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ORIGINAL ARTICLE—ALIMENTARY TRACT

Genetic polymorphisms of *SCN10A* are associated with functional dyspepsia in Japanese subjects

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Abstract
Background Visceral sensory impulses are transmitted via C-fibers from the gastrointestinal tract to the central nervous system. The tetrodotoxinresistant (TTX-r) sodium channel, Na(V) 1.8/SNS (sensory-neuron specific), encoded by *SCN10A*, has been identified on C-fibers. We attempted to clarify the association between functional dyspepsia (FD) and *SCN10A* non-synonymous polymorphisms (2884 A>G, 3218 C>T and 3275 T>C).
Methods The study was performed in 642 subjects (345 with no symptoms and 297 with FD). We employed a multiplex polymerase chain reaction single-strand confirmation polymorphism (PCR-SSCP) method to detect the gene polymorphisms.
Results The 3218 CC homozygotes had a reduced risk for the development of FD [odds ratio (OR) 0.589; 95 % confidence interval (CI) 0.402–0.864; $p = 0.0067$]. In addition, both 2884 A>G and 3275 T>C, which were in linkage disequilibrium, were also associated with the development of FD ($p = 0.039$ and 0.028, respectively). Each 2884 G carrier, 3218 CC homozygote, and 3275 C carrier had a reduced risk for the development of both epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS). The subjects with the 2884 G allele, 3275 C allele, and no 3218 T allele had a reduced risk for FD (OR 0.618; 95 % CI 0.448–0.853; $p = 0.0034$). This haplotype was associated with a reduced risk for both EPS and PDS ($p = 0.0011$ and 0.0056, respectively). In addition, there was a significant association between FD and this haplotype in *Helicobacter pylori*-negative subjects (OR 0.463; 95 % CI 0279–0.9768; $p = 0.0029$).
Conclusion We conclude that genetic polymorphisms of *SCN10A* are closely associated with FD (both EPS and PDS), especially in *H. pylori*-negative subjects, in Japanese.

Keywords *SCN10A* · Na(V) 1.8/SNS · Genetic polymorphism · Functional dyspepsia · Visceral sensation

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Introduction

Functional dyspepsia (FD) is clearly the most common cause of dyspeptic symptoms in the West and the condition is becoming increasingly common in other parts of the world [1], affecting about 25 % of the population [2]. FD is characterized by the presence of recurrent or chronic upper abdominal symptoms, such as epigastric pain, early satiety, and fullness, without anatomical or biochemical abnormality identifiable by conventional diagnostic tests, including upper gastrointestinal endoscopy [3]. FD is a heterogeneous condition, indicated by the variety of different pathophysiologic mechanisms that have been demonstrated in this disorder [4], so FD does not have a well-established pathophysiology. Gastrointestinal motor abnormalities [5], altered visceral sensation [6], and psychosocial factors [7] are thought to be essential in the pathophysiology of FD. Recently, Locke et al. [8] reported familial clustering of FD. In addition, it has been reported that a G-protein beta3 subunit gene polymorphism was associated with FD [9]. These facts suggest that genetic

factors may play a significant role in the development of FD.

Gastrointestinal primary afferent nociceptive nerves can transduce mechanical, chemical, and thermal stimuli from nerve terminals and transmit impulses via spinal and vagal pathways to the central nervous system to induce nociception [10]. These processes are enhanced by peripheral and central sensitization under certain conditions, such as gastrointestinal motor abnormality and inadequate acid secretion, and may result in visceral discomfort. Unmyelinated C-fibers are considered to play important roles in the visceral afferent nerve system [11]. The tetrodotoxin-resistant (TTX-r) sodium channel Na(V) 1.8 (sensory neuron-specific/SNS) is preferentially expressed on C-fibers [12], and a major role for Na(V) 1.8/SNS in visceral nociceptive pathways has been revealed [13, 14]. Recently, it has been reported that a non-synonymous single-nucleotide polymorphism (SNP), rs6795970 (3218 C>T, V1073A) in *SCN10A* encoding Nav1.8/SNS, is closely associated with the PR interval, a marker of cardiac atrioventricular conduction [15]; this study has also revealed that the mutant allele of this SNP is a gain-of-function allele. From these observations, we hypothesize that genome variation in *SCN10A* may affect the development of dyspepsia symptoms. Several polymorphisms have been identified in *SCN10A*. Interestingly, a non-synonymous SNP cluster, including rs6795970, is present from exons 15 to 18 in *SCN10A*.

In the present study, we investigated the association between rs6795970 in *SCN10A* and FD diagnosed according to the Roma III criteria. In addition, we also investigated the associations of the rs77049337 (2431 G>A, G811K), rs57326399 (2884 A>G, I962V), and rs12632942 (3275 T>C, L1092P) non-synonymous SNPs in *SCN10A*.

Subjects, materials, and methods

Clinical samples

We studied patients attending the endoscopy center of Fujita Health University Hospital or Kanazawa Medical University Hospital from January 2007 to October 2010. After undergoing a barium X-ray examination in the health check, all the patients underwent upper gastrointestinal endoscopy to screen for gastric cancer, or because of complaints of abdominal discomfort. Patients with significant upper gastrointestinal findings such as peptic ulcer disease, reflux esophagitis, or malignancies were excluded from this study. Patients with severe systemic diseases or malignancies in other organs, and those who had received non-steroidal anti-inflammatory drugs, antibiotics, and *Helicobacter pylori* eradication treatment were also excluded. After the exclusion of these patients, the study population consisted of 642 subjects.

Two hundred and ninety-seven of the subjects (FD group) were identified as having a primary complaint of at least 3 months duration of either continuous or intermittent dyspepsia, predominantly located in the upper abdomen, irrespective of medical treatment, including treatment with proton pump inhibitory drugs or histamine H2-receptor antagonists. In these 297 dyspeptic patients, 149 and 98 patients were diagnosed with epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS), respectively. The 345 subjects who had had no dyspeptic symptoms within the past 12 months were considered as non-dyspepsia subjects (non-symptom group). The DNA of the 642 subjects in the final study population was clearly analyzed. None of the subjects had diarrhea or constipation and none were morbidly obese or excessively lean [mean body mass index (BMI) values in the non-symptom group ($n = 73$) and FD group ($n = 43$), whose data of height and weight were obtained, 24.53 ± 3.20 and 24.75 ± 3.56, respectively]. The subjects were divided into two groups according to genotype as follows:

L-SNS group: subjects with the 2884 G and 3275 C alleles and no 3218 T allele
H-SNS group: subjects not included in the L-SNS group

The Ethics Committees of Fujita Health University and Kanazawa Medical University approved the study protocol, and written informed consent was obtained from all of the participating subjects.

Detection of *H. pylori* infection

Helicobacter pylori infection status was assessed by serology, histological examination, or the urea breath test. Patients were diagnosed as having the infection when at least one of the diagnostic tests was positive.

Histological evaluation

In 190 of the 642 subjects, biopsy specimens were taken from the antral mucosa (85 in the non-symptom group and 105 in the FD group). Each specimen was fixed in 10 % buffered formalin and embedded in paraffin. The severity of chronic gastritis was classified according to the updated Sydney system [16] by a pathologist who had no access to any clinical information.

Genotyping of polymorphisms

Genomic DNA was isolated from peripheral blood using a FlexiGene DNA Kit (QIAGEN, Hilden, Germany).

Polymorphism was genotyped by a one-tube multiplex polymerase chain reaction single strand confirmation polymorphism (PCR-SSCP) method as reported previously [17, 18]. To detect 2431 G>A and 3275 T>C, using the primer pairs (2431 forward: 5′-tcaccatcatcctggccatcattg-3′ and 2431 reverse: 5′-tcttcatggggcgcggagatatttt-3′, and 3275 forward: 5′-acacaagctcctctgagggcagca-3′ and 3275 reverse: 5′-tctggccctaattaacatcagtgtgagg-3′, respectively), one-tube multiplex PCR was carried out in a volume of 20 μL containing 0.1 μg of genomic DNA. The DNA was denatured at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 53 °C for 40 s, and 72 °C for 30 s, with final extension at 72 °C for 5 min. Then 2 μL of the PCR product was denatured with 10 μL of formamide (Sigma-Aldrich, St. Louis, USA) at 90 °C for 5 min. SSCP was carried out at 6 °C, using a GenePhor DNA separation system with GeneGel Excel 12.5/24 (Amersham Biosciences, Piscatway, NJ, USA), after which the denatured single-strand DNA bands were detected using a DNA Silver Staining Kit (Amersham Biosciences).

To detect 2884 A>G and 3218 C>T, using the primer pairs (2884 forward: 5′-cctgagctggtggtgaaactccca-3′ and 2884 reverse: 5′-acccacacagtcggattagcgatga-3′, and 3218 forward: 5′-ctgggtgagacgtggaaagatgagtc-3′ and 3218 reverse: 5′-actgctcagtgtctggctggctg-3′, respectively), one-tube multiplex PCR was carried out in a volume of 20 μL containing 0.1 μg of genomic DNA. The DNA was denatured at 95 °C for 3 min, followed by 35 cycles at 96 °C for 15 s, 50 °C for 40 s, and 72 °C for 30 s, with final extension at 72 °C for 5 min. Then SSCP (at different temperature from that used to detect detect 2431 G>A and 3275 T>C [18 °C]) and silver staining were carried out in the same manner as that described above.

Statistical analysis

The data were expressed as means ± SD. Differences in mean ages between 2 groups were compared by Student's *t*-test. Allele and genotype frequencies were calculated by direct counting. The allele counts were compared by a 2×2 table using Fisher's exact test. The strength of association between genotype frequency and the disease was assessed by calculating the adjusted odds ratio (OR) and 95 % confidence intervals (CIs) by logistic regression analysis after adjustment for age, gender, and *H. pylori* infection status. The differences between 2 groups in each of the updated Sydney system scores were compared by the Mann–Whitney *U*-test. Concerning the power of the study, the β value was calculated when the α value was set at 0.05. For all analyses, the level of significance was set at $p < 0.05$.

Results

Characteristics of the subjects and the frequencies of genotypes

As shown in Fig. 1, single-strand DNAs of each polymorphism were clearly identified by SSCP. The characteristics of the subjects and the frequencies of the genotypes are shown in Table 1. The variation of 2431 G>A was not detected in Japanese. The other genotypes were in Hardy–Weinberg equilibrium (2884 A>G, $p = 0.48$; 3218 C>T, $p = 0.43$; 3275 T>C, $p = 0.18$). In addition, there was a strong allelic association between 2884 A>G and 3275 T>C. The mean age was significantly lower in the patients with EPS than in the non-symptom groups. The male/female ratio was significantly lower in FD and its subgroups than in the non-symptom group (FD, $p = 0.0001$; EPS, $p = 0.0031$; PDS, $p = 0.0021$ vs. non-symptom group). The *H. pylori*-positive ratio was also significantly lower in the FD and EPS groups than in the non-symptom group (FD, $p = 0.021$; EPS, $p = 0.0005$ vs. non-symptom group). The ratios of 2884 AA and 3275 TT homozygotes were significantly higher and that of 3218 CC homozygotes was significantly lower in the FD group than the non-symptom group ($p = 0.028$, $1 - \beta$power $= 0.60$; $p = 0.013$, $1 - \beta$power $= 0.69$; $p = 0.010$, $1 - \beta$power $= 0.74$, respectively). The frequency of the 3275 C allele was significantly lower and that of the 3218 T allele was significantly higher in the FD group than the non-symptom group ($p = 0.014$, $1 - \beta$power $= 0.69$;

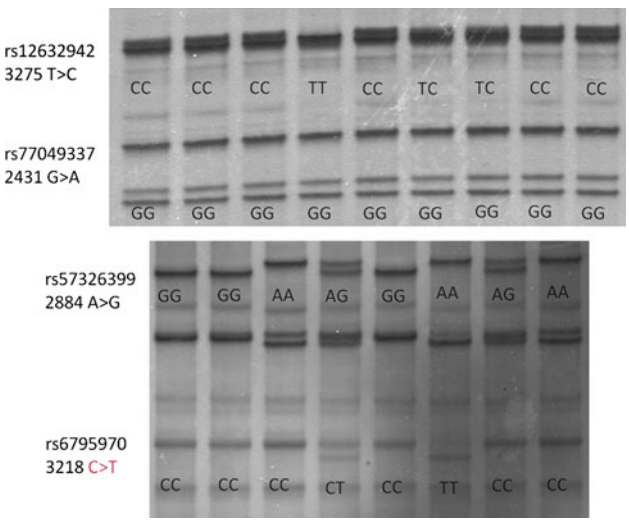

Fig. 1 Images of multiplex polymerase chain reaction single-strand confirmation polymorphism (PCR-SSCP) using clinical samples. Single-strand DNAs were clearly separated by SSCP. The variation of 2431 G>A was not detected

Table 1 Characteristics of the subjects and frequency of genotypes

	Non-symptom	FD	EPS	PDS
Number of subjects	345	297	149	98
Mean age ± SD (years)	60.0 ± 13.2	58.9 ± 14.4	55.3 ± 14.8[a]	60.7 ± 14.0
Male:female	206:139	132:165[b]	67:82[c]	45:53[d]
Helicobacter pylori-infected	208/345	152/297[e]	64/149[f]	49/98
rs57326399 (Ile962Val, 2884 A>G)				
A/A	84	96[g]	53[h]	35[i]
A/G	176	135	65	43
G/G	85	66	31	20
G allele frequency (%)	50.1	44.9	42.6[j]	42.3
rs6795970 (Val1073Ala, 3218 C>T)				
C/C	282	217[k]	102[l]	69[m]
C/T	59	74	43	27
T/T	4	6	4	2
T allele frequency (%)	9.71	14.5[n]	17.1[o]	15.8[p]
rs12632942 (Leu1092Pro, 3275T>C)				
T/T	80	95[q]	48[r]	35[s]
T/C	169	135	69	41
C/C	96	67	32	22
C allele frequency (%)	52.3	45.3[t]	44.6[u]	43.4[v]

FD functional dyspepsia, *PDS* postprandial distress syndrome, *EPS* epigastric pain syndrome

[a] $p = 0.0005$, [b]$p = 0.0001$, [c]$p = 0.0031$, [d]$p = 0.0021$ versus non-symptom group, [e]$p = 0.021$, [f]$p = 0.0005$ versus non-symptom group

The ratio of AA homozygotes: [g]$p = 0.028$, [h]$p = 0.012$; [i]$p = 0.028$ versus non-symptom group

The frequency of the 2844G allele: [j]$p = 0.032$ versus non-symptom group

The ratio of CC homozygotes: [k]$p = 0.010$, [l]$p = 0.0015$; [m]$p = 0.023$ versus non-symptom group

The frequency of the 3218T allele: [n]$p = 0.0095$, [o]$p = 0.0013$, [p]$p = 0.020$ versus non-symptom group

The ratio of TT homozygotes: [q]$p = 0.013$, [r]$p = 0.044$; [s]$p = 0.018$ versus non-symptom group

The frequency of the 3275C allele: [t]$p = 0.014$; [u]$p = 0.032$, [v]$p = 0.029$ versus non-symptom group

$p = 0.0095$, $1 - \beta$power $= 0.74$, respectively). In analyses by subtypes of FD, the ratios of 2884 AA and 3275 TT homozygotes were significantly higher and that of the 3218 CC homozygote was significantly lower in both the EPS and PDS groups than the non-symptom group; in particular, the ratio of the 3218 CC homozygotes was very low in the EPS group ($p = 0.0015$, $1 - \beta$power $= 0.88$).

Association between functional dyspepsia and *SCN10A* polymorphisms

By logistic regression analysis after adjustment for age, gender, and *H. pylori* infection status, *SCN10A* 2884 G carriers, 3218 CC homozygotes, and 3275 C carriers had a significantly reduced risk for FD; in particular, the 3218 CC homozygotes had a strongly reduced risk (OR 0.589; 95 % CI 0.402–0.864; $p = 0.0067$, Table 2). When assessing by subgroups of FD, 2884 G allele carriers, 3218 CC homozygotes, and 3275 C carriers had a significantly reduced risk for both EPS and PDS; in particular, the 3218 CC homozygotes had a strongly reduced risk for EPS (OR 0.495; 95 % CI 0.313–0.783; $p = 0.0027$, $1 - \beta$power $= 0.85$, Table 2).

Association between functional dyspepsia and L- or H-SNS groups

Because 2884 A>G and 3275 T>C polymorphisms were in linkage disequilibrium and the 2884 G–3218 C–3275 C allele was considered to be a loss-of-function allele, the subjects with the 2884 G allele, 3275 C allele, and no 3218 T allele were classified in the L-SNS group and the other subjects were classified in the H-SNS group. In the L-SNS group, the number of subjects was 371,the mean age was 58.7 ± 13.8 years, the male/female ratio was 203/168, and the *H. pylori*-positive ratio was 208/371, whereas, in the H-SNS group, the number of subjects was 271, mean age was 60.6 ± 13.6 years, the male/female ratio was 135/136, and the *H. pylori*-positive ratio was 152/271 (all data, not significant).

The subjects in the L-SNS group had a significantly reduced risk for FD (OR 0.618; 95 % CI 0.448–0.853;

Table 2 Risk of *SCN10A* polymorphisms for the development of FD

FD					
2884 A>G	AA	AG	GG	G car. versus AA homo.; OR (95 % CI)	*p* value
Non-symptom (345)	84	176	85	Reference	–
FD (297)	96	135	66	0.689 (0.484−0.908)	0.039
3218 C>T	CC	CT	TT	CC homo. versus T car.; OR (95 % CI)	
Non-symptom (345)	282	59	4	Reference	–
FD (297)	217	74	6	0.589 (0.402−0.864)	0.0067
3275 T>C	TT	TC	CC	C car. versus TT homo.; OR (95 % CI)	
Non-symptom (345)	80	169	96	Reference	–
FD (297)	95	135	67	0.670 (0.469–0.959)	0.028
EPS					
2884 A>G	AA	AG	GG	G car. versus AA homo.; OR (95 % CI)	*p* value
Non-symptom (345)	84	176	85	Reference	–
EPS (149)	53	65	31	0.574 (0.372−0.887)	0.012
3218 C>T	CC	CT	TT	CC homo. versus T car.; OR (95 % CI)	
Non-symptom (345)	282	59	4	Reference	–
EPS (149)	102	43	4	0.495 (0.313−0.783)	0.0027
3275 T>C	TT	TC	CC	C car. versus TT homo.; OR (95 % CI)	
Non-symptom (345)	80	169	96	Reference	–
EPS(149)	48	69	32	0.641 (0.411−1.00)	0.0498
PDS					
2884 A>G	AA	AG	GG	G car. versus AA homo.; OR (95%C.I.)	*p* value
Non-symptom (345)	84	176	85	Reference	–
PDS (98)	35	43	20	0.608 (0.372−0.994)	0.047
3218 C>T	CC	CT	TT	CC homo. versus T car.; OR (95 % CI)	
Non-symptom (345)	282	59	4	Reference	–
PDS (98)	69	27	2	0.516 (0.306−0.869)	0.013
3275 T>C	TT	TC	CC	C car. versus TT homo.; OR (95 % CI)	
Non-symptom (345)	80	169	96	Reference	–
PDS (98)	35	41	22	0.575 (0.350−0.943)	0.028

By logistic regression analysis after adjustment for age, gender, and *H. pylori* infection status

Car. Carrier, *homo* homozygote, *CI* confidence interval, *OR* odds ratio

$p = 0.0034$; $1 - \beta$power $= 0.84$, Table 3), and for both EPS and PDS (OR 0.509; 95 % CI 0.339–0.764; $p = 0.0011$; $1 - \beta$power $= 0.89$ and OR 0.519; 95 % CI 0.326–0.825; $p = 0.0056$; $1 - \beta$power $= 0.82$, respectively). In addition, in the L-SNS group, *H. pylori*-negative subjects had a significantly reduced risk for the development of FD (OR 0.463; 95 % CI 0.279–0.768; $p = 0.0029$; $1 - \beta$power $= 0.80$, Table 4), whereas no significant risk was seen in the *H. pylori*-positive subjects.

Histological evaluation of chronic gastritis

There were no significant differences in any of the Sydney system scores between the non-symptom and FD groups (Table 5). In both *H. pylori*-negative and -positive subjects, the degree of histological gastritis in the FD group was not different from that in the non-symptom group.

Discussion

In the present study, we investigated the association of *SCN10A* polymorphisms with FD and its subgroups. We found that *SCN10A* 3218 CC homozygosity with the 2884 G and 3275 C alleles was significantly associated with a reduced risk for the development of FD, both EPS and PDS, especially in *H. pylori*-negative subjects.

Functional gastrointestinal disorders are defined by chronic or recurrent abdominal symptom patterns in the absence of identifiable organic causes. Although various pathogenic mechanisms underlie the symptoms of functional disorders [19], it is, ultimately, sensory neurons that notify the brain of pathological events in the gastrointestinal tract. If the afferent sensory nervous system responds inadequately to physiological phenomena in the gastrointestinal tract, it may create anomalous sensations itself.

Table 3 Risk of L-SNS for the development of functional dyspepsia

	L-SNS	H-SNS	L- versus H-SNS, OR (95 % CI)	*p* value
Non-symptom (345)	218	127	Reference	–
FD (297)	153	144	0.618 (0.448–0.853)	0.0034
EPS (149)	71	78	0.509 (0.339–0.764)	0.0011
PDS (98)	46	52	0.519 (0.326–0.825)	0.0056

By logistic regression analysis after adjustment for age, gender, and *H. pylori* infection status

H-SNS group: subjects not included in the L-SNS group *L-SNS* group: subjects with the 2884 G and 3275 C alleles and no 3218 T allele

Table 4 Risk of L-SNS for the development of dyspepsia in *H. pylori*-positive and -negative subjects

	L-SNS	H-SNS	L- versus H-SNS; OR (95 % CI)	*p* value
H. pylori-negative				
Non-symptom (137)	91	46	Reference	–
FD (145)	72	73	0.463 (0.279–0.768)	0.0029
H. pylori-positive				
Non-symptom (208)	127	81	Reference	–
FD (152)	81	71	0.724 (0.473–1.10)	0.14

By logistic regression analysis after adjustment for age and gender

Table 5 Comparisons of each updated Sydney system score

	Non-symptom (85)	FD (105)	
Overall			
Activity score	0.638 ± 0.833	0.624 ± 0.913	NS
Inflammation score	1.412 ± 0.930	1.371 ± 0.891	NS
Atrophy score	0.929 ± 0.936	0.895 ± 0.887	NS
Metaplasia score	0.518 ± 0.946	0.438 ± 0.808	NS
	Non-symptom (36)	FD (51)	
H. pylori-negative			
Activity score	0.056 ± 0.232	0.196 ± 0.530	NS
Inflammation score	0.528 ± 0.609	0.754 ± 0.659	NS
Atrophy score	0.139 ± 0.351	0.275 ± 0.603	NS
Metaplasia score	0	0.176 ± 0.555	NS
	Non-symptom (49)	FD (54)	
H. pylori-positive			
Activity score	1.041 ± 0.999	1.056 ± 0.856	NS
Inflammation score	2.061 ± 0.475	1.963 ± 0.634	NS
Atrophy score	1.510 ± 0.794	1.481 ± 0.693	NS
Metaplasia score	0.898 ± 1.104	0.685 ± 0.928	NS

Values in parentheses indicate the numbers of subjects

NS not significant

Thus, visceral hypersensitivity is, in part, responsible for functional gastrointestinal disorders [20]. It is still unclear what induces visceral hypersensitivity in patients with functional gastrointestinal disorders. We previously reported that the G315C polymorphism of the *TRPV1* gene was associated with the development of FD, both EPS and PDS [21]. This led us to investigate the association between *SCN10A* polymorphisms and the development of FD, in the present study.

Sensitization of primary afferent nociceptors plays a key role in many forms of visceral pain. TTX-r currents are present in the majority of visceral afferents [22]. The Na(V) 1.8/SNS, which is expressed preferentially in small dorsal root ganglia (DRG) and trigeminal neurons, produces a sodium current which is relatively resistant to TTX [12, 23]. Many previous studies have revealed that Na(V) 1.8 plays important roles in primary hyperalgesia [24–26]. In addition, Laird et al. [27] showed an essential

role for Na(V) 1.8 in mediating spontaneous activity in sensitized nociceptors. In their study, they reported that Na(V) 1.8-null mice showed markedly reduced pain responses and no referred hyperalgesia in response to intracolonic capsicin. Furthermore, A-803467, a potent and selective Na(V) 1.8 sodium channel blocker, produced a dose-related analgesic effect in rat distal colon/rectum distended by a balloon [28]. These data suggested that Na(V) 1.8 function may affect visceral sensation.

Our epidemiological study cannot reveal how the polymorphisms of *SCN10A* are associated with FD. Recently, a genome-wide association study, carried out by Chambers et al. [15] in an additive genetic model, revealed a strong association of rs6795970 (3218 C>T) in *SCN10A*, encoding Na(V) 1.8, with prolonged PR intervals, a marker of cardiac atrioventricular conduction. That report also revealed that the PR interval was shorter in *Scn10A*−/− mice than in their wild-type littermates. Therefore, Chambers et al. concluded that rs6795970 in *SCN10A* was a gain-of-function variant. In our present study, rs6795970 was most closely associated with FD in 3 *SCN10A* polymorphisms, which were associated with FD. That is, 3218 CC homozygotes, the subjects without the gain-of-function allele, had a reduced risk for FD. In addition, 3218 C>T was more closely associated with EPS than with PDS, although this polymorphism was associated with both subtypes of FD. These findings suggested that the up-regulated function of Na(V) 1.8 might be associated with FD and that this up-regulated function was more closely associated with visceral pain than with postprandial distress symptoms. Further study using other methods and techniques will be necessary to prove this hypothesis, because we have no evidence for visceral hyperalgesia in the subjects with each 3218 C>T genotype. Our results indicated that the 2884 A>G and 3275 T>C polymorphisms were in linkage disequilibrium, and we considered the 2884 G–3218 T–3275 C allele to be a loss-of-function allele. Therefore, the L-SNS group(the subjects with the 2884 G allele, 3275 C allele, and no 3218 T allele) was considered to be a loss-of-Na(V) 1.8-function group. L-SNS was more closely associated than 3218 CC homozygosity with a reduced risk for the development of FD, both EPS and PDS.

There have been studies investigating the role of the Na(V) 1.8 current under the influence of inflammation. Hillsley et al. [29] have reported that DRG neuron hyperexcitability induced by *Nippostrongylus brasiliensis* infection was not observed in Na(V) 1.8-null mice, but it was still present in their wild-type littermates. That study indicates that visceral inflammation evokes hyperexcitability in nociceptive DRG neurons and that these changes are associated with increased Na(V) 1.8 current density. It is well known that infection with *H. pylori* first induces chronic superficial gastritis, which can progress to chronic atrophic gastritis and intestinal metaplasia [30]. Previously, we demonstrated that up-regulated function of TRPV1 prevented the development of dyspepsia symptoms under the influence of *H. pylori* infection [21]. That is, TRPV1 stimulation seemed to maintain gastric function under conditions of inflammation via the vagal afferent nerve system [31]. In the present study, L-SNS had a reduced risk for the development of dyspepsia in *H. pylori*-negative subjects, but not in *H. pylori*-positive subjects. When we investigated the degree of gastritis in the non-symptom group and the FD group, we found no significant differences in the degree of histological gastritis between the two groups. This result was supported by our previous observation [32]. The severity of gastritis was not significantly different in the *H. pylori*-positive and *H. pylori*-negative subjects in the H-SNS and L-SNS groups , although each of the Sydney system scores was significantly higher in the *H. pylori*-positive than the *H. pylori* negative subjects. King et al. [33] have shown that experimental mouse colitis causes an increase in Na(V) 1.8 protein in colonic DRG neurons, suggesting that increased availability of these channels contributes to the increased Na(V) 1.8 currents and associated visceral hyperalgesia. If severe inflammation induced by *H. pylori* infection produces increased Na(V) 1.8 protein, the increased protein may conceal the up-regulated function of Na(V) 1.8. Therefore, in *H. pylori*-positive subjects, L-SNS might have no risk for the development of dyspepsia.

In conclusion,we found that subjects with the 2884 G allele, 3275 T allele, and no 3218 T allele of *SCN10A* had a reduced risk for FD, both the EPS and PDS subtypes, especially in *H. pylori*-negative subjects. We expect that further studies will reveal the associations between *SCN10A* polymorphisms and other functional gastrointestinal disorders, such as non-erosive reflux disorder and irritable bowel syndrome.

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Conflict of interest None.

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