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胃幽門螺旋菌感染病人的前發炎因子多型性與胃腸及食道逆流的研究

Investigation of pro-inflammatory polymorphisms in *Helicobacter pylori*-infected patients with gastrointestinal and reflux esophagitis diseases

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Abstract

Infection of *Helicobacter pylori*, a group I carcinogen, induces chronic gastritis and may further develop into peptic ulcers or gastric carcinoma. Additionally, infection with virulent strains is proposed as a protective factor against the development of gastroesophageal reflux disease. Several cytokine genes of given polymorphisms are linked to the level of inflammation in *H. pylori*-infected patients and increased gastric cancer risk. In this study, we investigated the association of cytokine polymorphisms in *H. pylori*-infected patients with gastritis, peptic ulcer, and gastroesophageal reflux disease (GERD) in Taiwan. Our results showed that the frequencies of IL-1 β -511 CT and -31 CT genotypes were associated with increased risk of the development of GERD. Additionally, the high-produced IL-10 alleles (IL-10-819 CC/CT and IL-10-592 CC/CA) were associated with increased risk of the development of gastritis.

Keywords: *Helicobacter pylori*, gastroesophageal reflux disease, cytokine polymorphism

Introduction

In 1983, Warren JR and Marshall BJ discovered *Helicobacter pylori* in gastric mucosa from chronic gastritis (1). In the second year, they published that this bacteria characterized as being a new Gram-negative, microaerophilic species related to the genus *Campylobacter* stay in stomach from chronic gastritis and peptic ulcer patients again, and isolated in pure culture (2). After two decades, *H. pylori* is recognized as the major etiologic agent in the development of chronic gastritis and duodenal ulcer, and also associated with gastric ulcer and gastric cancer (3). This unique species infects approximately half of the human population, in which it may persist for a lifetime, making it one of the most successful pathogens of mankind (2,4,5). Persistent infection of this peculiar microbe induces chronic inflammation in gastric epithelial cells, which may further develop into peptic ulcers, gastric atrophy and is considered as a risk factor for gastric adenocarcinoma and low-grade B-cell lymphoma (4). In 1994, the International Agency for Research on Cancer (IARC) has declared *H. pylori* as a group I carcinogen.

Two complete genomic sequences and other results demonstrate that *H. pylori* is a species of unexpectedly high heterogeneous genetic sequences (6,78). In accord with strains found in Western countries, we demonstrate high genetic heterogeneity (9-14). These results together shed light on the extraordinary ability of *H. pylori* to adapt into different ecological niches created by the diversity of humans, their ancestors, their environments and their diets. It is thus considered that bacterial heterogeneity, host polymorphisms and environmental factors result in differential pathology during chronic infection that underlies the development of various clinical sequelae (15).

Apart from gastrointestinal diseases, the relationship of *H. pylori* infection with gastro-esophageal reflux disease (GERD) that is a common disorder characterized by abnormal exposure of the esophageal mucosa to acid gastric contents has been a subject of great interest in recent years. This is because the prevalence of *H. pylori* infection as well as the rates of duodenal ulcer and distal gastric carcinoma are declining, the incidence of GERD is increasing in developed countries (16-18). The association between *H. pylori* infection and GERD is not evident; controversial data have been reported (17,19-26). A milder GERD symptom is more frequently observed for patients with strains, particularly virulent isolates (19-23,27). Cure of *H. pylori* infection also associates with occurrence of both de novo and rebound/exacerbated GERD (17,28,29). In contrast, there are evidences surrounding the *H. pylori* infection with no clinically significant effect on GERD symptoms (17,24-26). In some reports, virulent strains did not protect against the development of GERD (30,31). One potential explanation for the controversial results among studies is due to other confounding factors, particularly predisposition of host factors. Thus, differences of host genetics need to be better evaluated in the genesis of GERD.

Persistent colonization of *H. pylori* induces chronic inflammation. As a result, infected and infiltrating cells produce pro-inflammatory factors including interleukin 8 (IL-8), interleukin 1-beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α). IL-8 recruits lymphocytes and neutrophils, whereas IL-1β activates macrophages and polymorphonuclear

leukocytes and stimulates interleukin 6 (IL-6) release and COX-2 expression. IL-6 activates and differentiates macrophages, increases the phagocytic activity of neutrophils, and increases ICAM-1 expression on endothelial cells and activates B-cell differentiation. TNF-α activates and differentiates macrophages, mediates apoptosis of epithelial cells and disrupts the epithelial barrier, inhibits microvascular epithelial cell proliferation and wound healing (32). Chronic antral-predominant inflammation is associated with increased stimulated acid production and predisposes to duodenal ulceration. On the other hand, corpus-predominant or pan-gastritis is associated with reduced acid production and predisposes to gastric ulceration and gastric atrophy (33).

Polymorphisms of several cytokine genes are identified to contribute to the immune and inflammatory response to *H. pylori* infection. Studies have linked genotypes with increased expression of IL-1β (34,35) or TNF-α (36) to the increased risk of gastric adenocarcinoma. The presence of IL-1β and TNF-α has been shown to inhibit gastric acid, which may cause hypochlorhydria (37). For *IL-1β* gene, there are three diallelic polymorphisms at positions −511, −31, and +3953 base pairs (bp) from the transcriptional start site (38). Polymorphism at positions −511 and +3954 are C–T base transitions, while polymorphism at position −31 is a T–C transition. The IL-1β-511-T/T and C/T genotypes are associated with increased IL-1β production in comparison with the IL-1β-511-C/C genotype. The frequency of IL-1β-511T alleles was significantly higher in reflux esophagitis patients than in controls in Japan(39). IL-1β-31-C/C and C/T were less frequently in patients with GERD in Brazil(23). The human *TNF-α* gene has a polymorphism has been found at position -308 within the promoter region, in which the 308A-allele haplotype is associated with higher transcriptional level of TNF-α (40).

Genetic variation of an anti-inflammatory cytokine, IL-10 that modulates a number of immunoregulatory activities (41) is also found to link to increased risk of cancer (42,43). The *IL-10* gene has three diallelic polymorphisms at positions -1082, -819, and-592 bp from the transcriptional start site. Polymorphism at position -1082 is a G–A transition, polymorphism at position -819 is a C–T transition, and polymorphism at position -592 is a C–A transition. All of these SNPs were associated with the increase probability of gastric cancer. The carriers of the IL-10-1082G, -819C, and -592C alleles had higher mucosal IL-10 mRNA than ATA haplotype carriers (44). In Taiwan studies, the IL-10 genotype containing the G allele at -1082, C allele at -819 or C allele at -592 positions was more frequent in GC than in controls (45).

The *IL-8* gene polymorphisms are found at position -251 bp (from the transcriptional start site) as a T–A transition. The IL-8-251-A/A genotype is associated with increased IL-8 production. IL-8-251-A/A was significantly associated with elevated risk of atrophic gastritis in Japan (46). IL-8-251-A/T was higher in DU patients and IL-8-251-T/T was lower in DU patients in Hungary (47).

In this investigation, we presented the relationship between cytokine polymorphism (IL-8, IL-10, IL-1β and TNF-α) and different gastro-intestinal diseases (gastritis, peptic ulcer, and GERD) in Taiwan. Our results showed that IL-1*β*-511 CT and -31 CT alleles were associated

with increased risk of the development of GERD. Furthermore, the high-production IL-10 was associated with increased risk of the development of gastritis.

Results and discussion

From December 2005 through January 2008, 865 consecutive patients who had routine physical examination and underwent upper gastrointestinal endoscopies in the Division of Gastroenterology at Taichung Veterans General Hospital were analyzed in this study. Sixty-one patients were excluded from the study: previous esophageal or gastric surgery (14), esophageal, gastric, or duodenal cancer (12), and concomitant use of proton pump inhibitors or prior *H. pylori* eradication (3). In total, there were 804 patients recruited in this investigation ranging in age from 18 to 85 years (51.5 ± 12.1 years) and 480 patients (59.7%) were male. Of these patients, 208 were with GERD, 154 with gastritis, 133 with gastric ulcers, 133 with duodenal ulcers, and 176 with healthy controls.

The characteristics of the patients in this study are shown in Table 1. The mean age of patients in the normal group is lower that that of patients with gastritis ($p < 0.001$), gastric ulcer ($p < 0.001$) and duodenal ulcer ($p < 0.001$), respectively (Table 1). As compared with the healthy control group (71 males and 105 females, male/female = 0.68), there were significantly more males than females among patients with GERD (150 males and 58 females, male/female = 2.59, $p < 0.001$), gastritis (84 males and 70 females, male/female = 1.23, $p = 0.007$), gastric ulcer (82 males and 51 females, male/female = 1.61, $p < 0.001$), and duodenal ulcer (93 males and 40 females, male/female = 2.33, $p < 0.001$) (Table 1). The prevalence of *H. pylori* infection among patients with gastric ulcer (53.4%, $p = 0.004$) and duodenal ulcer (63.9%, $p < 0.001$) were significantly higher than that of the normal group (Table 1). The frequencies of cytokine genotypes in gastroduodenal diseases are shown in Table 2.

IL-1β allele frequencies were associated with increased risk of the *development of GERD*

We first evaluated the frequency of a given gene polymorphism between different groups. As seen in Table 3, the frequency of IL-1β-511 TT genotype was significantly higher in the GERD group (23.1%) than in the control group (15.3%) (OR, 1.384; 95% CI, 1.023-1.871; $P = 0.034$). The frequency of IL-1β-31 CC genotype was significantly higher in the GERD group (24.0%) than in the control group (15.9%) (OR, 1.388; 95% CI, 1.028-1.873; $P=0.031$, Table 3).

Of *H. pylori*-positive patients, the frequency of IL-1β-511 TT genotype was significantly higher in the GERD group (27.2%) than in the control group (17.1%) (OR, 1.522; 95% CI, 1.008-2.298; $P=0.044$, Table 4). The frequency of IL-1β-31 CC genotype was also higher in the GERD group (27.2%) than in controls (17.1%), but did not reach statistical significance (OR, 1.502; 95% CI, 0.993-2.270; $P=0.052$, Table 4). No significant differences were found for the frequency of the IL-1β-511 and -31 genotype between the peptic ulcer group and the control

group., For the other IL-1 β +3953 allele, group comparisons revealed no significant differences. .

IL-10 allele frequencies were associated with increased risk of the *development of gastritis*

The carriage of IL-10-819 C allele was significantly higher in patients with gastritis (63.0%) than in controls (51.1%) (OR, 1.626; 95% CI, 1.046-2.527; *P*=0.030, Table 5). The frequency of IL-10-819 CT genotype was also significantly higher in gastritis groups (52.6%) than in control groups (40.3%) (OR, 1.721; 95% CI, 1.084-2.733; *P*=0.021, Table 5). For the IL-10-592 polymorphism, the carriage of IL-10-592 C allele was significantly higher in patients with gastritis (63.0%) than in controls (50.6%) (OR, 1.664; 95% CI, 1.070-2.585; *P* = 0.023, Table 5). The frequency of IL-10-592 CA genotype was significantly higher in patients with gastritis (52.6%) than in control groups (39.8%) (OR, 1.766; 95% CI, 1.112-2.805; *P* = 0.016, Table 5).

Of *H. pylori*-negative patients, the carriage of IL-10-819 C allele was significantly higher in the gastritis group (65.6%) than in *H. pylori*-negative controls (49.5%) (OR, 1.938; 95% CI, 1.093-3.434; *P* = 0.023, Table 6). The frequency of IL-10-819 CT genotype was significantly higher in the gastritis group (54.4%) than in *H. pylori*-negative controls (38.7%) (OR, 2.059; 95% CI, 1.130-3.752; *P*=0.018, Table 6). The carriage of IL-10-592 C allele was significantly higher in gastritis groups (65.6%) than in *H. pylori*-negative control groups (49.5%) (OR, 1.938; 95% CI, 1.093-3.434; *P*=0.023, Table 6). The frequency of IL-10-592 CA genotype was significantly higher in gastritis groups (54.4%) than in H. pylori-negative control groups (38.7%) (OR, 2.059; 95% CI, 1.130-3.752; *P*=0.018, Table 6). There were no significantly differences in the frequencies of IL-10-819 and -592 genotype between GERD groups, peptic ulcer groups and control groups.

IL-8 and TNF-α allele frequencies had no association in gastro-intestinal diseases

There were no significant differences in the frequencies of IL-8-251 and TNF- α -308 genotype between all disease groups and the control group. However, the frequency of IL-8-251 AT genotype was higher in H. pylori-negative GERD groups (53.5%) than in H. pylori-positive GERD group (40.7%) (OR, 0.564; 95% CI, 0.308-1.033; *P* = 0.063).

Conclusion

In summary, our results showed that the frequencies of IL-1 β -511 CT and -31 CT genotype were associated with increased risk of the development of GERD. The frequencies of the genotypes that have higher production of IL-10 (IL-10-819 CC/CT alleles and IL-10-592 CC/CA alleles) were associated with increased risk of the development of gastritis. Our results together suggest that the higher-production IL-10 alleles were associated with increased risk for such disorders.

Experimental procedures

Study subjects

Patients who have routine physical examinations and completed a self-administered questionnaire with a clinical diagnosis of upper gastrointestinal endoscopies are analyzed in this study. The severity of reflux esophagitis is evaluated using the Los Angeles grading system (53,54). Hiatus hernia was diagnosed by the presence of gastric wall above the diaphragmatic hiatus as previously described (55). Barrett's esophagus is defined as the presence of a columnar epithelium lining the lower esophagus for more than 3 cm (56).

Patients are excluded from the investigation if they (i) had previous esophageal or gastric surgery, (ii) had esophageal or gastric cancer, (iii) refused endoscopy, and (iv) concomitantly took proton pump inhibitors, or have prior antibiotic therapy for *H. pylori*. Demographic factors of the patients included age, age group (> 70 yrs), gender, and concurrent medical conditions including diabetes mellitus. *H. pylori* status is assessed by histology (hematoxylin and eosin staining or Giemsa staining), rapid urease test on biopsies with Pronto Dry (Medical Inst. Corp., Solothurn, Switzerland) and bacterial culture. A patient is considered *H. pylori*-positive if one or more of the diagnostic methods applied were positive, and *H. pylori*-negative if all the applied methods are negative. Blood samples are collected.

Genotyping

The polymorphism at position -511, -31 and +3953 of the IL-1β gene and -1082, -819 and -592 of the IL-10 gene and -251 of the IL-8 gene and -308 of the TNF-α gene are genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method (57).

The IL-1β-511 PCR products were digested with *Ava*I at 37°C for 12 h. The genotypes were designated as follows: C/C, two bands of 114 and 190 bp; C/T, three bands of 114, 190 and 304 bp; and T/T, a single band of 304 bp(57). The IL-1β-31 PCR products were digested with *Alu*I at 37°C for 12 h. The genotypes were designated as follows: T/T, two bands of 102 and 137 bp; C/T, three bands of 102, 137 and 239 bp; and C/C, a single band of 239 bp(34). The IL-1β+3953 PCR products were digested with *Taq*$^{\alpha}$I at 65°C for 6 h. The genotypes were designated as follows: C/C, two bands of 85 and 97 bp; C/T, three bands of 85, 97 and 182 bp; and T/T, a single band of 182 bp(57).

For IL-10 typing, the IL-10-1082 PCR products were digested with *Bse*RI at 37°C for 12 h. The genotypes were designated as follows: G/G, two bands of 40 and 320 bp; G/A, three bands of 40, 320 and 360 bp; and A/A, a single band of 360 bp(58). The IL-10-819 PCR products were digested with *Mae*III at 55°C for 6 h. The genotypes were designated as follows: C/C, three bands of 79, 217 and 292 bp; C/T, four bands of 79, 217, 292, and 509 bp; and T/T, two bands of 79 and 509 bp(59). The IL-10-592 PCR products were digested with *Rsa*I at 37°C for 12 h. The genotypes were designated as follows: C/C, a single band of 302 bp; C/A, two bands of 240 and

302 bp; and A/A, a single band of 302 bp(43).

The PCR conditions used to determine IL-1β-511, IL-1β-31, IL-1β+3953, IL-10-1082, IL-10-592 and IL-8-251 polymorphisms were as follows: 94°C for 6 min, then 28 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min. The PCR conditions used to determine IL-10-819 polymorphism were as follows: 94°C for 6 min, then 35 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min. The PCR conditions used to determine TNF-a-308 polymorphism were as follows: 94°C for 6 min, then 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min. The IL-8-251 PCR products were digested with *Mfe*I at 37°C for 12 h. The genotypes were designated as follows: A/A, two bands of 296 and 520 bp; A/T, three bands of 296, 520 and 816 bp; and T/T, a single band of 816 bp(43). The TNF-α-308 PCR products were digested with *Nco*I at 37°C for 12 h. The genotypes were designated as follows: G/G, a single band of 151 bp; G/A, two bands of 151 and 177 bp; and A/A, a single band of 177 bp(43).

Statistical analysis

The role of the cytokine genotypes in the genesis of various diseases is evaluated before and after stratification by *H. pylori* status in logistic regression models controlling for confounding factors. The relationship of between-group comparisons was performed using the χ^2 test and Fisher's exact test ($n < 5$). Differences in age mean between the two groups were compared using the mann-Whitney U test. Univariate analysis followed by multiple regression analysis is performed to evaluate whether any of associated factors (including gender and *H. pylori* infection) affected the severity of diseases. Statistical analysis is performed with the SPSS (version 13.0, SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

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Table 1. Characteristics of the study subjects

Characteristics	**Healthy control**	**Esophaitis**	**Gastritis**	**Gastric ulcer**	**Duodenal ulcer**
No. patients	176	208	154	133	133
mean age ± SD	47.7 ± 12.7	50.6 ± 11.5	53.5 ± 12.3[a]	53.7 ± 11.6[b]	53.6 ± 11.3[c]
Gender (male/female)	71/105	150/58[d]	84/70[e]	82/51[f]	93/40[g]
The prevalence of *H. pylori* infection	36.9%	38.9%	41.6%	53.4%[h]	63.2%[i]

[a]age compared between the gastritis group and control group, $p < 0.001$

[b]age compared between the gastric ulcer group and control group, $p < 0.001$

[c]age compared between the duodenal ulcer group and control group, $p < 0.001$

[d]gender compared between the esophaitis group and control group, $p < 0.001$

[e]gender compared between the gastritis group and control group, $p = 0.007$

[f]gender compared between the gastric ulcer group and control group, $p < 0.001$

[g]gender compared between the duodenal ulcer group and control group, $p < 0.001$

[h]the prevalence of *H. pylori* infection compare between the gastric ulcer group and control group, $p = 0.004$

[i]the prevalence of *H. pylori* infection compare between the duodenal ulcer group and control group, $p < 0.001$

Table 2. The frequencies of cytokine genotypes in gastro-intestinal diseases

	HC[a]		GERD[b]		GS[c]		GU[d]		DU[e]	
Genotype	n	%	n	%	n	%	n	%	n	%
IL-1b-511										
CC	56	31.8	52	25.0	47	30.5	42	31.6	36	27.1
CT	93	52.8	108	51.9	76	49.4	62	46.6	75	56.4
TT	27	15.3	48	23.1	31	20.1	29	21.8	22	16.5
T allele carrier	120	68.2	156	75.0	107	69.5	91	68.4	97	72.9
IL-1b-31										
TT	55	31.3	51	24.5	45	29.2	42	31.6	35	26.3
CT	93	52.8	107	51.4	78	50.6	62	46.6	76	57.1
CC	28	15.9	50	24.0	31	20.1	29	21.8	22	16.5
C allele carrier	121	68.8	157	75.5	109	70.8	91	68.4	98	73.7
IL-1b+3953										
CC	169	96.0	199	95.7	147	94.8	128	96.2	128	96.2
CT	7	4.0	9	4.3	7	4.5	5	3.8	5	3.8
TT	0	0.0	0	0.0	1	0.6	0	0.0	0	0.0
T allele carrier	7	4.0	9	4.3	8	5.2	5	3.8	5	3.8
IL-10-1082										
AA	162	92.0	188	90.4	133	86.4	123	92.5	123	92.5
AG	14	8.0	20	9.6	21	13.6	9	6.8	10	7.5
GG	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0
G allele carrier	14	8.0	20	9.6	21	13.6	10	7.5	10	7.5
IL-10-819										
TT	86	48.9	92	44.2	57	37.0	64	48.1	58	43.6
CT	71	40.3	92	44.2	81	52.6	56	42.1	54	40.6
CC	19	10.8	24	11.5	16	10.4	13	9.8	21	15.8
C allele carrier	90	51.1	116	55.8	97	63.0	69	51.9	75	56.4
IL-10-592										
AA	87	49.4	92	44.2	57	37.0	64	48.1	58	43.6
CA	70	39.8	93	44.7	81	52.6	56	42.1	54	40.6
CC	19	10.8	23	11.1	16	10.4	13	9.8	21	15.8
C allele carrier	89	50.6	116	55.8	97	63.0	69	51.9	75	56.4
IL-8-251										
TT	64	36.4	80	38.5	52	33.8	46	34.6	48	36.1
AT	86	48.9	101	48.6	79	51.3	63	47.4	65	48.9
AA	26	14.8	27	13.0	23	14.9	24	18.0	20	15.0
A allele carrier	112	63.6	128	61.5	102	66.2	87	65.4	85	63.9
TNF-a-308										
GG	142	80.7	168	80.8	125	81.2	111	83.5	114	85.7
AG	31	17.6	37	17.8	28	18.2	21	15.8	17	12.8

AA	3	1.7	3	1.4	1	0.6	1	0.8	2	1.5
A allele carrier	34	19.3	40	19.2	29	18.8	22	16.5	19	14.3

[a]healthy control; [b]esophaitis; [c]gastritis; [d]gastric ulcer; [e]duodenal ulcer

Table 3. Risks of cytokine genotype frequencies for the development of GERD

	HC		GERD				
Genotype	n	%	n	%	OR	95 % CI	*P*-value
IL-1b-511							
CC	56	31.8	52	25.0	1.000		
CT	93	52.8	108	51.9	1.251	0.783-1.998	0.349
TT	27	15.3	48	23.1	1.384	1.023-1.871	**0.034**
T allele carrier	120	68.2	156	75.0	1.400	0.896-2.187	0.139
IL-1b-31							
TT	55	31.3	51	24.5	1.000		
CT	93	52.8	107	51.4	1.241	0.774-1.988	0.370
CC	28	15.9	50	24.0	1.388	1.028-1.873	**0.031**
C allele carrier	121	68.8	157	75.5	1.399	0.893-2.192	0.142
IL-1b+3953							
CC	169	96.0	199	95.7	1.000		
CT	7	4.0	9	4.3	1.092	0.398-2.994	0.864
TT	0	0.0	0	0.0			
T allele carrier	7	4.0	9	4.3	1.092	0.398-2.994	0.864
IL-10-1082							
AA	162	92.0	188	90.4	1.000		
AG	14	8.0	20	9.6	1.231	0.602-2.515	0.568
GG	0	0.0	0	0.0			
G allele carrier	14	8.0	20	9.6	1.231	0.602-2.515	0.568
IL-10-819							
TT	86	48.9	92	44.2	1.000		
CT	71	40.3	92	44.2	1.211	0.790-1.856	0.379
CC	19	10.8	24	11.5	1.087	0.777-1.519	0.627
C allele carrier	90	51.1	116	55.8	1.205	0.805-1.802	0.364
IL-10-592							
AA	87	49.4	92	44.2	1.000		
CA	70	39.8	93	44.7	1.256	0.820-1.925	0.294
CC	19	10.8	23	11.1	1.070	0.764-1.499	0.694
C allele carrier	89	50.6	116	55.8	1.233	0.824-1.844	0.309
IL-8-251							
TT	64	36.4	80	38.5	1.000		
AT	86	48.9	101	48.6	0.940	0.607-1.454	0.780
AA	26	14.8	27	13.0	0.911	0.665-1.250	0.564
A allele carrier	112	63.6	128	61.5	0.914	0.604-1.385	0.672
TNF-a-308							
GG	142	80.7	168	80.8	1.000		
AG	31	17.6	37	17.8	1.009	0.596-1.709	0.974
AA	3	1.7	3	1.4	0.919	0.410-2.062	1.000[a]
A allele carrier	34	19.3	40	19.2	0.994	0.598-1.654	0.983

[a]calculated by Fisher's exact test

Table 4. Risks of cytokine genotype frequencies in *H. pylori*-negative control and *H. pylori*-positive GERD

	HC-N[a]		GERD				
Genotype	n	%	n	%	OR	95 % CI	*P*-value
IL-1b-511							
CC	38	34.2	19	23.5	1.000		
CT	54	48.6	40	49.4	1.481	0.746-2.942	0.260
TT	19	17.1	22	27.2	1.522	1.008-2.298	**0.044**
T allele carrier	73	65.8	62	76.5	1.699	0.890-3.242	0.106
IL-1b-31							
TT	37	33.3	19	23.5	1.000		
CT	55	49.5	40	49.4	1.416	0.713-2.815	0.320
CC	19	17.1	22	27.2	1.502	0.993-2.270	0.052
C allele carrier	74	66.7	62	76.5	1.632	0.853-3.119	0.137
IL-1b+3953							
CC	107	96.4	81	100.0			
CT	4	3.6	0	0.0			
TT	0	0.0	0	0.0			
T allele carrier	4	3.6	0	0.0			
IL-10-1082							
AA	102	91.9	72	88.9	1.000		
AG	9	8.1	9	11.1	1.417	0.536-3.744	0.481
GG	0	0.0	0	0.0			
G allele carrier	9	8.1	9	11.1	1.417	0.536-3.744	0.481
IL-10-819							
TT	56	50.5	40	49.4	1.000		
CT	43	38.7	35	43.2	1.140	0.623-2.083	0.671
CC	12	10.8	6	7.4	0.837	0.492-1.422	0.508
C allele carrier	55	49.5	41	50.6	1.044	0.589-1.851	0.884
IL-10-592							
AA	56	50.5	40	49.4	1.000		
CA	43	38.7	35	43.2	1.140	0.623-2.083	0.671
CC	12	10.8	6	7.4	0.837	0.492-1.422	0.508
C allele carrier	55	49.5	41	50.6	1.044	0.589-1.851	0.884
IL-8-251							
TT	37	33.3	37	45.7	1.000		
AT	59	53.2	33	40.7	0.559	0.300-1.044	0.067
AA	15	13.5	11	13.6	0.856	0.546-1.344	0.499
A allele carrier	74	66.7	44	54.3	0.595	0.330-1.072	0.083
TNF-a-308							
GG	91	82.0	63	77.8	1.000		

AG	19	17.1	17	21.0	1.292	0.623-2.679	0.490
AA	1	0.9	1	1.2	1.202	0.298-4.850	1.000[b]
A allele carrier	20	18.0	18	22.2	1.300	0.637-2.653	0.470

[a]*H. pylori*-negative healthy control

[b]calculated by Fisher's exact test

Table 5. Risks of cytokine genotype frequencies for the development of GS

	HC		GS				
Genotype	n	%	n	%	OR	95 % CI	*P*-value
IL-1b-511							
CC	56	31.8	47	30.5	1.000		
CT	93	52.8	76	49.4	0.974	0.595-1.593	0.915
TT	27	15.3	31	20.1	1.170	0.847-1.615	0.341
T allele carrier	120	68.2	107	69.5	1.062	0.666-1.695	0.799
IL-1b-31							
TT	55	31.3	45	29.2	1.000		
CT	93	52.8	78	50.6	1.025	0.624-1.683	0.922
CC	28	15.9	31	20.1	1.163	0.842-1.606	0.358
C allele carrier	121	68.8	109	70.8	1.101	0.687-1.764	0.689
IL-1b+3953							
CC	169	96.0	147	94.8	1.000		
CT	7	4.0	7	4.5	1.158	0.397-3.377	0.789
TT	0	0.0	1	0.6			
T allele carrier	7	4.0	8	5.2	1.323	0.468-3.736	0.596
IL-10-1082							
AA	162	92.0	133	86.4	1.000		
AG	14	8.0	21	13.6	1.827	0.895-3.731	0.094
GG	0	0.0	0	0.0			
G allele carrier	14	8.0	21	13.6	1.827	0.895-3.731	0.094
IL-10-819							
TT	86	48.9	57	37.0	1.000		
CT	71	40.3	81	52.6	1.721	1.084-2.733	**0.021**
CC	19	10.8	16	10.4	1.127	0.777-1.636	0.528
C allele carrier	90	51.1	97	63.0	1.626	1.046-2.527	**0.030**
IL-10-592							
AA	87	49.4	57	37.0	1.000		
CA	70	39.8	81	52.6	1.766	1.112-2.805	**0.016**
CC	19	10.8	16	10.4	1.134	0.781-1.645	0.508
C allele carrier	89	50.6	97	63.0	1.664	1.070-2.585	**0.023**
IL-8-251							
TT	64	36.4	52	33.8	1.000		
AT	86	48.9	79	51.3	1.131	0.702-1.821	0.614
AA	26	14.8	23	14.9	1.043	0.746-1.459	0.803
A allele carrier	112	63.6	102	66.2	1.121	0.712-1.764	0.622
TNF-a-308							
GG	142	80.7	125	81.2	1.000		
AG	31	17.6	28	18.2	1.026	0.583-1.805	0.929
AA	3	1.7	1	0.6	0.613	0.197-1.913	0.626[a]
A allele carrier	34	19.3	29	18.8	0.969	0.559-1.681	0.911

[a]calculated by Fisher's exact test

Table 6. Risks of cytokine genotype frequencies in *H. pylori*-negative control and GS

	HC-N		GS				
Genotype	n	%	n	%	OR	95 % CI	*P*-value
IL-1b-511							
CC	38	34.2	29	32.2	1.000		
CT	54	48.6	47	52.2	1.140	0.613-2.124	0.678
TT	19	17.1	14	15.6	0.983	0.645-1.498	0.935
T allele carrier	73	65.8	61	67.8	1.095	0.606-1.977	0.763
IL-1b-31							
TT	37	33.3	27	30.0	1.000		
CT	55	49.5	49	54.4	1.221	0.652-2.288	0.533
CC	19	17.1	14	15.6	1.005	0.657-1.537	0.982
C allele carrier	74	66.7	63	70.0	1.167	0.641-2.124	0.614
IL-1b+3953							
CC	107	96.4	84	93.3	1.000		
CT	4	3.6	5	5.6	1.592	0.415-6.114	0.515[a]
TT	0	0.0	1	1.1			
T allele carrier	4	3.6	6	6.7	1.911	0.522-6.990	0.348
IL-10-1082							
AA	102	91.9	77	85.6	1.000		
AG	9	8.1	13	14.4	1.913	0.778-4.706	0.152
GG	0	0.0	0	0.0			
G allele carrier	9	8.1	13	14.4	1.913	0.778-4.706	0.152
IL-10-819							
TT	56	50.5	31	34.4	1.000		
CT	43	38.7	49	54.4	2.059	1.130-3.752	**0.018**
CC	12	10.8	10	11.1	1.227	0.764-1.970	0.396
C allele carrier	55	49.5	59	65.6	1.938	1.093-3.434	**0.023**
IL-10-592							
AA	56	50.5	31	34.4	1.000		
CA	43	38.7	49	54.4	2.059	1.130-3.752	**0.018**
CC	12	10.8	10	11.1	1.227	0.764-1.970	0.396
C allele carrier	55	49.5	59	65.6	1.938	1.093-3.434	**0.023**
IL-8-251							
TT	37	33.3	32	35.6	1.000		
AT	59	53.2	45	50.0	0.882	0.478-1.626	0.687
AA	15	13.5	13	14.4	1.001	0.645-1.555	0.996
A allele carrier	74	66.7	58	64.4	0.906	0.505-1.626	0.741
TNF-a-308							
GG	91	82.0	71	78.9	1.000		
AG	19	17.1	18	20.0	1.214	0.594-2.483	0.595
AA	1	0.9	1	1.1	1.132	0.281-4.566	1.000[a]
A allele carrier	20	18.0	19	21.1	1.218	0.604-2.453	0.581

[a]calculated by Fisher's exact test