1062613	CC	CT	TT	CC	CT	TT	CC	CT	TT	C	T	C	T	C	T
Case (n = 500)	299	155	46	112	51	10	187	104	36	753	247	275	71	478	176
Control	340	144	16	115	39	1	225	105	15	824	176	269	41	555	135
P value	< 0.001			0.018			0.003			< 0.001		0.013		0.001	
rs62625044	GG	GA	AA	GG	GA	AA	GG	GA	AA	G	A	G	A	G	A
Case	429	71	0	151	22	0	278	49	0	929	71	324	22	605	49
Control	453	47	0	133	22	0	320	25	0	953	47	288	22	665	25
P value	0.019			0.695			0.001			0.023		0.706		0.002	

Table 3. Risk analyses on D-IBS between different genotype

Genotypes	D-IBS	Control	OR (95% CI)	P
HTR3A				
CC	299	340	1	
CT	155	144	1.207 (0.918-1.588)	0.178
TT	46	16	3.269 (1.813-5.896)	< 0.001
HTR3E				
GG	429	453	1	
GA	71	47	1.595 (1.078-2.360)	0.019
AA	0	0	-	-

Compared to rs1062613 CC genotype, TT genotype carriers have increased risk for D-IBS (OR = 3.269, 95% CI: 1.813-5.896). Similarly, compared to rs62625044 GG genotype, GA genotype carriers have increased risk for D-IBS (OR = 1.595, 95% CI: 1.078-2.360) (**Table 3**).

Discussion

In the present study, we found genetic polymorphisms in HTR3A and HTR3E gene were associated with D-IBS in Chinese population.

IBS is a common functional gastrointestinal disorder. In developed countries, the incidence rate of IBS is10% to 15% [13-15]. In developing countries, the incidence rate of IBS also presents increasing trend [14]. However, the pathogenesis of IBS is unclear. It is generally agreed that the brain - gut interaction may involve in the pathogenesis of IBS [16, 17]. 5-HT is a key neurotransmitter to contact brain - gut axis. 95% of the 5-HT distribute in the gastrointestinal tract and about 5% exist in the brain. The changes in the central nervous levels will lead to mental and behavioral abnormalities, such as insomnia, anxiety, etc [18]. However, the level changes in the gastrointestinal tract will cause abdominal pain, diarrhea and other symptoms. In all of the 5-HT receptor, 5-HT3 receptors are not only ligand-gated ion channel protein, but important mediums for 5-HT. It has been shown 5-HT3 receptors play a key role in sensory function of the gut.

There are five 5-HT3 receptor subtypes (HTR3A, HTR3B, HTR3C, HTR3D, and HTR3E). Among these subtypes, HTR3A plays a key role in the formation of 5-HT3 receptor function. HTR3E only expressed in gastrointestinal tissues, such as the colon, small intestine and stomach, while other subtypes (HTR3B, HTR3C, and HTR3D) express in a wider range of the body, which suggest HTR3E may play a special role in the human gastrointestinal tract function. Kapeller et al [19] found HTR3A and HTR3E untranslated region polymorphisms are major predisposing factor of D-IBS. However, HTR3A genetic polymorphisms have not been validated in Germany population. In this study we analyzed the association between D-IBS risk and HTR3A and HTR3E gene polymorphisms and found that HTR3A genetic polymorphism was associated with D-IBS in both men and women. Our results are consistent with the Kapeller et al's findings [19].

Our results indicated that the T allele of HTR3A may be a predisposing factor of D-IBS in Chinese population. Previous study suggested that HTR3A receptor density increased in T allele carriers [19]. Previous studies also found that, T allele was associated with female bipolar disorder, avoiding injuries and regulation of amygdala activities [20, 21]. Therefore, T allele may affect the composition and density of 5-HT3 receptor in various body tissues.

This study also showed the A allele of HTR3E frequency was significantly higher in female D-IBS patients compared with female control group. Whereas there were no differences in either A allele or GA genotype between male patients and male control subjects. This result was consistent with the findings of Kapeller et al [19]. Therefore, our results indicated that A allele may be an important factor in women's susceptibility to D-IBS. The reason for why there is no association to be found in male may be explained by the affection of ovarian hormones on visceral sensitivity, although, which needs verify by further research.

In conclusion, the present study indicated that genetic polymorphisms in HTR3A and HTR3E were associated with the risk for D-IBS in Chinese population, especially in women.

Disclosure of conflict of interest

None.

Address correspondence to: Jun Zhang, Department of Gastroenterology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, Shaanxi Province, P. R. China. Tel: +86-029-87679305; Fax: +86-029-87679305; E-mail: jun3z@163.com

References

[1] Vanuytsel T, Tack JF, Boeckxstaens GE. Treatment of abdominal pain in irritable bowel syndrome. J Gastroenterol 2014; 49: 1193-205.

[2] Camilleri M. Current and future pharmacological treatments for diarrhea-predominant irritable bowel syndrome. Expert Opin Pharmacother 2013; 14: 1151-60.

[3] Lovell RM, Ford AC. Effect of gender on prevalence of irritable bowel syndrome in the community: systematic review and meta-analysis. Am J Gastroenterol 2012; 107: 991-1000.

[4] Zhan DW, Sun JH, Luo KT, Xu LZ, Zhou JL, Pei LX, Chen L, Wu XL, Zhang JW, Zhang W, Jiao DY, Zhu L. Effects and efficacy observation of acupuncture on serum 5-HT in patients with diarrhea-predominant irritable bowel syndrome. Zhongguo Zhen Jiu 2014; 34: 135-8.

[5] Itagaki R, Koda K, Yamazaki M, Shuto K, Kosugi C, Hirano A, Arimitsu H, Shiragami R, Yoshimura Y, Suzuki M. Serotonin (5-HT3) receptor antagonists for the reduction of symptoms of low anterior resection syndrome. Clin Exp Gastroenterol 2014; 7: 47-52.

[6] Barbara G. Revival of 5-HT3 antagonism as treatment of IBS-D? Gut 2014; 63: 1530-2.

[7] Kapeller J, Möller D, Lasitschka F, Autschbach F, Hovius R, Rappold G, Brüss M, Gershon MD, Niesler B. Serotonin receptor diversity in the human colon: Expression of serotonin type 3 receptor subunits 5-HT3C, 5-HT3D, and 5-HT-3E. J Comp Neurol 2011; 519: 420-32.

[8] Schuhmacher A, Mössner R, Quednow BB, Kühn KU, Wagner M, Cvetanovska G, Rujescu D, Zill P, Möller HJ, Rietschel M, Franke P, Wölwer W, Gaebel W, Maier W. Influence of 5-HT3 receptor subunit genes HTR3A, HTR3B, HTR3C, HTR3D and HTR3E on treatment response to antipsychotics in schizophrenia. Pharmacogenet Genomics 2009; 19: 843-51

[9] Montagnana M, Danese E, Lippi G. Genetic risk factors of atherothrombosis. Pol Arch Med Wewn 2014; 124: 474-82.

[10] Kilpatrick LA, Labus JS, Coveleskie K, Hammer C, Rappold G, Tillisch K, Bueller JA, Suyenobu B, Jarcho JM, McRoberts JA, Niesler B, Mayer EA. The HTR3A polymorphism c. -42C> T is associated with amygdala responsiveness in patients with irritable bowel syndrome. Gas troenterology 2011; 140: 1943-51.

[11] Zhang Y, Huang Y, Bo P. Association between diarrhea-predominant irritable bowel syndrome and HTR3A, HTR3E gene polymorphism in Yangzhou, Jiangsu province, China. Zhonghua Liu Xing Bing Xue Za Zhi 2013; 34: 721-4.

[12] Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006; 130: 1480-91.

[13] Scanu AM1, Bull TJ, Cannas S, Sanderson JD, Sechi LA, Dettori G, Zanetti S, Hermon-Taylor J. Mycobacterium avium subspecies paratuberculosis infection in cases of irritable bowel syndrome and comparison with Crohn's disease and Johne's disease: common neural and immune pathogenicities. J Clin Microbiol 2007; 45: 3883-90.

[14] Canavan C, Card T, West J. The incidence of other gastroenterological disease following diagnosis of irritable bowel syndrome in the UK: a cohort study. PLoS One 2014; 9: e106478.

[15] Herman J, Pokkunuri V, Braham L, Pimentel M. Gender distribution in irritable bowel syndrome is proportional to the severity of constipation relative to diarrhea. Gend Med 2010; 7: 240-6

[16] Okumura T. Brain-gut interaction in the pathophysiology of IBS. Nihon Shokakibyo Gakkai Zasshi 2014; 111: 1334-44.

[17] De Palma G, Collins SM, Bercik P. The microbiota-gut-brain axis in functional gastrointestinal disorders. Gut Microbes 2014; 5: 419-29.

[18] Saletu-Zyhlarz GM, Anderer P, Arnold O, Saletu B. Confirmation of the neurophysiologically predicted therapeutic effects of trazodone on

its target symptoms depression, anxiety and insomnia by postmarketing clinical studies with a controlled-release formulation in depressed outpatients. Neuropsychobiology 2003; 48: 194-208.

[19] Kapeller J, Houghton LA, Mönnikes H, Walstab J, Möller D, Bönisch H, Burwinkel B, Autschbach F, Funke B, Lasitschka F, Gassler N, Fischer C, Whorwell PJ, Atkinson W, Fell C, Büchner KJ, Schmidtmann M, van der Voort I, Wisser AS, Berg T, Rappold G, Niesler B. First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. Hum Mol Genet 2008; 17: 2967-77.

[20] Niesler B, Flohr T, Nöthen MM, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold GA. Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. Pharmacogenetics 2001; 11: 471-5.

[21] Iidaka T, Ozaki N, Matsumoto A, Nogawa J, Kinoshita Y, Suzuki T, Iwata N, Yamamoto Y, Okada T, Sadato N. A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. J Neurosci 2005; 25: 6460-6.