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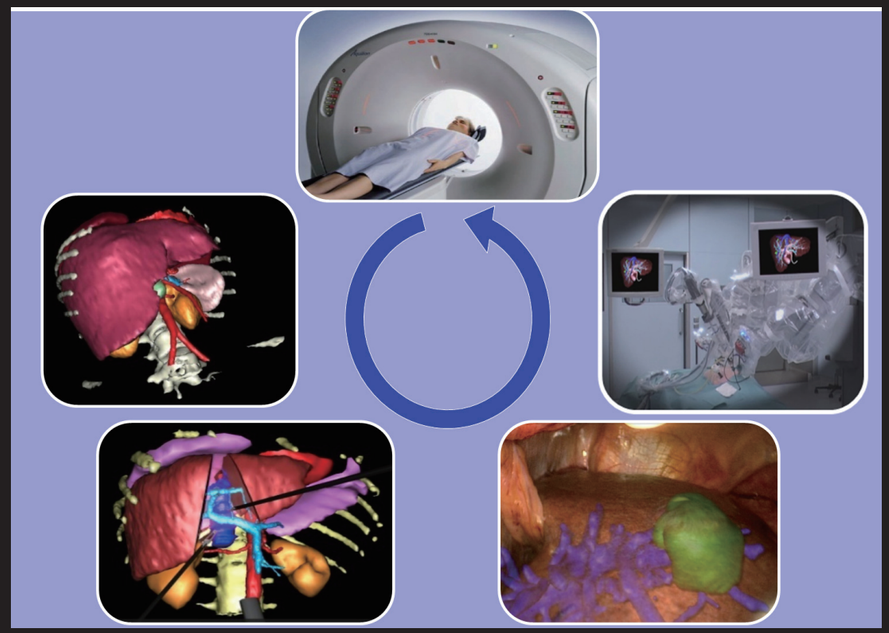

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ORIGINAL ARTICLE

Association Between Brain-gut Peptide Polymorphisms and Irritable Bowel Syndrome

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ABSTRACT

AIM: Associations between calcitonin gene-related peptide (CGRPα, encoded by CALCA), transient receptor potential vanilloid-1 (TRPV1) and transcription factor 7-like2 (TCF7L2) polymorphisms in IBS were evaluated.

METHODS: DNA was obtained from 108 IBS patients [53 diarrhea-type (IBS-D), 31 constipation-type (IBS-C), 8 mixed-type (IBS-M), and 16 unsubtyped (IBS-U)] and 61 controls. For all analyses, IBS-M and IBS-U patients were combined into one group (NonDNonC). CALCA, TRPV1 and TCF7L2 polymorphisms were detected by the polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method.

RESULTS: The CALCA, TRPV1 and TCF7L2 genotype frequencies were not significantly different between IBS patients and controls. The genotype distributions were not significantly different between IBS-D, IBS-C and NonDNonC. TRPV1 genotype frequencies were significantly different between patients with <65 years old (y.o.) and ≥65 y.o. Particularly, TRPV1 C/C genotype with ≥65 y.o. in male or disease duration <3 years were trend to be fewer than in female or disease duration ≥3 years. CALCA and TCF7L2 genotype frequencies were not significantly different between patients with <65 y.o. and ≥65 y.o.

CONCLUSIONS: TRPV1 polymorphisms in IBS patients would be associated with age. TRPV1 C/C genotype with ≥65 y.o. might be associated with gender and disease duration.

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Key words: Irritable bowel syndrome; Brain-gut peptide; Transient receptor potential vanilloid-1 (TRPV1); Calcitonin gene-related peptide (CALCA); Transcription factor 7-like2 (TCF7L2)

Toya Y, Chiba T, Sugai T, Habano W, Suzuki K. Association Between Brain-gut Peptide Polymorphisms and Irritable Bowel Syndrome. *Journal of Gastroenterology and Hepatology Research* 2013; 2(5): 576-580 Available from: URL: http://www.ghrnet.org/index./joghr/

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional disease characterized by symptoms such as abdominal pain, discomfort, and bowel movement disturbance, although the cause has not yet been identified. IBS is currently a major healthcare burden because it represents one of the most common disorders encountered in gastrointestinal practice and highly affects quality of life[1]. However, the precise mechanisms underlying IBS pathogenesis remain unclear. Multiple factors appear to be involved, further complicating the elucidation of disease mechanisms. Recent studies have suggested that the functional relationship between the brain and the gut (brain-gut interaction) plays a key role in the pathophysiology of IBS[2,3], and that environmental factors are also likely to play an important role in the pathogenesis and clinical manifestations of IBS.

Several recent studies have suggested a genetic basis for IBS, either with respect to etiology or predicting response to therapy[4]. Analysis of polymorphisms in toll-like receptor 9 (TLR9), Cadherin-1 (CDH1) and IL-6 genes associated with IBS, which have been clinical risk factors in post-infectious IBS (PI-IBS)[5].

Reactivity in cerebral function has been reported to differ in association with gene polymorphism. For example, serotonin transporter gene polymorphism has been shown to influence development of visceral pain and negative emotions in IBS patients[6]. Polymorphisms in three genes that encode brain-gut peptides, calcitonin gene-related peptide (CGRPα, encoded by CALCA), transient receptor potential vanilloid-1 (TRPV1) and transcription factor 7-like 2 (TCF7L2) have been characterized. CGRP is expressed throughout the central and peripheral nervous

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systems, consistent with control of vasodilatation and nociception. CGRPα is prominently localized in primary spinal afferent C and Aδ fibers of sensory ganglia[7]. The rs1553005 CGRP polymorphism has been reported to be associated with migraine onset[8-9]. Furthermore, CGRP is thought to participate in colonic hypersensitivity due to the observation that treatment with a CGRP receptor antagonist reduces the incidence of colonic hypersensitivity in rats[10].

TRPV1 is expressed in the sensory nerves of the intestinal tract. While participating in membrane protection, it also functions in the hyperalgia and abdominal pains that accompany inflammation. An increase in TRPV1-positive nerves has been reported in the esophageal mucosa of patients with non-erosive reflux disease (NERD)[11], and TRPV1 expression is increased in patients with rectal hypersensitivity[12]. The rs222747 TRPV1 gene polymorphism is associated with a reduced risk of functional dyspepsia (FD)[13].

TCF7L2 may regulate glucagon gene expression in the intestinal L cells that secrete glucagon-like peptide-1 (GLP-1)[14]. Many reports have suggested that the T allele of the TCF7L2 polymorphism (rs7903146) is a risk factor for type 2 diabetes[15-20], and TCF7L2 gene polymorphism has been reported to participate in gastric function[21].

Since the relationship between CGRP, TRPV1 and TCF7L2 in IBS remains unknown, in the present study, we evaluated the association between the CGRP (rs1553005), TRPV1 (rs222747) and TCF7L2 (rs7903146) polymorphisms in IBS.

PATIENTS AND METHODS

Study participants

A total of 108 IBS patients (53 males, 55 females; median age, 59.2 y.o.) diagnosed by Rome III criteria were enrolled. Sixty-one outpatients (31 males, 30 females; median age, 63.3 y.o.) without abdominal symptoms and with normal bowel habits served as controls (Table 1). Among the IBS patients, 53 patients had diarrhea-type disease (IBS-D; 33 males, 20 females; median age, 54.8 y.o.), 31 had constipation-type disease (IBS-C; 13 males, 18 females; median age, 63.7 y.o.), 8 had mixed-type disease (IBS-M; 2 males, 6 females; median age, 62.8 y.o.), and 16 had unsubtyped disease (IBS-U; 5 males, 11 females; median age 63.1 y.o.). For all of the analyses, IBS-M and IBS-U patients were combined into one group (NonDNonC), consisting of 24 patients (7 males, 17 females; median age, 63 y.o.) (Table 2). The disease duration of IBS was <3 years in 59 patients (32 males, 27 females; median age, 57.9 y.o.) and ⩾3 years in 49 patients (21 males, 28 females; median age, 60.7 y.o.).

Table 1 Demographic data of study subjects.

	Control group	IBS group	*P* value
	(*n*=61)	(*n*=108)	
Male, *n* (%)	31(50.8)	53(49.1)	NS
Female, *n* (%)	30(49.2)	55(50.9)	NS
Age, years (mean±SD)	63.3±11.5	59.2(16.4)	NS
BMI, kg/m^2 (mean±SD)	23.1±3.8	22.1±3.6	NS
Complication, n (%)			
Diabetes mellitus	0(0)	5(4.6)	NS
Psychiatric medicine	1(1.6)	8(7.4)	NS

Table 2 Demographic data of study subjects with IBS by subtype.

	IBS-D	IBS-C	NonDNonC
	(*n*=53)	(*n*=31)	(*n*=24)
Male, *n* (%)	33(62.3)	13(41.9)	7(29.2)
Female, *n* (%)	20(37.7)	18(58.1)	17(70.8)
Age, years (mean±SD)	54.8±17.2	63.7±11.8	63.0±17.6
BMI, kg/m^2 (mean±SD)	22.8±3.9	21.1±2.9	21.8±3.5
Complication, *n* (%)			
Diabetes mellitus	4(7.5)	1(3.2)	0(0)
Psychiatric medicine	3(5.7)	2(6.5)	3(12.5)

IBS-D: diarrhea type; IBS-C: constipation type; NonDNonC: mixed type+unsubtyped

We previously reported genetic association between β3-adrennoceptor (β3-AR) and cholinergic receptor muscarinic 3 (CHRM3) polymorphisms in IBS patients. According to this report, IBS patients were divided into two groups at the disease duration (<3 years or ⩾3 years) and the age (<65 y.o. or ⩾65 y.o.)[22].

The demographics of IBS subjects and controls are shown in table 1. No statistical differences in age, gender, or BMI were observed between the IBS and control groups.

The study was approved by the institutional review board at Iwate Medical University, and all subjects provided written consent to participate in the clinical trial.

Sample collection and genotyping

A sample of 10 mL EDTA-anticoagulated blood from each individual was collected for genomic DNA isolation. Polymorphisms in the CALCA, TRPV-1, and TCF7L2 genes were investigated using the allele-specific polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method. In brief, PCR was carried out in 25 μL containing 200 ng genomic DNA, 10 pmol each primer, 200 ng each dNTP, and 0.6U Taq DNA polymerase. The following primers were used: CALCA2 (rs1553005), forward (5'-TAGCTGGTATTACCCACAGAG-3') and reverse (5'-CCCATTTCAAAGATGAGTACCCTG-3'); TRPV-1 (rs222747), forward (5'-GGCCGACAACACGAAGTTTGT-3') and reverse (5'-AGTGAGTCAGGCAGTCCTCTC-3'); and TCF7L2 (rs7903146), forward (5'-TAGAGAGCTAAGCACTTTTTAGGTA-3') and reverse (5'-TGTAGCAGTGAAGTGCCCAA-3'). DNA was denatured at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 40 sec, and 72°C for 40 sec. The final extension step was 7 min. After digestion with Bsu36I for CALCA, MvaI for TRPV-1 and RsaI for TCF7L2, PCR products were visualized by electrophoresis on a 3.5% agarose gel and stained with ethidium bromide.

Statistical analysis

Demographic comparisons used chi-square tests for differences in allele and genotype frequencies for each polymorphism amongst cases and controls. Odds ratios for each genotype were determined by chi-square tests. A P value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 20 software for Windows.

RESULTS

Influence of genotypes on IBS risk

The genotype frequencies of CALCA, TRPV1 and TCF7L2 in IBS patients and controls are shown in table 3. The genotype frequencies were not significantly different between IBS patients and controls. The distribution of the genotypes was not significantly different between IBS-D, IBS-C, and NonDNonC in IBS patients. Furthermore, the genotype frequencies were not significantly different between males and females in IBS patients.

Genotype frequencies by disease duration

The genotype frequencies of CALCA, TRPV1 and TCF7L2 in IBS patients were not significantly different between patients with a disease duration <3 years versus ⩾3 years (Table 4).

Genotype frequencies by age

The genotype frequencies of CALCA and TCF7L2 in IBS patients were not significantly different between patients with <65 y.o. and ⩾65 y.o. TRPV1 genotype frequencies were significantly different between the two age groups (P<0.05): patients with ⩾65 years had

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Table 3 Influence of genotypes on IBS risk.

Variables, (%)		Genotype / *n* (%)			OR (95%CI)	*P*	OR (95%CI)	*P*
CALCA		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
Controls	*n*=61	3(4.9)	21(34.4)	37(60.7)	Reference		Reference	
IBS	*n*=108	4(3.7)	46(42.6)	58(53.7)	0.7(0.2-3.4)	0.70	0.8(0.4-1.4)	0.38
TRPV1		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
Controls	*n*=61	18(29.5)	25(41.0)	18(29.5)	Reference		Reference	
IBS	*n*=108	38(35.2)	42(38.9)	28(25.9)	1.3(0.7-2.6)	0.45	0.8(0.4-1.7)	0.62
TCF7L2		C/C	C/T	T/T	C/C *vs* Others		T/T *vs* Others	
Controls	*n*=61	57(93.4)	3(4.9)	1(1.6)	Reference		Reference	
IBS	*n*=108	93(86.1)	14(13.0)	1(0.9)	0.4(0.1-1.4)	0.15	0.6(0.3-9.1)	0.68

Table 4 Genotype frequencies by disease duration.

Disease duratation years, (%)		Genotype / *n* (%)			OR (95%CI)	*P*	OR (95%CI)	*P*
CALCA		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
<3	*n*=59	2(3.4)	27(45.8)	30(50.8)	Reference		Reference	
⩾3	*n*=49	2(4.1)	19(38.8)	28(57.1)	1.2(0.2-8.9)	0.85	1.3(0.6-2.8)	0.51
TRPV1		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
<3	*n*=59	20(33.9)	22(37.3)	17(28.8)	Reference		Reference	
⩾3	*n*=49	18(36.7)	20(40.8)	11(22.4)	1.1(0.5-2.5)	0.76	0.7(0.3-1.7)	0.45
TCF7L2		C/C	C/T	T/T	C/C *vs* Others		T/T *vs* Others	
<3	*n*=59	52(88.1)	7(11.9)	0(0)	Reference		Reference	
⩾3	*n*=49	41(83.7)	7(14.3)	1(2.0)	0.7(0.2-2.1)	0.50	0.9(0.9-1.0)	0.27

Table 5 Genotype frequencies by age.

Age years, (%)		Genotype / *n* (%)			OR (95%CI)	*P*	OR (95%CI)	*P*
CALCA		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
<65	*n*=60	2(3.3)	26(43.3)	32(53.3)	Reference		Reference	
⩾65	*n*=48	2(4.2)	20(41.7)	26(54.2)	1.3(0.2-9.3)	0.82	1.0(0.5-2.2)	0.93
TRPV1		C/C	C/G	G/G	C/C *vs* Others		G/G *vs* Others	
<65	*n*=60	30(50.0)	18(30.0)	12(20.0)	Reference		Reference	
⩾65	*n*=48	8(16.7)	24(50.0)	16(33.3)	0.2(0.1-0.5)	<0.01	2.0(0.8-4.7)	0.12
TCF7L2		C/C	C/T	T/T	C/C *vs* Others		T/T *vs* Others	
<65	*n*=60	53(88.3)	6(10.0)	1(1.7)	Reference		Reference	
⩾65	*n*=48	40(83.3)	8(16.7)	0 (0)0.9	(0.2-2.0)	0.46	1.0(0.9-1.1)	0.37

Table 6 Detailed examination about C/C genotype of TRPV1 by age.

TRPV1 C/C					
Age years, (%)					
		gender / *n* (%)		OR (95%CI)	*P*
		male	fmale	male *vs* female	
<65	*n*=30	20(66.7)	10(33.3)	Reference	
⩾65	*n*=8	3(37.5)	5(62.5)	0.3(0.6-1.5)	0.06
		Subgroup of IBS/ *n* (%)			*P*
		IBS-C	IBS-D	NonDNonC	
<65	*n*=30	6(20.0)	19(63.3)	5(16.7)	
⩾65	*n*=8	4(50.0)	4(50.0)	0(0.0)	0.16
		Disease duration years / *n* (%)		OR (95%CI)	*P*
		<3	⩾3	<3 vs ⩾3	
<65	*n*=30	18(60.0)	12(40.0)	Reference	
⩾65	*n*=8	2(25.0)	6(75.0)	4.5(0.8-26.1)	0.08

a lower incidence of the C/C genotype (odds ratio, 0.2; 95%CI, 0.1-0.5) (Table 5).

C/C genotype of TRPV1 by age

Furthermore, we investigated the C/C genotype polymorphism of TRPV1 association between gender, subgroup of IBS and disease duration in IBS patients which was divided into two groups by age. TRPV1 C/C genotype with ⩾65 y.o. in male IBS patients were trend to be fewer than in female patients, and TRPV1 C/C genotype with ⩾65 y.o. in disease duration <3 years of IBS patients also tended to be fewer than in disease duration ⩾3 years patients (Table 6).

DISCUSSION

Recently, several studies have shown the importance of gene polymorphisms in IBS. Serotonin [5-hydroxytryptamine (5-HT)] is a neurotransmitter that functions in the enteric nervous system, promoting gut motility, visceral sensation and secretion[23,24]. The serotonin transporter gene (SLC6A4) polymorphism and higher 5-HT levels have been shown to be significantly associated with IBS, particularly in patients with diarrhea and abdominal pain[25]. Carriers of the rare G allele of the serotonin transporter polymorphism rs25531 have an approximately three-fold increased risk of developing IBS compared to healthy controls[26]. Furthermore, polymorphisms in genes involved in serotonin reuptake and the transporter protein (SERT) promoter influence responses to a serotonin type 3 (5-HT3) antagonist in diarrhea-type IBS[27]. The rs4263839 G allele of the tumor necrosis factor superfamily member 15 (TNFSF15) gene has been reported to be associated with a risk of Crohn's disease[28]. Risk of IBS, particularly the constipation-predominant subtype, is also influenced by polymorphisms in the

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TNFSF15 gene, which is involved in the regulation of immune and inflammatory responses[29].

We previously reported genetic association between β3-AR and CHRM3 polymorphisms in IBS[22], then we also examined the association between brain-gut peptides such as CGRPα, TRPV1 and TCF7L2 in this study. As shown the results, both CALCA and TCF7L2 genotypes were not significantly different between IBS and controls, and types of IBS.

TRPV1 is a member of a sensory ion channel superfamily[30-33] that was initially cloned and molecularly characterized as the capsaicin or vanilloid receptor[34]. NERD and erosive esophagitis (EE) patients have increased TRPV1 receptor mRNA and protein levels, although no correlation with acid exposure has been demonstrated. Increased TRPV1 in the esophageal mucosa may contribute to symptoms both in NERD and EE patients and may possibly account for peripheral mechanisms responsible for esophageal hypersensitivity in NERD patients[35]. Tahara *et al*[13] reported a significant association between TRPV1 315cc (rs222747) genotype and FD [CC vs others; odds ratio(OR) = 0.40, 95% confidence interval (CI) = 0.38-0.82], the CC genotype of rs222747 TRPV1 gene polymorphism was associated with a lower incidence of FD.

In the present study, we found the significant association between TRPV1 polymorphism and age in IBS patients, and the C/C genotype of TRPV1 genotypes (rs222747) was significantly fewer in patients with ≥65 y.o. compared to in patients with <65 y.o. This result is the first report the association between TRPV1 polymorphism and age of IBS patients. Then, TRPV1 C/C genotype with ≥65 y.o. in male or in disease duration <3 years of IBS patients were trend to be fewer than in female or in disease duration ≥3 years patients. TRPV1 C/C genotype in IBS patients with ≥65 y.o. might be associated with a higher incidence in female and in disease duration ≥3 years, which could be associated with gender and gastrointestinal dysmotility of the long disease duration of IBS. It will be necessary to determine the association between TRPV1 genotypes and the therapeutic responses of TRPV1 agonists or antagonists in IBS patients.

PI-IBS develops in some of cases after an episode of acute infectious gastroenteritis, risk factors for PI-IBS would be female gender, adverse psychological factors and polymorphisms in TLR9, CDH1 and IL-6 genes. In the present study, we did not define to include PI-IBS, it will be also necessary to clarify the association between brain-gut peptide polymorphisms and PI- IBS.

Since IBS is a multifactorial functional disorder, and genotype/allele frequency differences were observed, a future study should investigate to confirm the validity and significance of these findings. Then, we should also determine the association between brain-gut peptide polymorphisms and PI-IBS.

In conclusion, TRPV1 polymorphisms in IBS patients would be associated with age. Particularly, TRPV1 C/C genotype with ≥65 y.o. might be associated with gender and disease duration. TRPV1 polymorphisms could be important factors in the pathophysiology of IBS, but an additional study is required to confirm these differences.

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