

The Role of RANTES Promoter Polymorphism in Functional Dyspepsia

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Summary Altered inflammatory immune responses have been shown to be associated with functional gastro intestinal disorder. We aimed to clarify the effect of functional promoter polymorphism of RANTES, which is a potent chemoattractant peptide for memory T lymphocytes and eosinophils, on the risk of functional dyspepsia in a Japanese population. RANTES promoter C-28G polymorphism was genotyped in 246 subjects including 134 FD patients according to Roma III criteria and 112 non-symptomatic healthy controls. Although frequency of RANTES promoter polymorphisms in overall dyspeptic patients and non-symptomatic healthy controls did not show any significant differences, a significant association was found between G carrier and reduced risk of PDS according to Roma III criteria (age, sex, *H. pylori* infection adjusted OR = 0.23, 95% CI = 0.06–0.80). We also found that the same genotype held a lower risk of PDS in *H. pylori* positive PDS subjects (age, sex adjusted OR = 0.11, 95% CI = 0.01–0.94). Our data suggest that RANTES promoter -28G carriers is associate with a reduced risk of PDS especially in *H. pylori* positive subjects.

Key Words: dyspepsia, polymorphism, Rome III, RANTES

Introduction

Functional dyspepsia (FD) is clearly the commonest cause of dyspeptic symptoms in the West and increasingly in other parts of the world [1], affecting about 25% of the population [2]. The latest definition of this includes the presence of “chronic or recurrent symptoms centred in the upper abdomen in the absence of any organic, systemic or metabolic disease that is likely to explain the symptoms” [3]. FD is a heterogeneous condition indicated by the variety of different pathophysiologic mechanisms that have been demonstrated in this disorder [4], gastrointestinal motor abnormalities [5],

altered visceral sensation [6] and psychosocial factors [7] have thought to be essential in the pathophysiology of FD. There have been data indicating that abnormal inflammatory immune response against various stimuli may be play a major role in developing functional gastro intestinal disorder. It has been reported that acute inflammation impairs nitergic innervation [8], and cause gastrointestinal motor dysfunction [9]. Furthermore, positive association of irritable bowel syndrome (IBS), FD between post infective condition has been also reported [10, 11].

Helicobacter pylori (*H. pylori*) infection is a powerful pathogenic factor and many studies have revealed a strong association between this organism infection and gastric disorders. *H. pylori* infection usually leads to chronic gastric inflammation. According to the Roma III criteria [12], *H. pylori* infected patients, who had some chronic or recurrent upper abdominal symptoms, with neither ulceration nor

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erosion in gastroduodenal mucosa were also diagnosed as FD. That is, there is a possibility that one of the FD subgroups may relate to the gastric mucosal inflammation induced by *H. pylori* [13–15].

RANTES (short for “regulated upon activation, normal T cell expressed and secreted”) is a member of the large and growing family of immunoregulatory cytokines called chemokines. RANTES belongs to the C-C chemokine subfamily. It is a potent chemotactic agent for T lymphocytes and monocytes [16] and is expressed after cellular activation in fibroblasts, T cells, monocytes, endothelial cells, and certain epithelial cells. RANTES has therefore been shown to contribute to the infiltration of lymphocytes in the gastric mucosa. Like that of IL-8, RANTES expression is increased in *H. pylori* infected gastric mucosa [17–19]. Persistent expression and secretion of RANTES are also related to residual infiltration of memory T lymphocytes for a prolonged period after *H. pylori* eradication [20].

Genetic studies on the RANTES gene have identified a number of polymorphisms, including one that causes a nucleotide substitution in promoter region, C-28G. The -28G allele of the RANTES promoter was associated with higher protein level than those of C allele [21].

Recent studies showed that RANTES promoter genotype was associated with diabetic nephropathy in type 2 diabetic subjects [22], late onset asthma [21] atopic dermatitis [23], and progression of AIDS [24, 25].

Because the RANTES plays a major role in inflammatory immune response in various condition, we hypothesized that RANTES promoter polymorphism may influence the susceptibility to FD. Here, we investigated the prevalence of RANTES promoter C-28G polymorphism in patients with FD according to Roma III in a Japanese population.

Materials and Methods

Study populations

We studied 246 subjects attending the Endoscopy Center of Fujita Health University Hospital from January 2005 to October 2007. The subjects underwent upper gastroscopy for their health check, secondary complete check up of stomach cancer following to barium X ray examination, or for the complaint of abdominal discomfort. Subjects who have significant upper gastrointestinal findings such as active peptic ulcer disease, reflux esophagitis and malignancies were excluded from this study. Patients with severe systemic diseases, with malignancies in other organs, and had received non-steroidal anti-inflammatory drugs, antibiotics, and *H. pylori* eradication treatment were also excluded by repeated face to face history and physical examination including blood test, abdominal US and ECG. According to the Roma III criteria, 134 FD patients were identified as having a primary complaint of either continuous or inter-

mittent dyspepsia for 3 months, onset at least 6 months before, predominantly located in upper abdomen irrespective of using H₂-receptor antagonists (H₂RAS) or proton-pump inhibitors (PPIs). Dyspeptic patients were also classified as epigastric pain syndrome (EPS) and postprandial syndrome (PDS) and others according to Roma III criteria. Subjects who were negative by upper gastroscopy and negative for dyspeptic symptom with in last 12 months were considered as non-dyspeptic healthy controls. Those who had received proton-pump inhibitory drugs or H₂RAS during the 4 week were excluded from healthy controls. The Ethics Committee of Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all of the subjects.

Detection of *H. pylori* infection

The *H. pylori* infection status was determined on the basis of histology, culture, the urease breath test (UBT), and antibodies to *H. pylori*. Infection was diagnosed when at least one of these 4 tests was positive.

Genotyping for RANTES promoter

Genomic DNA was extracted from non-neoplastic gastric biopsies or peripheral blood using the standard phenol/chloroform method. Then, the polymorphisms of -28 C/G in the RANTES gene promoter region was investigated by PCR-based RFLP assays as previously described [21].

Statistical analysis

Hardy-Weinberg equilibrium of the RANTES promoter allele in the non-dyspeptic healthy controls and FD patients were assessed by χ^2 statistics. Differences of RANTES promoter genotype frequencies between two groups were determined by the two-sided Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were also calculated. A probability value of less than 0.05 was considered statistically significant in all analyses.

Results

Study population

The characteristics of the subjects are summarized in Table 1. Age distribution and *H. pylori* infection positive ratio were not significantly different among those two groups. Meanwhile, female sex ratio was significantly higher in the dyspeptic patients than those of non-symptomatic healthy controls. Of all 134 FD patients 70 subjects and 41 subjects were diagnosed as EPS and PDS, respectively. The 70 EPS and 41 PDS patients contained 9 subjects, who were diagnosed as both PDS and EPS. Other 32 subjects were diagnosed as other dyspepsia.

Table 1. Characteristics of subjects

| | FD (<i>n</i> = 134) | Control (<i>n</i> = 112) |
|-----------------------------------------------|----------------------|---------------------------|
| Age (mean SD) | 60.1 ± 12.8 | 59.0 ± 13.9 |
| Sex (male/female) [#] | 59/75 | 69/43 |
| <i>H. pylori</i> infection positive ratio (%) | 53.7 | 58.9 |

[#]; FD vs Control, *p* = 0.007 (two-sided Fisher's exact test)

Table 2. RANTES promoter polymorphism and risk of FD

| Variables; <i>n</i> (%) | genotype/ <i>n</i> (%) | | | OR (95%CI) <i>p</i> |
|-------------------------|------------------------|-----------|---------|-----------------------|
| | CC | CG | GG | Gcarrier vs CC |
| Control (112) | 84 (75.0) | 24 (21.4) | 4 (3.6) | Reference |
| Over all FD (134) | 103 (76.9) | 29 (21.6) | 2 (1.5) | 0.61 (0.32–1.15) 0.77 |
| EPS (70) | 48 (68.6) | 20 (28.6) | 2 (2.8) | 1.38 (0.71–2.66) 0.39 |
| PDS (41) | 38 (92.7) | 3 (7.3) | 0 (0) | 0.24 (0.07–0.83) 0.02 |
| Others (32) | 24 (75) | 8 (25) | 0 (0) | 1.00 (0.40–2.48) 1.00 |

Note; G carriers, GG + CG. Statistical analysis was performed by two-sided Fisher's exact test. The 70 EPS and 41 PDS patients contained 9 subjects, who were diagnosed as both PDS and EPS.

Prevalence of RANTES promoter polymorphism in FD and control subjects

RANTES genotypes in the non-symptomatic healthy controls and FD patients did not deviate significantly from those expected under the Hardy–Weinberg equilibrium (*p* = 0.19, 0.98, respectively). In the non-symptomatic healthy controls, RANTES promoter genotype distribution was 84CC (75.0%), 24CG (21.4%), and 4GG (3.6%). Meanwhile, the RANTES promoter distribution in FD patients was 103CC (76.9%), 29CG (21.6%), and 2GG (1.5%). Because frequency of GG genotype was low, we considered CG and GG as G carrier and assessed the association between genotype frequency and risk of FD. Compared to healthy controls, frequencies of RANTES promoter polymorphisms in overall dyspeptic patients did not show any significant differences. Meanwhile, a significant association was found between G carrier and reduced risk of PDS according to Roma III criteria by Fisher's exact test (OR = 0.27, 95%CI = 0.07–0.83, *p* = 0.02; Table 2).

To further evaluate whether the RANTES promoter genotypes might influence the risk of FD, we also investigated the differences in RANTES promoter genotype frequencies between different *H. pylori* infection status and gender (Table 3). We found that the G carrier held a lower risk of PDS in *H. pylori* positive subjects (OR = 0.12, 95%CI = 0.02–0.99, *p* = 0.03).

Logistic regression analysis

Logistic regression analysis with adjustment for age and *H. pylori* infection status or gender was done for all PDS as well as *H. pylori* positive PDS subjects. It was revealed

that the significant association of the RANTES promoter -28G carriers with all PDS and *H. pylori* positive PDS subjects were remained after logistic regression analysis (all PDS; age, sex, *H. pylori* infection adjusted OR = 0.23, 95%CI = 0.06–0.80, *p* = 0.02, *H. pylori* positive PDS; age, sex adjusted OR = 0.11, 95% CI = 0.01–0.94, *p* = 0.04).

Discussion

Recent evidence supports the relevance of a genetic milieu in FD. A case-control study by Holtmann *et al.* suggested that there is a significant link between GNβ3 (C825T) CC genotype and functional dyspepsia [26]. The association has been independently confirmed [27]. However, there are few reports demonstrated the relationship between the polymorphisms of molecules associated with inflammation and FD.

In the present study, we investigated the prevalence of RANTES promoter polymorphism in dyspeptic patients in a Japanese population. The -28G carrier was associated with a reduced risk of PDS according to Rome III criteria.

Concerning the association between inflammatory immune response and functional gastrointestinal disorder, it has been reported that acute inflammation impairs nitergic innervation [8], and cause gastrointestinal motor dysfunction [9]. Positive association of irritable bowel syndrome (IBS), FD between post infective condition has been also documented [10, 11], suggesting that abnormal inflammatory immune response may be one of the major pathogenesis of functional gastro intestinal disorder.

RANTES is a powerful chemotactic agent for T lympho-

Table 3. RANTES promoter genotypes and risk of FD in different gender and *H. pylori* infection status

| Variables; n | genotype/n | | | OR (95% CI) <i>p</i> |
|------------------|------------|----|----|-----------------------|
| | CC | CG | GG | Gcarrier vs CC |
| <i>Hp</i> (-) | | | | |
| Control (46) | 37 | 9 | 0 | Reference |
| Over all FD (62) | 52 | 9 | 1 | 0.79 (0.29–2.14) 0.80 |
| EPS (38) | 29 | 8 | 1 | 1.28 (0.45–3.62) 0.79 |
| PDS (20) | 18 | 2 | 0 | 0.46 (0.09–2.34) 0.48 |
| Others (11) | 11 | 0 | 0 | ND |
| <i>HP</i> (+) | | | | |
| Control (66) | 47 | 15 | 4 | Reference |
| Over all FD (72) | 51 | 20 | 1 | 1.02 (0.49–2.13) 1.00 |
| EPS (32) | 19 | 12 | 1 | 1.13 (0.42–3.06) 0.80 |
| PDS (21) | 20 | 1 | 0 | 0.12 (0.02–0.99) 0.03 |
| Others (21) | 13 | 8 | 0 | 1.52 (0.54–4.26) 0.43 |
| <i>Male</i> | | | | |
| Control (69) | 54 | 14 | 1 | Reference |
| Over all FD (59) | 51 | 7 | 1 | 0.56 (0.22–1.44) 0.26 |
| EPS (33) | 28 | 4 | 1 | 0.64 (0.21–1.95) 0.60 |
| PDS (19) | 18 | 1 | 0 | 0.20 (0.02–1.62) 0.18 |
| Others (10) | 8 | 2 | 0 | 0.90 (0.17–4.69) 1.00 |
| <i>Female</i> | | | | |
| Control (43) | 30 | 10 | 3 | Reference |
| Over all FD (75) | 52 | 22 | 1 | 1.02 (0.45–2.30) 1.00 |
| EPS (37) | 20 | 16 | 1 | 1.96 (0.78–4.91) 0.17 |
| PDS (22) | 20 | 2 | 0 | 0.23 (0.05–1.13) 0.07 |
| Others (22) | 16 | 6 | 0 | 0.87 (0.28–2.71) 1.00 |

Note; G carriers, GG + CG. Statistical analysis was performed by two-sided Fisher's exact test. The *Hp*(-) FD patients contained 7 subjects, who were diagnosed as both PDS and EPS. The *HP*(+) FD patients contained 2 subjects, who were diagnosed as both PDS and EPS. The male FD patients contained 3 subjects, who were diagnosed as both PDS and EPS. The female FD patients contained 5 subjects, who were diagnosed as both PDS and EPS.

cytes and monocytes [16]. Therefore, RANTES has also been shown to contribute to the inflammatory response in the stomach especially for *H. pylori*-induced gastritis [17–20].

Changes in gene expression in human monocytes after stimulation of RANTES have been examined by the oligonucleotide array method, showing that RANTES activates the transcription of cytokine genes (MCP-1, pro interleukin-1 β , IL-8, etc.), membrane receptors (oxidized LDL receptor, etc.), regulators of extracellular matrix proteins (MMP-9 etc.), and enzymes regulating intracellular signal transduction (MAPK, etc.) [28]. Four binding sites for nuclear factor- κ B in the RANTES promoter are critical for induction by the proinflammatory cytokines tumor necrosis factor- α and interleukin-1 β and induction through the CD28 costimulatory pathway [29]. The RANTES promoter C-28G polymorphism is located immediately downstream of the first of these nuclear factor- κ B sites (-40 to -31). Recent reports show quantitative differences in RANTES protein

expression between different RANTES promoter genotypes [21]. Although we did not investigate the serum or gastric mucosal RANTES expression in FD patients, it is possible that RANTES promoter genotypes would also influence the inflammatory immune response in the gastrointestinal tract, and therefore modify the risk of FD.

We have also shown that the -28 G carrier is associated with a reduced risk of PDS in *H. pylori* infected subjects. It is well known that *H. pylori* plays a major role in the pathogenesis of gastro-duodenal inflammation. *H. pylori* infection has also been reported to be more frequent in patients with non-ulcer dyspepsia than control population, although the role of *H. pylori* infection in functional dyspepsia is still controversial [30]. Many trials evaluating the efficacy of *H. pylori* eradication treatment for FD have given conflicting results but there is a clear indication that *H. pylori* eradication treatment is effective in at least a subset of patients with FD. The recently published meta-analysis

suggests that *H. pylori* eradication at 12 months has a small but statistically significant benefit in the treatment of FD [31]. Depending on the population under study, between 30%–65% of patients diagnosed with functional dyspepsia has *H. pylori*-induced gastritis [32, 33]. These studies suggest that *H. pylori*-induced gastric mucosal inflammation may play an important role on the pathophysiology of FD.

Increased RANTES production is a feature of *H. pylori* induced gastric inflammation [17–20]. RANTES mRNA expression is also thought to play an important role in maintaining residual memory T lymphocytes and eosinophils in gastric mucosa following *H. pylori* eradication [20]. Our data indicate that RANTES polymorphism may modify the risk of *H. pylori* infected FD by altering the inflammatory response against *H. pylori*.

In the functional perspective, -28G allele of the RANTES promoter have shown to have the higher level of mRNA and protein expression than those of C allele [21]. In our study, high producing genotype of -28G carriers was reduced the risk of PDS especially in *H. pylori* infected subjects. It is possible that altered immune response against *H. pylori* by RANTES promoter polymorphism may alter the inflammatory immune response. But why the high producing genotype of -28G carriers reduced the risk of PDS it remained to be explained.

Abbreviations

FD, functional dyspepsia; *H. pylori*, *Helicobacter pylori*; EPS, epigastric pain syndrome; PDS, postprandial syndrome.

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