Indian J Gastroenterol (September–October 2017) 36(5):343–352
DOI 10.1007/s12664-017-0788-7

ORIGINAL ARTICLE

Genetic variants of immune-related genes *IL17F* and *IL10* are associated with functional dyspepsia: A case–control study

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Received: 10 January 2017 / Accepted: 11 September 2017 / Published online: 30 September 2017

Abstract
Background Low-grade inflammation may play an important role in pathogenesis of functional dyspepsia (FD). Since cytokines may influence gastric mucosal inflammation, which is associated with FD, we evaluated singe nucleotide polymorphisms (SNPs) of pro-inflammatory *IL17F* and anti-inflammatory *IL10* cytokine genes in patients with FD and healthy subjects (HS).
Methods Two hundred and thirty-seven consecutive patients with FD (Rome III) and 250 HS were genotyped for *IL17F* (rs2397084: A/G, rs763780: T/C) and *IL10* (rs1800896: G/A, rs1800871: C/T) (PCR-RFLP).
Results Patients with FD [173 (73%) men, age 38.4±12 years] were comparable with HS [195 (78%) men, age 37.3±12 years] with respect to age and gender. Out of 237 patients, 26 (11%) had epigastric pain, 55 (23.2%) had postprandial distress syndromes (EPS, PDS), and 156 (65.8%) had EPS–PDS overlap. Among 237 patients with FD, GG (variant) genotype of *IL17F* (rs2397084) was more common than HS [15 (6.3%) vs. 4 (1.6%), p=0.015, odds ratio (OR)=4.0, 95% confidence interval (CI)=1.3–12.3]. *IL17F* (rs763780) and *IL10* (rs1800896) were comparable among patients and HS (p=0.56, 0.28), respectively. However, TT (variant) genotype of *IL10* (rs1800871) was more common among patients than HS [39 (16.5%) vs. 32 (12.8%), p=0.06, OR=1.7, 95% CI=0.98–2.98]. SNPs of *IL17F* and *IL10* (rs2397084, rs763780, rs1800896 and rs1800871) were comparable among patients among sub-types of FD (p=0.80 and 0.44).
Conclusion SNPs of *IL17F* (rs2397084) and *IL10* (rs1800871) genes are associated with FD.

Keywords Cytokines · Functional gastrointestinal disorders · Genes · Inflammation · Polymorphism

Introduction

Functional dyspepsia (FD) is a common clinical syndrome characterized by presence of recurrent or chronic upper abdominal symptoms, such as epigastric pain, early satiety, and fullness, without any structural abnormalities on upper gastrointestinal endoscopy (UGI-endoscopy) [1]. FD is a heterogeneous disorder, in which different pathophysiological mechanisms underlie specific symptom patterns [2–4]. It may result from a combination of visceral hypersensitivity, gastric motor dysfunction and low-grade mucosal inflammation [5–7]. Accumulating evidence including familial aggregation recently suggested that functional gastrointestinal (GI) disorders, including FD, might be contributed by genetic factors; identification of which may improve understanding of underlying pathophysiological mechanisms [8–11]. However, genetic factors contributing to development of FD are largely unknown. Since, FD might result from gastric mucosal inflammation, genes influencing it might contribute to occurrence of FD.

Genes encoding cytokines have been reported to be associated with FD [9]. Pro-inflammatory cytokines, such as tumor necrosis factor (*TNF*), interferon γ, and interleukin *17F* (*IL17F*), and anti-inflammatory cytokines, such as interleukin 10 (*IL10*) and transforming growth factor β1 (*TGF-β1*), are

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involved in the regulation of immune and inflammatory responses [12, 13]. Moreover, these cytokine genes have polymorphic sites, which are considered to alter gene transcription influencing the degree of inflammatory response [9]. Presence of some alleles in the genes is associated with high or low production of the cytokine. Therefore, individuals can be classified by their allele status as high, intermediate or low producers of a particular cytokine. Those who are homozygous for the high-producer allele make the highest amount; those who are homozygous for the low producers make the least, whereas those who are heterozygous produces intermediate quantity. A genetic predisposition to produce high or low amounts of a particular cytokine might alter the individual's susceptibility to FD [9, 14, 15]. The genetic basis for FD in relation to cytokine production is poorly understood.

IL17F, a pro-inflammatory molecule, belongs to IL17 family, which plays a role in coordinating tissue inflammation by inducing release of other pro-inflammatory and neutrophil-mobilizing cytokines [16, 17]. This cytokine is important in neutrophil recruitment and activation causing gastric mucosal inflammation [18]. Since gastritis is associated with symptoms of FD, it is important to study the role of immune-related gene polymorphism, such as that of *IL17F* in pathophysiology of FD. Cytogenetic location of *IL17F* is on 6p12 [19]. This gene exhibits common single nucleotide polymorphisms (SNPs) rs2397084: A/G and rs763780: T/C. The functional role of *IL17F* (rs2397084) polymorphism is not well-known. However, some studies suggest that it may alter *IL17F* expression [19]. *IL17F* (rs763780) polymorphism causes His-to-Arg substitution at amino acid 161 (H161R), which leads to the reduction of *IL17-F* cytokine level [17].

IL10 is an immune-regulatory cytokine that inhibits cell-mediated immune response [20]. It has been shown to exert potent anti-inflammatory activity by down-regulating pro-inflammatory cytokines such as *TNF-*α, *IL1*, *IL12* and chemokines [20, 21]. A genetic predisposition to produce low levels of anti-inflammatory cytokines may result in inability to control inflammatory response, which may be associated with development of some types of FGIDs including FD [9, 15].

IL10 gene is located on chromosome 1q31–32, consisting of five exons and four introns [22]. Two commonly SNPs studied of *IL10* gene are rs1800896: G/A and rs1800871: C/T [22, 23]. *IL10-819 C/T* and *IL10-592 A/C* showed 100% linkage disequilibrium in its genotype distribution [22]. It has been reported that *IL10* SNPs markedly influenced mucosal *IL10* expression in the course of chronic *Helicobacter pylori* (*H. pylori*) infection: GCC haplotype carriers (*IL10*-1082G, *IL10*-819C, *IL10*-592C) were associated with high *IL10* expression and ATA carriers with low *IL10* expression [22].

The genetic basis for FD in relation to cytokine production is poorly understood in relation to FD. Also, there are scanty data that show significant association between FD and genetic factors. Therefore, we aimed to study genetic polymorphism of *IL17F* (rs2397084: A/G and rs763780: T/C) and *IL10* (rs1800896: G/A and rs1800871: C/T) genes among patients with FD and healthy subjects (HS).

Methods

Sample size calculation

Sample size for SNPs *IL17F* (rs2397084, rs763780) and *IL10* (rs1800896, rs1800871) was calculated using software Quanto, version 1.1.1 (http://hydra.usc.edu/gxe), with minor allele frequency data from HapMap (http://www.hapmap.org/). Parameters used were as follows: significance level (2-tailed test) 0.05, mode of inheritance was log additive (which is the most suitable model for the polygenic diseases), odds ratio (OR) of larger or equal to 1.5, study power 80% and matched case to control ratio. Two hundred and thirty-seven patients with FD and two hundred and fifty HS were adequate to give the power of 80% (probability of not making a type II error).

Study participants

Two hundred and thirty-seven patients with FD attending the Gastroenterology outpatient clinic of a university hospital in northern India were included for the study during a 3-year period (2010 to 2012). The diagnosis of FD was based on Rome III criteria. Further, patients were sub-classified into epigastric pain syndrome (EPS), post-prandial distress syndrome (PDS) and EPS-PDS overlap. Patients who had organic or metabolic disorders were excluded from the study.

HS were invited from the community. A total of 250 age- and gender-matched unrelated HS of similar ethnicity, apparently free from any significant GI symptoms were included as controls from the same population. The Institutional Ethics Committee approved the study protocol, and written consent was obtained from all the subjects.

Esophagogastroduodenoscopy

Esophagogastroduodenoscopy (EGD) was performed in patients with FD using a video endoscope (Olympus Optical Co Ltd.; Tokyo, Japan). Two to four biopsies were collected from patients with FD (each from antrum and body) from normal-looking areas of the stomach for histology examination of *H. pylori* infection-associated gastritis.

Characterization of phenotype

Each patient was interviewed using a standard questionnaire to record demographic and clinical symptoms of FD.

Sample collection

Genomic DNA, extracted from venous blood in ethylene diamine tetra acetic acid (EDTA) using commercially available kit (Qiagen Inc., Valencia, CA, USA), was used for genotyping of *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) polymorphisms.

Quantitation and storage of DNA

The quality and purity of DNA were checked by measuring optical density (OD) at 260 and 280 nm, respectively. The ratio of absorbance at 260 and 280 nm of DNA was around 1.7–1.9. DNA quality was checked in few DNA samples by 1% agarose gel electrophoresis to validate the technique.

Genotyping of *IL17F* (rs2397084 and rs763780) polymorphisms

Genotyping of *IL17F* (rs2397084 and rs763780) was performed using PCR followed by restriction digestion. PCR amplification was carried out in a total volume of 25 μL with 0.4 μM of each primer as described previously [19]. For *IL17F* (rs2397084), forward primer used was 5′-GTGT AGGAACTTGGGCTGCATCAAT-3′ and reverse primer was 5′-AGCTGGGAATGCAAACAAAC-3′. Genomic DNA (100–150 ng), 1× 500 mM KCl buffer (pH 8.4), 15 mM $MgCl_2$, 0.2 mM dNTP set, and 1 U Taq DNA polymerase (Bangalore Genei, India) were used in the PCR reaction mixture. PCR amplification was performed as follows: initial denaturation at 94 °C for 5 min followed by 34 cycles of denaturation at 94 °C for 40 s, annealing at 54.6 °C for 40 s and extension at 72 °C for 45 s. The final extension at 72 °C was for 10 min. The PCR product was digested with restriction enzyme AvaII (10 U/μL, New England BioLabs, Germany) at 37 °C overnight, and fragments were analyzed on 3% agarose gels stained with ethidium bromide. The A allele was defined by the presence of one fragment of 470 bp and the G allele by the presence of two fragments of 395 and 75 bp. For genotyping of *IL17F* (rs763780), forward primer used was 5′-GTTCCCATCCAGCAAGAGAC-3′ and reverse primer was 5′-AGCTGGGAATGCAAACAAAC-3′. PCR amplification was performed as follows: initial denaturation at 95 °C for 5 min followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 59.4 °C for 25 s and extension at 72 °C for 30 s. The final extension at 72 °C was for 5 min. The PCR product was digested with restriction enzyme NlaIII (5 U/μL, New England BioLabs, Germany) at 37 °C overnight, and fragments were analyzed on 3% polyacrylamide gels stained with ethidium bromide. The T allele was defined by the presence of two fragments of 288 and 124 bp and the C allele by the presence of one fragment of 412 bp.

Genotyping of *IL10* (rs1800896 and rs1800871) polymorphisms

Genotyping was performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) as described previously [24]. For each polymorphism, two separate reactions were performed, each of which included one of the two alleles specific for forward primers and a generic anti-sense primer. For *IL10* (rs1800896) genotyping, primers used were sense (G) 5′-CTACTAAGGCTTCTTTGGGAG-3′, sense (A) 5′-ACTA CTAAGGCTTCTTTGGGAA-3′ and generic (anti-sense) 5′-CAGTGCCAACTGAGAATTTGG-3′. For *IL10* (rs1800871), primers used were sense (C) 5′-CCCT TGTACAGGTGATGTAAC-3′, sense (T) 5′-ACCC TTGTACAGGTGATGTAAT-3′ and generic (anti-sense) 5′-AGGATGTGTTCCAGGCTCCT-3′. DNA was amplified in a 15 μL reaction. Final concentrations of reagents were 10× PCR buffer, 200 μM for each dNTP, 500 mM KCl, 15 mM $MgCl_2$, 5 μM specific primer mix and 2.5 μM for each internal control primer and 25–100 ng DNA. Reaction buffer with cycling conditions as follows: 95 °C for 1 min, followed by 29 cycles at 95 °C for 30 s, 60 °C for 1 min, 72 °C for 50 s and finally 8 min extension at 72 °C. An internal control region was amplified from the β-globin for ensuring the PCR success (primers used for internal control were the following: forward primer 5′-ACACAACTGTGTTCACTAGC-3′, reverse primer 5′-CAACTTCAT CCACGTTCACC-3′). The PCR products were checked on 2% agarose gel. Alleles were written as GG and CC for homozygous wild genotype, AA and TT for homozygous variant genotype and GA and CT for heterozygous; if amplification was seen in G, C and A, T allele-specific primers for −1082 G/A and −819 C/T polymorphisms respectively.

Ten percent of samples from patients and HS was repeated to evaluate the quality of genotyping, which showed 100% concordance.

Statistical analysis

Genotype frequency in patients with FD and HS was compared using 2×2 contingency table using χ^2 test with Yates' correction, as applicable. The direct gene counting method was used to determine the frequency of genotypes. The χ^2 goodness-of-fit test was used to check for any deviation from Hardy-Weinberg equilibrium in HS. Continuous data were analyzed using unpaired "*t*" test. *P*-values less than 0.05 were considered significant. The association between *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) SNPs and FD was analyzed using binary logistic regression. The OR was adjusted for confounding variables such as age and gender. Post-hoc analysis and Bonferroni's

correction (a multiple-comparison correction) were applied to significant association in sub-group analysis. Data were analyzed using the statistical software SPSS, version 15.0 (SPSS, Inc., Chicago, IL, USA). Haplotype analysis was performed using online software SNPstats (http://bioinfo.iconcologia.net/SNPstats).

Results

Demographic and clinical parameters of study participants

Patients with FD [173 (73%) male, age 38.4±12 years] were comparable with HS [195 (78%) male, age 37.3±11.5 years] with respect to age and gender. In all the patients tested, erythrocyte sedimentation rate (ESR), blood glucose, and thyroid-stimulating hormone (TSH) levels were within normal range. Symptoms and clinical profile of patients with FD are summarized in Table 1.

Table 1 Demographic and clinical profile of patients with functional dyspepsia

Parameters	FD (n = 237)
Age (years)[a]	38.4±12
Gender (male)[b]	173 (73%)
Sub-types of FD (Rome III)[b]	
EPS	26 (11%)
PDS	55 (23%)
EPS-PDS overlap	156 (66%)
Symptoms[b]	
Epigastric pain [n (%)]	161 (67.9)
Epigastric burning [n (%)]	156 (65.8)
Post-prandial fullness [n (%)]	185 (78.1)
Early satiety [n (%)]	162 (68.4)
Abdominal bloating [n (%)]	174 (73.4)
Nausea [n (%)]	99 (41.8)
Belching after meal [n (%)]	183 (77.2)
Duration of symptoms months[a]	43.5±48.3
Hemoglobin (g/dL)[a]	12.3±1.4
ESR (mm/h)[a]	20.4±10.6
Blood glucose fasting (mg/dL)[a]	91.2±20.3
Blood glucose post-prandial (mg/dL)[a]	120.5±45.2
TSH (IU/L)[a]	2.8±3.4

FD functional dyspepsia, *HS* healthy subjects, *EPS* epigastric pain syndrome, *PDS* post-prandial distress syndrome, *TSH* thyroid-stimulating hormone

[a] Independent sample t test

[b] Chi-squared test

Sub-types of FD

Patients with FD were sub-typed in EPS, PDS and EPS-PDS overlap. A total of 26/237 (11%) had EPS, 55 (23.2%) PDS and 156 (65.8%) EPS-PDS overlap.

Histology of gastric biopsy for *H. pylori* infection

Histology examination was done in 228 biopsy samples from patients with FD. On histology, of 89/228 were found with *H. pylori*-associated gastritis, of 135/228 samples histology was normal without *H. pylori* infection and 4/228 were with intestinal metaplasia.

Association between *IL17F* (rs2397084) and FD

The genotype distribution in patients with FD and HS is shown in Table 2. Among HS, the frequency of genotypes was in Hardy–Weinberg equilibrium (p=0.08). Representative gel pictures for *IL17F* (*rs2397084*) are shown in Fig. 1.

Among 237 patients with FD and 250 HS, GG (variant) genotype of *IL17F* (rs2397084) was more common among patients than HS [15 (6.3%) vs. 4 (1.6%), p=0.02]. However, frequency of other genotypes i.e. AA and AG was similar among patients and HS (Table 2). Subjects having GG genotypes were more often associated with FD than those having AA and AG [odds ratio (OR)=4.0,95% confidence interval (CI)=1.31–12.3].

Association between *IL17F* (rs763780) and FD

The genotype distribution in patients with FD and HS is shown in Table 2. In HS, the frequency of genotypes was in Hardy–Weinberg equilibrium (p=0.23). Representative gel picture for *IL17F* (rs763780) is shown in Fig. 1.

IL17F (rs763780) polymorphism was comparable among patients with FD and HS [genotypes: TT 183 (77.2%), TC 51 (21.5%), CC 3 (1.3%) vs. TT 199 (79.6%), TC 46 (18.4%), CC 5 (2%), p=0.56].

Association between *IL17F* (rs239708 and rs763780) haplotype and FD

Haplotypes were evaluated for *IL17F* (rs239708 and rs763780). Four haplotypes AT, AC, GT and GC were constructed. Haplotype frequency was comparable among patients with FD and HS as shown in Table 3.

Table 2 Genotype frequency of *IL17F* (rs2397084: A/G and rs763780: T/C) and *IL10* (rs1800896: G/A and rs1800871: C/T) gene polymorphisms in patients with functional dyspepsia and healthy subjects

Genotypes	Patients with FD n^a (%)	Healthy subjects n^a (%)	*p*-Value	OR (95% CI)
IL17F (rs2397084) gene polymorphism				
AA (wild homozygous)	197 (83.2)	211 (84.4)	–	Reference
AG (heterozygous)	25 (10.5)	35 (14)	0.34	0.76 (0.44–1.32)
GG (variant-homozygous)	15 (6.3)	4 (1.6)	0.02	4.0 (1.31–12.3)
Allele				
A allele	419 (88.4)	457 (91.4)	–	Reference
G allele	55 (11.6)	43 (8.6)	0.12	1.4 (0.9–2.1)
IL17F (rs763780) gene polymorphism				
TT (wild homozygous)	183 (77.2)	199 (79.6)	–	Reference
TC (heterozygous)	51 (21.5)	46 (18.4)	0.42	1.2 (0.7–1.8)
CC (variant-homozygous)	3 (1.3)	5 (2.0)	0.56	0.65 (0.15–2.7)
Allele				
T allele	417 (88)	444 (88.8)	–	(Reference)
C allele	57 (12)	56 (11.2)	0.68	1.08 (0.73–1.6)
IL10 (rs1800896) gene polymorphism				
GG	35 (14.8)	43 (17.2)	–	Reference
GA	108 (45.6)	122 (48.8)	0.75	1.08 (0.69–1.8)
AA	94 (39.7)	85 (34)	0.26	1.3 (0.79–2.3)
Allele				
G (high-producer allele)	178 (37.5)	208 (41.6)	–	Reference
A (low-producer allele)	296 (62.4)	292 (58.4)	0.19	1.18 (0.91–1.5)
IL10 (rs1800871) gene polymorphism				
CC	72 (30.4)	101 (40.4)	–	Reference
CT	126 (53.2)	117 (46.8)	0.04	1.5 (1.01–2.23)
TT	39 (16.5)	32 (12.8)	0.06	1.7 (0.98–2.98)
Allele				
C (high-producer allele)	270 (57)	319 (63.8)	–	Reference
T (low-producer allele)	204 (43)	181 (36.2)	0.03	1.3 (1.02–1.72)

n^a = total number of patients with FD (237) and healthy subjects (250)

Statistical test: Binary logistic regression model was used to calculate age and gender adjusted odds ratio (OR) and 95% confidence interval (95% CI)

Association between *IL10* (rs1800896) polymorphism and FD

The genotype distribution among patients with FD and HS is shown in Table 2. Among HS, the frequency of genotypes was in Hardy–Weinberg equilibrium (p=0.9). Representative gel pictures for *IL10* (rs1800896) are shown in Fig. 2.

IL10 (rs1800896) polymorphism was comparable among patients with FD and HS [genotypes: GG 35 (14.8%), GA 108 (45.6%), AA 94 (39.7%) vs. GG 43 (17.2%), GA 122 (48.8%), AA 85 (34%), p=0.28].

Association between *IL10* (rs1800871) polymorphism and FD

The genotype distribution among patients with FD and HS is shown in Table 2. Among HS, the frequency of genotypes was in Hardy–Weinberg equilibrium (p = 0.83). Representative gel pictures for *IL10* (rs1800871) are shown in Fig. 2.

Among 237 patients with FD and 250 HS, TT (variant) genotype of *IL10* (rs1800871) tended to commoner among patients than HS [39 (16.5%) vs. 32 (12.8%), p=0.06]. Subjects having TT genotypes were more often associated with FD than CC and CT [odds ratio (OR)=1.7,95% confidence interval (CI)=0.98–2.98]. On applying dominant model, T allele carriage (CT+TT) was also commoner in patients with FD than HS [204 (43%) vs. 181 (36.2%), p=0.030, OR 1.3, 95% CI 1.02–1.72] in reference to C allele carriage (Table 2).

Association between *IL10* haplotype and FD

Haplotypes were evaluated for IL-10-1082 G/A, -819 C/T and -592 C/A (-819 and -592 are in linkage disequilibrium; allele

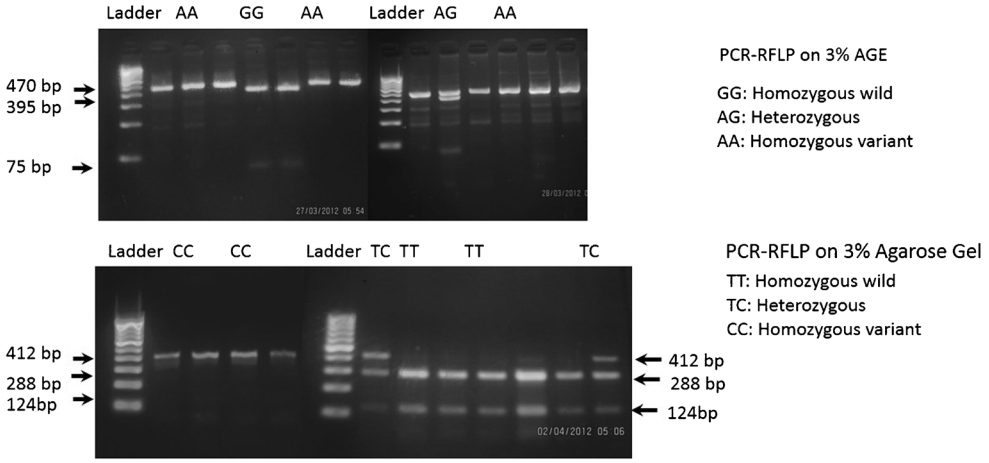

Fig. 1 Representative gel pictures of *IL17F* (rs2397084 and rs763780) polymorphisms: (**a**) PCR-restriction fragment length polymorphism products of *IL17F* (rs2397084) polymorphism were run on 3% agarose gel electrophoresis (AGE): lanes 2–8 and 10–15 show various genotypes, and lanes 1 and 9 show a 100-bp DNA ladder. (**b**) PCR-restriction fragment length polymorphism products of *IL17F* (rs763780) polymorphism were run on 3% AGE: lanes 2–5 and 7–13 show various genotypes, and lanes 1 and 6 show a 100-bp DNA ladder

C at -592 is always present when at position -819 is allele C and allele A is always present when at position -819 is allele T) polymorphisms located in the same gene with emphasis on combination of variants, which might be more likely to influence change in IL-10 level. For this purpose, four haplotypes, over-producer (GCC), over-producer (ACC), intermediate (GTA) and under-producer (ATA) were constructed. Frequency of haplotypes was comparable among patients with FD and HS as shown in Table 4.

Il17F and *IL10* genetic polymorphisms in sub-types of FD

The genotype distribution among patients with different sub-types of FD is shown in Table 5. *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) SNPs were comparable among patients with different sub-types of FD.

Association between *IL17F* and *IL10* gene polymorphisms and risk of FD in relation to *H. pylori* infection

The genotype distribution among patients with FD in the presence and absence of *H. pylori* infection is shown in Table 6. *IL17F* (rs2397084, rs763780) and *IL10* (rs1800896, rs1800871) SNPs were comparable among patients with FD with or without *H. pylori* infection.

Discussion

The present study on a large number of patients with FD and HS suggests a possible role of *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) genotypes in the pathogenesis of FD. The presence of the GG (variant genotype) of *IL17F* (rs2397084) and that of the TT (under-producers) genotype of *IL10* (rs1800871) were associated with FD. However, *IL17F* (rs763780) and *IL10* (rs1800896) did not show any significant association. We found no relationship between these genotypes and various sub-types of FD. In addition, these SNPs were comparable among patients with and without *H. pylori* infection.

A number of studies implicated inflammatory mechanisms in the pathogenesis of FD [25, 26]. One study showed that peripheral blood mononuclear cells from patients with FD had elevated levels of *TNF*, *IL-1β* and *IL10*, and an increased

Table 3 Association between *IL17F* (rs2397084 and rs763780) haplotypes and functional dyspepsia

Haplotypes[a]	FD *n* = 474 (%)	HS *n* = 500 (%)	*p*-Value	OR (95% CI)
AT	366 (77.2)	407 (81.5)	–	Reference
AC	52 (11.1)	50 (10.0)	0.41	0.84 (0.55–1.28)
GT	51 (10.8)	36 (7.1)	0.09	0.70 (0.47–1.06)
GC	5 (0.9)	7 (1.4)	0.56	1.58 (0.34–7.31)

[a] Haplotype number represents total number of chromosomes; frequency of combination equivalent to zero in any cell for *IL17F* (rs2397084 and rs763780) was not included in analysis; OR = age and gender adjusted odds ratio; 95% CI = 95% confidence interval

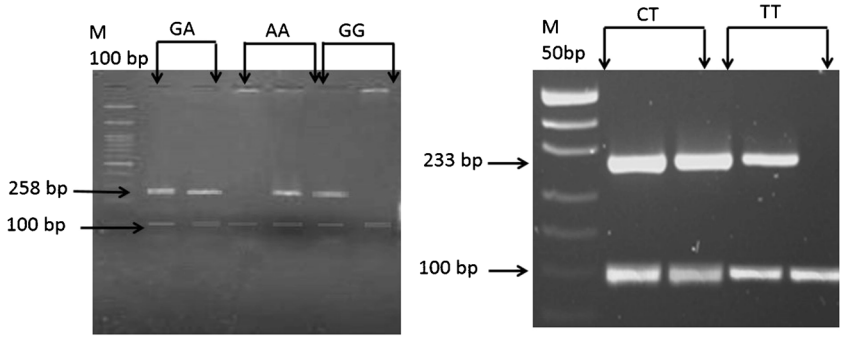

Fig. 2 Representative gel pictures of *IL10* (rs1800896 and rs1800871) polymorphisms: (**a**) PCR products of *IL10* (rs1800896) polymorphism were run on 2% AGE: lanes 2–7 show various genotypes, and lane 1 shows a 100-bp DNA ladder. (**b**) PCR products of *IL10* (rs1800871) polymorphism were run on 2% AGE: lanes 2–5 show various genotypes, and lane 1 shows a 50-bp DNA ladder

number of gut-homing T cells compared with HS [13]. In addition, cytokine release and the number of gut-homing T cells positively correlated with symptom intensity of pain, cramps, nausea and vomiting and with the presence of delayed gastric emptying [13]. In keeping with the concept of a duodenal inflammatory component in FD, increased duodenal eosinophil counts were reported in patients with FD in a population-based case–control study [27]. Also, transient mucosal inflammation is now believed to be an important trigger for the manifestation of FD [28]. Some studies showed presence of inflammation and reduced duodenal mucosal integrity in patients with FD [26, 29, 30]. Functional evidence of increased permeability and altered expression of several cell-to-cell adhesion proteins had been observed [26]. In another study, authors showed that patients with FD were characterized by low-grade inflammation, demonstrated by increased numbers of mast cells and eosinophils [31]. Thus, impaired barrier function can facilitate the passage of luminal antigens through the epithelium, by which they can enter the lamina propria [32, 33]. This may lead to low-grade inflammation, which can then contribute to the generation and persistence of GI symptoms.

We found that *GG* (variant) genotype of *IL17F* (rs2397084) was associated with FD as compared with the presence of AA (wild) genotype. Such high frequency of variant genotype in FD suggests that high production of *IL17F* (pro-inflammatory cytokine) may have some risk for FD. Such genetic predisposition to higher pro-inflammatory cytokine production among patients with FD might mean that exaggerated inflammatory response may predispose to FD. This is perhaps the first study on *IL17F* (rs2397084) SNP in FD. This SNP has been studied in other inflammatory disorders such as lung cancer and rheumatoid arthritis; however, no significant association was found with disease [19, 34]. Our data supported the recently proposed hypothesis that FD may be related to low-grade inflammation. Thus, a genetic predisposition to produce high amounts of pro-inflammatory cytokine might alter the individual's susceptibility to FD.

The findings of our study showed that *IL17F* (rs763780) SNP was comparable among patients with FD as compared with HS. This polymorphism causes His-to-Arg substitution at amino acid 161 (H161R), which leads to reduction of *IL17F* cytokine level [18]. In contrast to our study, one Japanese study showed *T* allele of *IL17F* (rs763780) was significantly associated with EPS, a sub-group of FD [18]. Another study from Japan showed that *IL17F* (rs763780) polymorphism was comparable among patients with duodenal ulcer disease and HS [35]. Also, some studies reported association between *IL17F* (rs763780) and ulcerative colitis, another inflammatory GI condition [36].

Our study showed that *IL10* (rs1800896) polymorphism was comparable among patients with FD and HS. However, TT (under-producers) genotype of *IL10* (rs1800871) was more common among patients than HS. Such high frequency of the under-producer genotype in FD suggests that lower production of *IL10* (anti-inflammatory cytokine) may have

Table 4 Association between *IL10* (rs1800896 and rs1800871) haplotypes and functional dyspepsia

Haplotypes[a]	FD *n* = 474 (%)	HS *n* = 500 (%)	*p*-Value	OR (95% CI)
ACC (over-producer)	166 (35.0)	179 (35.8)	–	Reference
GCC (over-producer)	103 (21.9)	140 (28.0)	0.28	1.25 (0.83–1.87)
GTA (intermediate)	75 (15.7)	68 (13.6)	0.41	0.84 (0.56–1.27)
ATA (under-producer)	130 (27.4)	113 (22.6)	0.27	0.80 (0.53–1.20)

[a] Haplotype number represents total number of chromosomes; frequency of combination equivalent to zero in any cell for *IL10* (rs1800896 and rs1800871) was not included in analysis; OR = age and gender adjusted odds ratio; 95% CI = 95% confidence interval

Table 5 Genotype frequency of *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) gene polymorphism among patients with different subtypes of functional dyspepsia

Genotypes	EPS n^a (%)	PDS n^b (%)	EPD-PDS overlap n^c (%)	*p*-Value
IL17F (rs2397084) gene polymorphism				
AA	22 (84.6)	52 (94.5)	123 (78.8)	0.08
AG	4 (15.4)	35 (5.5)	18 (11.5)	
GG	0 (0)	0 (0)	15 (9.6)	
IL17F (rs763780) gene polymorphism				
TT	20 (76.9)	43 (78.2)	120 (76.9)	0.7
TC	5 (19.2)	11 (20)	35 (22.4)	
CC	1 (3.8)	1 (1.8)	1 (0.6)	
IL10 (rs1800896) gene polymorphism				
GG	3 (11.5)	5 (9.1)	27 (17.3)	0.2
GA	10 (38.5)	32 (58.2)	66 (42.3)	
AA	13 (50)	18 (32.7)	63 (40.4)	
IL10 (rs1800871) gene polymorphism				
CC	10 (38.5)	18 (32.7)	44 (28.2)	0.4
CT	15 (57.7)	29 (52.7)	82 (52.6)	
TT	1 (3.8)	8 (14.5)	30 (19.2)	

n^a = total number of patients with epigastric pain syndrome (26)

n^b = total number of patients with post-prandial distress syndrome (55)

n^c = total number of patients with EPS-PDS overlap (156)

some risk for FD. Several studies have shown that serum *IL10* levels are significantly lower among patients with IBS (another common FGID) than HS, suggesting that altered *IL10* levels may be involved in the pathogenesis of FGIDs [15, 23, 37]. Also, it has been reported that *IL10* SNPs markedly influenced mucosal IL-10 expression in the course of chronic

Table 6 Genotype frequency of *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) gene polymorphisms in patients with functional dyspepsia in the presence and absence of *H. pylori* infection

Genotypes	*H. pylori* infection Present n^a = 89 (%)	*H. pylori infection* Absent n^b = 135 (%)	*p*-Value	OR (95% CI)
IL17F (rs2397084) gene polymorphism				
AA (wild homozygous)	73 (82.0)	113 (83.7)	–	Reference
AG (heterozygous)	11 (12.4)	13 (9.6)	0.53	1.3 (0.5–3.0)
GG (variant-homozygous)	5 (5.6)	9 (6.7)	0.79	0.86 (0.2–2.7)
IL17F (rs763780) gene polymorphism				
TT (wild homozygous)	64 (71.9)	108 (80.0)	–	(Reference)
TC (heterozygous)	23 (25.8)	26 (19.3)	0.22	1.4 (0.7–2.8)
CC (variant-homozygous)	2 (2.2)	1 (0.7)	0.32	3.3 (0.3–37.9)
IL10 (rs1800896) gene polymorphism				
GG	11 (12.4)	23 (17)	–	(Reference)
GA	47 (52.8)	57 (42.2)	0.21	1.6 (0.7–3.7)
AA	31 (34.8)	53 (39.3)	0.64	1.2 (0.5–2.8)
IL10 (rs1800871) gene polymorphism				
CC	25 (28.1)	43 (31.9)	–	
CT	47 (52.8)	71 (52.6)	0.67	1.1 (0.6–2.1)
TT	17 (19.1)	21 (15.6)	0.42	1.3 (0.6–3.1)

n^a = total number of patients with FD having *H. pylori* infection (89)

n^b = total number of patients with FD without *H. pylori* infection (135)

Statistical test: Binary logistic regression model was used to calculate age and gender adjusted odds ratio (OR) and 95% confidence interval (95% CI)

H. pylori infection: GCC haplotype carriers (*IL-10*-1082G, *IL-10*-819C, *IL-10*-592C) were associated with high *IL10* expression and ATA carriers with low *IL10* expression [22]. A study by Wang et al. showed that *IL10*-genetic polymorphism at positions -819 and -592 in the promoter region, -819 T/T and -592 A/A genotypes, was associated with diarrhea-predominant irritable bowel syndrome (D-IBS) [38]. In another study, lower frequency of high-producer genotype of IL-10 in patients with IBS suggested that low levels of IL10 might be more likely to develop IBS than individuals who are predisposed to produce high amounts of this cytokine might have some protective role [15, 39, 40]. A genetic predisposition to produce low levels of anti-inflammatory cytokines could mean that control of the inflammatory response might be compromised in some individuals, which may lead to continuing problems.

H. pylori infection is another important factor in the manifestation of FD symptoms that is localized to the stomach [41]. According to the Rome criteria, *H. pylori* infection status does not affect the diagnosis of FD, and both *H. pylori* infection positive and negative types of FD exist [42, 43]. The most common approach to investigating the relationship between *H. pylori* and FD had been to examine improvements in FD symptoms resulting from the eradication of *H. pylori*. However, the results on the effect of *H. pylori* eradication therapy on improvement in FD symptoms are not consistent. Although there have been many rigorous, high-quality clinical studies, the conclusions remain highly controversial [44–47]. As far as gastritis is concerned, no consistent data demonstrated that such an inflammatory status of gastric mucosa may be associated with dyspeptic symptoms. A certain genetic predisposition to develop dyspeptic symptoms in patients with *H. pylori*-associated gastritis has been suggested [18, 48–50]. In a study from Japan, it showed that RANTES promoter -28G carriers were associated with a reduced risk of PDS especially in *H. pylori*-positive subjects [48]. Also, Arisawa et al. had reported that IL-17F 7488T and macrophage migration inhibitory factor -173C alleles were significantly associated with the development of FD, particularly epigastric pain syndrome, a sub-group of FD, in *H. pylori*-infected subjects [18]. In our study, we did not find significant association between *IL17F* and *IL10* gene polymorphisms among patients with FD having with or without *H. pylori* infection.

Moreover, our study did not find significant association among *IL17F* (rs2397084 and rs763780) and *IL10* (rs1800896 and rs1800871) SNPs and different sub-types of FD. In contrast, one study from Japan showed significant association between *IL17F* (rs763780) SNP and EPS, a sub-type of FD [18].

In conclusion, *IL17F* (rs2397084) was significantly associated with FD. However, *IL17F* (rs763780) was comparable among patients with FD and HS. In addition, we did not observe any risk associated with *IL10* (rs1800896) and FD. However, homozygous variant (TT) of *IL10* (rs1800871) was more common among patients with FD than HS and up to two-fold higher odds of FD in reference to homozygous wild (CC) genotype. Our study provided evidence that the genetic polymorphisms of molecules associated with inflammation or immune response may be involved in the development of FD for the first time in an Indian population. In addition, these results provide some support to the concept that "low-grade inflammation" may contribute to the pathogenesis of this condition. Lack of evaluation of expression and/or activity of the *IL17F* and *IL10* is a limitation of this study. More studies addressing this issue are needed to strengthen our hypothesis.

Acknowledgements Rajan Singh thanks the Indian Council of Medical Research (ICMR) for junior and senior research fellowships.

Compliance with ethical standards

Conflict of interest RS, UCG, SK, and BM declare that they have no conflict of interest to disclose.

Ethics statement The Institutional Ethics Committee approved the study protocol, and written consent was obtained from all the subjects. The authors declare that the study was performed in a manner that conformed with the Helsinki Declaration of 1975, as revised in 2000 and 2008, concerning human and animal rights.

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