Lack of association between gene polymorphisms of Angiotensin converting enzyme, Nod-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of Helicobacter pylori-induced premalignant gastric lesions and gastric cancer in Caucasians

Juozas Kupcinskas†, Thomas Wex‡*, Jan Bornschein2, Michael Selgrad2, Marcis Leja3, Elona Juozaityte4, Gediminas Kiudelis1, Laimas Jonaitis1 and Peter Malfertheiner2

Abstract

Background: Several polymorphisms of genes involved in the immunological recognition of Helicobacter pylori and regulating apoptosis and proliferation have been linked to gastric carcinogenesis, however reported data are partially conflicting. The aim of our study was to evaluate potential associations between the presence of gastric cancer (GC) and high risk atrophic gastritis (HRAG) and polymorphisms of genes encoding Angiotensin converting enzyme (ACE), Nod-like receptor 1 (NOD1), Toll-like receptor 4 (TLR4) and FAS/FASL.

Methods: Gene polymorphisms were analyzed in 574 subjects (GC: n = 114; HRAG: n = 222, controls: n = 238) of Caucasian origin. ACE I/D (rs4646994), NOD1 796G>A (rs5743336), TLR4 3725G>C (rs11536889), FAS 1377G>A (rs2234767), FAS 670A>G (rs1800682) and FASL 844T>C (rs763110) were genotyped by different PCR approaches and restriction fragment length polymorphism analysis.

Results: Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. There was a tendency for NOD1 796G/G genotype to be associated with increased risk of HRAG (62.4% vs 54.5% in controls, p = 0.082). FAS 670G/G genotype was more frequent in HRAG when compared to controls, 23.9% and 17.2% respectively, however it failed to reach significance level (p = 0.077). We did not find any significant associations for all polymorphisms in relation to GC or HRAG. NOD1 796G>A and TLR4 3725G>C gene polymorphisms were also not associated with Helicobacter pylori infection.

Conclusions: ACE, NOD1, TLR4 and FAS/FASL gene polymorphisms are not linked with gastric carcinogenesis in Caucasians, and therefore they should not be considered as potential biomarkers for identifying individuals with higher risk for GC.
Background

Based on the current paradigm, gastric carcinogenesis is considered as multistep process involving complex interplay between Helicobacter pylori (H. pylori) infection, environmental and host genetic factors [1]. In gastric carcinogenesis two distinct pathways have been suggested for intestinal and diffuse type of gastric cancer (GC) [1]. Correa et al. [2] demonstrated that H. pylori infection induces chronic inflammation of the gastric mucosa, leading to atrophic gastritis (AG) and intestinal metaplasia (IM), which are regarded as essential predisposing factors in intestinal-type GC development. Many researchers have shown a close relationship between H. pylori not only for intestinal-type GC, but also for diffuse-type GC [3,4]. Furthermore, although AG and IM are considered as obligatory predisposing factors in intestinal-type GC, the accumulating evidence suggests that AG and IM are at least partially associated with GC of the diffuse-type as well [3]. Up to date, no molecular screening methods are available for sporadic GC, therefore pathogenetic mechanisms involved in different stages of gastric carcinogenesis need to be further elucidated to allow identification of individuals with increased risk for GC. Recent studies have revealed several genetic polymorphisms related to immune-recognition of H. pylori, cell proliferation, and apoptosis pathways that were linked with premalignant gastric conditions and GC, but further research is needed to establish their role in gastric carcinogenesis [5].

Angiotensin I-converting enzyme (ACE) is expressed by different cell types [6] and generates Angiotensin II, which is a key effector in renin-angiotensin system. Recent reports provided evidence that Angiotensin II type 1 receptor is expressed by tumor and endothelial cells [7-9]. Insertion (I) or deletion (D) polymorphism of ACE gene was shown to have functional relevance, since the carriers of D allele have higher ACE activity [10]. ACE I/D polymorphism was linked with early development and spread of GC [11], but the studies report null association with the overall risk for GC [12,13]. ACE I/D polymorphism was not linked with gastric atrophy in Asian subjects [14]. There are however no reports that evaluate the role ACE I/D polymorphism in premalignant gastric conditions in Caucasians and studies addressing the role of ACE I/D polymorphisms in gastric carcinogenesis are limited.

Nucleotide-binding oligomerisation domain 1 (NOD1) is a member of the Nod-like receptors, which is expressed in the cytoplasm of antigen presenting cells and gastric epithelial cells and is involved in recognition of gram-negative bacteria [15]. It is known that stimulation of gastric epithelial cells with NOD1 ligands leads to production of proinflammatory cytokines [16,17] and NOD1 participates in host defense against mucosal infection with H. pylori infection [17,18]. Recently NOD1 was found to respond to peptidoglycan delivered by H. pylori cagPAI [19]. Given the significant role of H. pylori in gastric carcinogenesis, it is hypothesized that genetic variations in gene encoding NOD1 receptor could be related to different outcomes. Gene polymorphism 796G>A of NOD1 has been linked with peptic ulcer disease in H. pylori-positive patients [20] and a significant association with very high odds ratios has been recently reported for the risk of premalignant lesions in the antrum of the stomach [21]. Up to date, the data on NOD1 796G>A gene polymorphism remain very scarce and there are no reports concerning the role of this polymorphism in patients with GC.

Another important receptor for the recognition of H. pylori is Toll-like receptor 4 (TLR4), a member of Toll receptors family. TLR4 recognizes lipopolysaccharide of gram-negative bacteria, and it is thought to interact with macrophage/monocyte in response to H. pylori infection [22]. Expression of TLR4 by gastric epithelium is upregulated in H. pylori-induced gastritis compared to non-inflamed gastric mucosa [23]. Single nucleotide polymorphisms (SNPs) of TLR4 gene are thought to disrupt the normal structure of the extracellular region of the TLR4 and are therefore hypothesized to decrease responsiveness to lipopolysaccharide through alterations in binding. TLR4 896A>G polymorphism was found to be associated with higher risk of H. pylori-induced GC and its precursors [24]. Recently, a novel polymorphism in TLR4 gene 3725G>C (rs11536889) with functional relevance was identified [25] and linked with severe gastric atrophy in Asian population [26]. This is however the only report on TLR4 3725G>C polymorphism and the potential link with premalignant gastric lesions or GC has not been evaluated yet.

The role of apoptosis in tumor genesis has been well established [27]. The FAS and FASL system plays an important role in regulating apoptotic cell death, initiating the extrinsic pathway of apoptosis [28]. Decreased expression levels of FAS and FASL are associated with different malignancies as well as the progression of gastric carcinoma [29]. Several single nucleotide polymorphisms (SNPs) FAS 1377G>A (rs2234767), FAS 670A>G (rs1800682) and FASL 844T>C (rs763110) were shown to affect gene expression [30,31]. Liu et al. showed an association between these FAS/FASL gene polymorphisms and higher risk of GC [32], while other studies reported null associations [33,34]. These polymorphisms were also associated with the risk of atrophic gastritis [35], however the data published on the association with risk for GC are still scarce and there are no reports on the role FAS/FASL polymorphisms with respect to GC in Caucasians.
Given the pathophysiological significance of ACE, NOD1, TLR4, FAS and FASL genes in gastric carcinogenesis, it is intriguing to assess the role of these polymorphisms for the development of GC and H. pylori-associated premalignant gastric lesions. Therefore, the aim of our study was to evaluate potential links between the presence of GC or HRAG and ACE I/D, NOD1 796G>A, TLR4 3725G>C, FAS 1377G>A, FAS 670A>G and FASL 844T>C gene polymorphisms in 114 patients with GC, 222 patients with HRAG and 238 controls of Caucasian origin. This study provides further insights in the puzzle of genetic susceptibility for GC and its precursors.

Methods

Study population
Subjects included in the study came from our previous research groups on IL-1B, IL-1RN and NOD2 gene polymorphisms [36-38]. Patients were recruited at three gastroenterological centers in Germany, Lithuania and Latvia. In Germany, patients with GC, HRAG and healthy controls were recruited from the Department of Gastroenterology of the Otto-von-Guericke University Magdeburg between 1998 and 2008. Patients with HRAG were included from the Out-patient Department, and from a clinical study aimed at the long-term follow up of H. pylori infection. Controls were recruited from clinical studies with healthy volunteers and subjects from the Out-patient Department with dyspeptic symptoms.

In Lithuania and Latvia, patients with HRAG and controls were included from the Out-patient Departments of University Hospitals in Kaunas and Riga. All the individuals were referred for upper endoscopy because of dyspeptic symptoms during the period of 2005-2006. The inclusion criteria of HRAG and controls were no history of malignancy, gastrointestinal disease or surgery. GC was determined by histology. From all participants, DNA for genotyping was available. In total, 346 individuals (114 GC, 140 HRAG, 92 controls) from Germany and 228 individuals from Lithuania and Latvia (82 HRAG, 146 controls) were included. All patients were of Caucasian ethnicity. The study was approved by the Ethics Committees of the OvG University Magdeburg, Lithuanian University of Health Sciences and University of Latvia, and informed consent to participate in the study was obtained from all subjects included.

Histological analysis and H. pylori status
Scoring of atrophic gastritis and intestinal metaplasia was done according to the modified Sydney classification [39]. Histological evaluation for GC type was carried out according to the Laurén classification [40]. HRAG was defined as pan-gastritis (similar inflammatory scores in antrum and corpus), corpus-predominant gastritis with or without the presence of gastric atrophy, and intestinal metaplasia either in antrum or corpus as described by Uemura et al. and Meining et al. [41,42]. H. pylori status was determined by testing for anti-H. pylori IgG antibodies in sera.

Genotyping
Genomic DNA was extracted from peripheral blood mononuclear cells using the QiAamp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

ACE I/D
The ACE I/D genotype (rs4646994) was determined by PCR using primers: forward 5′-CTGGAGACCACCCATCCCTTCT-3′ and reverse, 5′-GATGTGGCCATCACATTCGTCAGAT-3′. An initial 15 minute denaturation at 95° was followed by 40 cycles of 1 min at 64°C, 1 min at 72°C, and 35 s at 95.5°C. Amplified ACE gene polymorphisms were separated on 1.0% agarose gels, and visualized by ethidium bromide staining. D and I alleles were determined by the presence of 190 or 490 bp fragments (Figure 1).

![Figure 1](https://www.biomedcentral.com/1471-2350/12/112)
The NOD1 796G>A SNP (rs5743336) was analyzed by PCR restriction fragment length polymorphism analysis (PCR-RFLP) with the following primers: forward 5'-TGA GAC CAT CTT CAT CCT GG-3' and reverse 5'-CTT CCC ACT GAG CAG GTT G-3'. An initial 15 minute denaturation at 95° was followed by 40 cycles of 1 min at 64°C, 1 min at 72°C, and 35 s at 95.5°C. For RFLP analysis, the PCR products were digested with AvaI restriction enzyme (Fermentas, Vilnius, Lithuania) at 37°C overnight, studied by gel electrophoresis on a 2% agarose gel and visualized with ethidium bromide staining. The presence of G allele was indicated by cleavage of the 379 bp amplified PCR product to yield fragments of 209 bp and 170 bp (Figure 1).

The TLR4 3725G>C SNP (rs11536889) was genotyped using confronting two-pair primers PCR (PCR-CTPP). The primers were F1: TTT GAT GGA CCT CTG AAT CTC, R1: TTT TCT CAA TGA TAA CAT CCA CTG, F2: CTT GAC CAC ATT TTG GGA AC, and R2: TTC CAA TTC TCT TAT ATC CTT GAT GA. An initial 15 minute denaturation at 95° was followed by 40 cycles of 1 min at 64°C, 1 min at 72°C, and 35 s at 95.5°C. The amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining. The amplified DNA was 184 bp for G allele, 256 bp for C allele, and 397 bp for common band (Figure 1).

SNPs of FAS 1377G>A (rs2234767), FAS 670A>G (rs1800682) and FASL 844T>C (rs763110) were genotyped by using predesigned TaqMan assays with a BioRad CFX96™ real-time cycler, in accordance with the manufacturer’s instructions (Bio-Rad Laboratories Inc, Hercules, USA). Thermal cycling conditions for polymerase chain reaction (PCR) were, first, denaturing at 95°C for 10 min, followed by 40 cycles of 95.5°C for 15 s and 60°C for 1 min (Figure 2).

Concordance of genotype frequencies with the Hardy-Weinberg equilibrium was tested by online software http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Age is shown as means and standard deviations, and was compared using ANOVA and unpaired Student’s t-test. Categorical data (e.g. gender, distribution of genotypes or alleles) are presented as frequencies; comparisons were performed using the Chi-square test. Association between HRAG and GC with gene polymorphisms were calculated using unconditional multiple logistic regression analysis and expressed as odds ratios (OR) with 95% confidence intervals (CI). The ORs presented in the study were not adjusted for confounding factors. The relative risks for mutations were studied using recessive and dominant model that led to a comparison between wildtype+heterozygous vs. homozygous and wildtype vs. heterozygous+homozygous, respectively. Statistical analysis was done using statistical software SPSS Version 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The characteristics of the study groups are presented in Table 1. German and Baltic study groups did not differ significantly according to the allele distribution (data not shown), therefore the respective groups with HRAG as well as controls were combined and further analyzed together. Patients differed significantly according to age and gender distribution. Male made up for almost 2/3 of patients with GC, while both other groups demonstrated inverse findings. Furthermore, controls were significantly younger (6 or 9 years) than both other groups (Table 1). Notably, serological H. pylori status were

| Table 1 Characteristics of groups |
|-----------------------------------|
|                                | Controls (n = 238) | GC (n = 114) | HRAG (n = 222) | ANOVA (Age) Chi-squared test p value |
| Age Mean ± SD                  | 56.9 ± 16.1        | 65.51 ± 13.4 | 63.4 ± 10.4 | < 0.001 |
| Gender                          |                  |               |               |                          |
| Male                            | 79 (33.2%)        | 77 (67.5%)    | 87 (39.2%)    | < 0.001 |
| Female                          | 159 (66.8%)       | 37 (32.5%)    | 135 (60.8%)   |          |
| H. pylori                        |                  |               |               |                          |
| Positive                        | 141 (59.2%)       | 70 (61.4%)    | 133 (59.9%)   | 0.928   |
| Negative                        | 97 (40.8%)        | 44 (38.6%)    | 89 (40.1%)    |          |

Statistical analysis was performed globally for all three groups. GC, gastric cancer; HRAG, high risk atrophic gastritis

at 95°C for 10 min, followed by 40 cycles of 95.5°C for 15 s and 60°C for 1 min (Figure 2).
similar among the three groups, the proportion of *H. pylori*-positive subjects was between 59-62%, and did not differ significantly.

All individuals were successfully genotyped for ACE I/D, FAS 1377G>A, FAS 670A>G and FASL 844T>C polymorphisms. Four subjects were not genotyped for NOD1 796G>A polymorphism and five subjects for TLR4 3725G>C polymorphism. The genotype distributions for all six polymorphisms in control, HRAG and GC groups were similar to those expected for Hardy-Weinberg equilibrium.

**Table 2** Association of ACE, NOD1, TLR4, FAS and FASL gene polymorphisms with gastric cancer and high risk atrophic gastritis

| Genotypes       | Controls (n = 238) | GC (n = 114) | HRAG (n = 222) |
|-----------------|--------------------|--------------|----------------|
|                 | n (%)              | OR (95% CI)  | p              | n (%)              | OR (95% CI)  | p              |
| ACE I/D         |                    |              |                |                    |              |                |
| I/I             | 62 (26.1)          | 0.88 (0.52-1.48) | 0.632 | 62 (27.9)          | 1.10 (0.73-1.66) | 0.650 |
| I/D             | 110 (46.2)         | 1.25 (0.79-1.95) | 0.331 | 108 (48.6)         | 1.10 (0.76-1.59) | 0.602 |
| D/D             | 66 (27.7)          | 0.85 (0.51-1.42) | 0.529 | 52 (23.4)          | 0.79 (0.52-1.21) | 0.290 |
| Allele I        | 234 (49.2)         | 1.02 (0.74-1.39) | 0.921 | 232 (52.3)         | 1.13 (0.87-1.47) | 0.348 |
| Allele D        | 242 (50.8)         | 0.98 (0.72-1.35) | 0.921 | 212 (47.7)         | 0.88 (0.68-1.15) | 0.348 |
| NOD1 796G>A     |                    |              |                |                    |              |                |
| G/G             | 129 (54.4)         | 1.00 (0.64-1.57) | 0.995 | 138 (62.4)         | 1.39 (0.96-2.02) | 0.082 |
| G/A             | 85 (35.9)          | 0.99 (0.62-1.59) | 1.000 | 66 (29.9)          | 0.76 (0.52-1.3) | 0.172 |
| A/A             | 23 (9.7)           | 1.01 (0.48-2.16) | 0.973 | 17 (7.7)           | 0.78 (0.40-1.49) | 0.446 |
| Allele G        | 343 (72.4)         | 0.99 (0.70-1.42) | 0.991 | 342 (77.4)         | 1.31 (0.97-1.76) | 0.081 |
| Allele A        | 131 (27.6)         | 1.00 (0.70-1.73) | 0.991 | 100 (22.6)         | 0.77 (0.57-1.3) | 0.081 |
| TLR4 3725G>C    |                    |              |                |                    |              |                |
| G/G             | 190 (80.5)         | 0.95 (0.54-1.66) | 0.849 | 181 (82.3)         | 1.12 (0.70-1.80) | 0.629 |
| G/C             | 41 (17.4)          | 1.09 (0.61-1.94) | 0.782 | 33 (15.0)          | 0.84 (0.51-1.38) | 0.492 |
| C/C             | 5 (2.1)            | 0.83 (0.16-4.36) | 0.832 | 6 (2.7)            | 1.29 (0.39-4.31) | 0.672 |
| Allele G        | 421 (89.2)         | 0.97 (0.59-1.62) | 0.919 | 395 (89.8)         | 1.06 (0.69-1.62) | 0.776 |
| Allele C        | 51 (10.8)          | 1.03 (0.62-1.71) | 0.919 | 45 (10.2)          | 0.94 (0.62-1.44) | 0.776 |
| FAS 1377G>A     |                    |              |                |                    |              |                |
| G/G             | 197 (82.8)         | 1.04 (0.57-1.88) | 0.895 | 178 (80.2)         | 0.84 (0.52-1.35) | 0.471 |
| G/A             | 40 (16.8)          | 0.92 (0.51-1.70) | 0.809 | 41 (18.5)          | 1.12 (0.69-1.81) | 0.640 |
| A/A             | 1 (0.4)            | 2.09 (0.13-3.38) | 0.593 | 3 (1.4)            | 3.2 (0.33-3.14) | 0.282 |
| Allele G        | 434 (91.2)         | 1.00 (0.57-1.75) | 0.981 | 397 (89.4)         | 0.82 (0.52-1.27) | 0.366 |
| Allele A        | 42 (8.8)           | 0.99 (0.56-1.73) | 0.981 | 47 (10.6)          | 1.22 (0.79-1.90) | 0.366 |
| FAS 670A>G      |                    |              |                |                    |              |                |
| A/A             | 70 (29.4)          | 0.89 (0.54-1.47) | 0.666 | 68 (30.6)          | 1.06 (0.71-1.57) | 0.775 |
| A/G             | 127 (53.4)         | 1.04 (0.66-1.63) | 0.856 | 101 (45.5)         | 0.72 (0.51-1.05) | 0.091 |
| G/G             | 41 (17.2)          | 1.08 (0.60-1.94) | 0.783 | 53 (23.9)          | 1.50 (0.95-2.39) | 0.077 |
| Allele A        | 267 (56.1)         | 0.93 (0.68-1.28) | 0.699 | 237 (53.4)         | 0.89 (0.69-1.16) | 0.408 |
| Allele G        | 209 (43.9)         | 1.07 (0.78-1.47) | 0.699 | 207 (46.6)         | 1.12 (0.86-1.44) | 0.408 |
| FASL 844T>C     |                    |              |                |                    |              |                |
| T/T             | 124 (52.1)         | 0.86 (0.55-1.34) | 0.498 | 108 (48.6)         | 0.87 (0.60-1.25) | 0.459 |
| T/C             | 94 (39.5)          | 1.28 (0.81-2.01) | 0.275 | 91 (41.0)          | 1.06 (0.73-1.54) | 0.743 |
| C/C             | 20 (8.4)           | 0.71 (0.29-1.73) | 0.455 | 23 (10.4)          | 1.26 (0.67-2.36) | 0.471 |
| Allele T        | 342 (71.8)         | 0.96 (0.67-1.36) | 0.826 | 307 (69.1)         | 0.87 (0.66-1.17) | 0.368 |
| Allele C        | 134 (28.2)         | 1.04 (0.73-1.47) | 0.826 | 137 (30.9)         | 1.14 (0.86-1.51) | 0.368 |

GC, gastric cancer; HRAG, high risk atrophic gastritis; CI, confidence interval; OR, odds ratio. The ORs were calculated comparing each genotype vs. the other two genotypes, the first line for each gene polymorphism represents the dominant model and third line represents the recessive model.
Weinberg equilibrium (data not shown). The genotype frequencies of \textit{ACE I/D}, NOD1 796G>A, TLR4 3725G>C, FAS 1377G>A, FAS 670A>G and FASL 844T>C among different groups are presented in Table 2. Analysis of genotypes and allele frequencies showed similar distribution between GC, HRAG, and controls. We did not find any significant association between all polymorphisms included in our study with respect to GC and HRAG. We also performed an analysis comparing control group with a risk group (combined GC and HRAG) subjects; however it did not reveal significant differences in genotype frequencies (Additional file 1, Table S1).

We observed a tendency for NOD1 796G/G genotype for increased risk of atrophic gastritis (62.4% vs. 54.5% in controls, \( p = 0.082 \)). Similarly, NOD1 allele G was more common in patients with HRAG (77.4%) when compared to controls (72.4%), but without significance (\( p = 0.081 \)). FAS 670G/G genotype was more frequent in HRAG group when compared to controls, 23.9% and 17.2% respectively, however it failed to reach significance level (\( p = 0.077 \)). The FAS 670G/A genotype was less frequent in HRAG (45.5%) when compared to controls (53.4%), but the difference was not statistically significant (\( p = 0.091 \)).

We also analyzed the distribution of genotypes with respect to different histological GC types (Additional file 2, Table S2). Histological type of GC was intestinal, diffuse and mixed type in 47, 47 and 20 patients, respectively. In order to compare intestinal and diffuse type GC, mixed tumor types were excluded from subanalysis.

**Table 3 Distribution of NOD1 and TLR4 gene polymorphisms in Helicobacter pylori positive and negative subjects**

| Genotypes  | \( Hp \) negative | \( Hp \) positive | OR (95% CI)         | \( p \) |
|------------|--------------------|-------------------|---------------------|-------|
| **NOD1 796G>A** |              |                   |                     |       |
| G/G        | 133 (57.8)        | 195 (75.7)        | 0.98 (0.69-1.37)    | 0.910 |
| G/A        | 75 (32.6)         | 116 (45.1)        | 1.07 (0.75-1.52)    | 0.708 |
| A/A        | 22 (9.60)         | 29 (8.50)         | 0.88 (0.49-1.57)    | 0.670 |
| Allele G   | 341 (74.1)        | 506 (74.4)        | 1.01 (0.77-1.33)    | 0.915 |
| Allele A   | 119 (25.9)        | 174 (25.6)        | 0.98 (0.75-1.29)    | 0.915 |
| **TLR4 3725G>C** |              |                   |                     |       |
| G/G        | 182 (79.1)        | 279 (82.3)        | 1.22 (0.80-1.87)    | 0.343 |
| G/C        | 44 (19.1)         | 51 (15.0)         | 0.74 (0.48-1.16)    | 0.199 |
| C/C        | 4 (1.8)           | 9 (2.7)           | 1.54 (0.46-5.06)    | 0.549 |
| Allele G   | 408 (88.7)        | 609 (89.8)        | 1.12 (0.76-1.64)    | 0.545 |
| Allele C   | 52 (11.3)         | 69 (10.2)         | 0.88 (0.60-1.30)    | 0.545 |

\( Hp \), Helicobacter pylori; OR, odds ratio

The ORs were calculated comparing each genotype vs. the other two genotypes, the first line for each gene polymorphism represents the dominant model and third line represents the recessive model.

\textit{ACE D/D} polymorphism was found to be less prevalent in diffuse type GC, when compared to controls (27.7% vs. 12.8%, respectively; OR 0.38, 95% CI 0.15-0.94, \( p = 0.030 \)). There were no differences between different histological subtypes of GC with respect to the other gene polymorphisms. We also analyzed the seropositivity status of \textit{H. pylori} with respect to TLR4 3725G>C and NOD1 796G>A polymorphisms, but no significant differences in genotype distribution were observed (Table 3).

**Discussion**

Overall, in our study \textit{ACE I/D}, NOD1 796G>A, TLR4 3725G>C, FAS 1377G>A, FAS 670A>G and FASL 844T>C gene polymorphisms were not associated with the presence of GC or HRAG. Here, we evaluated six genetic polymorphisms related to the immune-recognition of \textit{H. pylori}, proliferation and apoptosis that were previously described to be associated with increased risk of GC or premalignant gastric lesions in different case-control studies; however reported data are partially conflicting or just based on one study [11-14,20,21,26,33-35]. Since \textit{ACE}, NOD1, TLR4, FAS, and FASL have been shown to be involved in gastric carcinogenesis pathways, we expected that the polymorphisms of genes encoding these proteins could be related to GC.

When analyzing \textit{ACE I/D} polymorphism we hypothesized that carriers of D allele, which was associated with higher \textit{ACE} activity [10], could have an increased risk of GC. Ebert \textit{et al.} [11] identified an association of \textit{ACE D/D} genotype with the development of early GC, and the same group of researchers showed that this genotype is related to the number of metastatic lymph nodes in GC, but not to the overall risk for GC in German subjects [12]. A Japanese study did not find an association between \textit{ACE I/D} polymorphism and susceptibility to GC [13]. Another Asian study [14] reported that \textit{ACE D/D} polymorphism carried a higher risk for GC with odds ratio of 1.59, however the same study did not find significant association of \textit{ACE} polymorphisms with gastric atrophy. \textit{ACE I/D} genotype frequencies in our study are in line with previous reports [12]. We did not observe significant link between \textit{ACE I/D} polymorphisms and the risk for GC or HRAG.

Considering the relationship between \textit{H. pylori}-induced chronic inflammation and carcinogenesis in the stomach it was tempting to speculate that genetic variation in NOD1 receptor, which is involved in bacterial recognition, could be associated with \textit{H. pylori}-induced diseases. NOD1 796A/A homozygous mutants were linked with increased risk for peptic ulcer disease in a Hungarian study [20]; however they did not find a significant association with atrophic gastritis. A recent
study from Turkey [21] found that subjects with NOD1 796A/A genotype had a significantly increased risk for gastric atrophy and antral intestinal metaplasia with very high odds ratios, 34.2 and 39.7 respectively. Such a strong association reported by Kara et al. [21] urged us to evaluate the possible association of this polymorphism in our subjects with GC and HRAG. The genotype frequencies of NOD1 796G>A in this study correspond to previous reports [20,21]. In our study we observed a tendency for NOD1 796G/G genotype and allele G for increased risk for atrophic gastritis; however the difference did not reach statistical significance. We did not observe an association between NOD1 796G>A gene polymorphism and HRAG or GC. H. pylori seropositivity status was also not linked with NOD1 796G>A genotypes.

Polymorphisms of TLR4 gene are hypothesized to decrease responsiveness to gram-negative bacteria lipopolysaccharide through alterations in binding. In a recent study TLR4 3725G>C polymorphism was identified as a risk factor for severe gastric atrophy in H. pylori sero-positive Japanese subjects with OR of 1.43 and 1.47 for G/C and C/C genotypes, respectively [26], however in the same study this polymorphism was not associated with the risk for GC. Another study showed a combined effect of TLR4 3725G>C and miR-146a G>C gene polymorphisms for risk of gastric atrophy, but not for GC [43]. Our data on TLR4 3725G>C genotype frequencies are comparable to the reported frequencies in leukemia study on Caucasian population [44]. We did not find significant association between TLR4 3725G>C genotypes and HRAG or GC. There was also no association between H. pylori seropositivity and TLR4 3725G>C genotypes.

There are only few studies that have evaluated the risk for GC in relation to FAS and FASL gene polymorphisms. Reported results are conflicting and cover Asian subjects only. In two Chinese case-control studies FAS and FASL genotypes had no significant associations with risk of GC [33,34]. Another study from China suggested that FASL 844T>T or T/C and FAS 1377A/A genotypes could be a risk factor for GC in combination with other gene polymorphisms [32]. Hsu et al. [35] have reported that FAS 1377 allele A was a protective factor for developing intestinal metaplasia in the antrum with odds ratio 0.3, while carrying the FASL 844 allele C was a risk factor for developing gastric atrophy in the corpus with OR of 9.4. The distribution of FAS and FASL genotypes in our cohorts corresponds to the frequencies reported on Caucasian subjects in a lung cancer study [45], but we could not provide any evidence that FAS and FASL gene polymorphism are linked with risk for GC or HRAG in Caucasians.

In the present study we evaluated potential links between the risk for GC and several carcinogenesis-related gene polymorphisms that have been rarely or not described, especially in Caucasian population. We did not find significant associations between the presence of GC or HRAG and ACE I/D, NOD1 796G>A, TLR4 3725G>C, FAS 1377G>A, 670A>G and FASL 844T>C gene polymorphisms. We also analyzed the genotype frequencies with respect to different histological GC types. ACE D/D genotype was less prevalent in diffuse-type GC than in controls, however the corresponding subgroups of intestinal and diffuse-type GC are rather small and therefore firm conclusions can not be drawn. Because the studies on ACE I/D, NOD1 796G>A, TLR4 3725G>C, FAS 1377G>A, 670A>G and FASL 844T>C polymorphisms remain limited, evaluation of the association with GC and pre-malignant gastric lesions requires additional research. Since the strength of the association may depend on the studied population, larger studies of different ethnic groups with different genetic profiles are required. The differences in current data on these polymorphisms may result from study design, H. pylori prevalence, and different histological subtypes of GC. In this study there was no difference in H. pylori positivity among GC, HRAG and control groups. The possible explanation for these findings could be higher prevalence of H. pylori in Baltic countries, when compared to Germany. HRAG and control groups were selected both in Germany and Baltic countries, while GC subjects were recruited only in the German centre, thus possibly affected the H. pylori status within the groups. Alterations in various genes, including oncogenes, tumor-suppressor genes, proinflammatory genes, bacterial recognition and cell-adhesion-related genes have been studied in gastric carcinogenesis [5,24,46]. In previous studies we also evaluated the role of IL-1B, IL-1RN and NOD2 gene polymorphisms with respect to risks for GC; however no significant associations were identified [36-38]. Some reports suggested that host genetic factors determine the severity of gastric damage and the eventual clinical outcome of H. pylori infection [47,48]. These findings however have not been transferred to daily clinical practice, and therefore applicable predisposing genetic factors remain still to be determined.

Conclusions
The study shows that the polymorphisms of ACE, NOD1, TLR4, FAS and FASL genes are not associated with H. pylori-induced premalignant gastric conditions and GC in subjects of Caucasian ethnicity. Based on the data available now, the investigated polymorphisms are not applicable for identifying individuals with higher risk for developing GC.
Additional material

Additional file 1: Table S1. Distribution of ACE, NOD1, TLR4, FAS and FASL gene polymorphisms in control and risk group (gastric cancer and high risk atrophic gastritis patients).

Additional file 2: Table S2. Distribution of ACE, NOD1, TLR4, FAS and FASL gene polymorphisms in controls, intestinal and diffuse type gastric cancer groups.

Abbreviations

GC, gastric cancer; HRAG, high risk atrophic gastritis; OR, odds ratio; CI, confidence interval; ACE, Angiotsin converting enzyme; NOD1: Nod-like receptor 1; TLR4: Toll-like receptor 4; H. pylori: Helicobacter pylori; AG: atrophic gastritis; IM: intestinal metaplasia; RFLP: restriction fragment length polymorphism analysis; SNP: single nucleotide polymorphisms.

Acknowledgements

We thank Ursula Stolz and Marion Holley for their support in this study. The study was partially co-funded by Ministries of Education and Research in Latvia and Lithuania, by Science Foundation of Lithuanian University of Health Sciences and State Research Program in Health in Latvia. The study was funded by the “LOM-Program” of the Medical Faculty of the Otto-von-Guericke University Magdeburg.

Author details

1Department of Gastroenterology, Lithuanian University of Health Sciences, Elivienė 2, 50009 Kaunas, Lithuania. 2Clinic of Gastroenterology, Hepatology and Infectious Diseases, Otto von Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany. 3Faculty of Medicine, University of Latvia, Digestive Diseases Center, Hospital Līzneris, 6 Linezeru iela, LV1006 Riga, Latvia. 4Department of Oncology, Lithuanian University of Health Sciences, Elivienė 2, 50009 Kaunas, Lithuania.

Authors’ contributions

JK performed acquisition of data, SNP genotyping, statistical analysis, drafted the manuscript, TW was involved in study design, coordination, drafting and final revision of the manuscript; JB, MS, EK, GJ, LI performed acquisition of data; ML was involved in study design and data collection; PM was involved in study design, and final revision of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 7 June 2011 Accepted: 24 August 2011 Published: 24 August 2011

References

1. Bornschein J, Kandulska A, Selgrad M, Malfeltener P. From gastric inflammation to gastric cancer. Dig Dis 2010, 28:609-614.
2. Correa P. A human model of gastric carcinogenesis. Cancer Res 1988, 48:3554-3560.
3. Bornschein J, Selgrad M, Warnecke M, Kuester D, Wes T, Malfeltener P. H. pylori infection is a key risk factor for proximal gastric cancer. Dig Dis Sci 2010, 55:3124-3131.
4. Komoto K, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, Kajiyama G, Tailley NJ. Helicobacter pylori infection and gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998, 93:1271-1276.
5. Hishida A, Matsuoka K, Goto Y, Hamajima N. Genetic predisposition to Helicobacter pylori-induced gastric precancerous conditions. World J Gastroenterol Oncol 2010, 2:2689-379.
6. Bauvois B. Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis? Oncogene 2004, 23:317-329.
7. Fujita M, Hayashi Y, Yamashita S, Itoman M, Majima M. Blockade of angiotsin II A1 receptor signaling reduces tumor growth, angiogenesis, and metastasis. Biochim Biophys Acta Commun 2002, 294:441-447.
8. Uemura H, Ishiguro H, Nakagawa N, Nagashima Y, Miyoshi Y, Fujimaki K, Sakaguchi A, Kubota Y. Angiotsin II receptor blocker shows antiproliferative activity in prostate cancer cells: a possibility of tyrosine kinase inhibitor of growth factor. Mol Cancer Ther 2003, 2:1139-1147.
9. Deshayes F, Nahmias C. Angiotsin receptors: a new role in cancer? Trends Endocrinol Metab 2005, 16:293-299.
10. Rigat B, Hubert C, Ahenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990, 86:1343-1346.
11. Ebert MP, Lendeckel U, Westphal S, Dierkes J, Gla J, Falwaczcy C, Roesner A, Stolte M, Malfeltener P, Rocken C. The angiotensin I-converting enzyme gene insertion/deletion polymorphism is linked to early gastric cancer. Cancer Epidemiol Biomarkers Prev 2005, 14:2987-2989.
12. Rocken C, Röhl FW, Diebler E, Lendeckel U, Pross M, Carl-McGrath S, Ebert MP. The angiotensin II/angiotensin II receptor system correlates with nodal spread in intestinal type gastric cancer. Cancer Epidemiol Biomarkers Prev 2007, 16:2106-2112.
13. Sugimoto M, Furuta T, Shira N, Ikuma M, Sugimura H, Hishida A. Influences of chymase and angiotsin I-converting enzyme gene polymorphisms on gastric cancer risks in Japan. Cancer Epidemiol Biomarkers Prev 2006, 15:1929-1934.
14. Goto Y, Ando T, Nishio K, Iida Y, Kawai S, Goto H, Hamajima N. The ACE gene polymorphism is associated with the incidence of gastric cancer among H. pylori seropositive subjects with atrophic gastritis. Asian Pac J Cancer Prev 2005, 6:4646-467.
15. Strober W, Murray PJ, Kitanai A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. Nat Rev Immunol 2006, 6:9-20.
16. Xu X, Lee SJ, Kogoff MF. Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria that avoid recognition by toll-like receptors. Infect Immun 2004, 72:1487-1495.
17. Masumoto J, Yang K, Varambally S, Hasegawa M, Tomlins SA, Qu S, Fujimoto Y, Kawaiaki A, Foster SJ, Foster SJ, Horie Y, Mak TW, Nühé G, Chinnayyan AM, Fukase K, Ishihara N. Nod1 acts as an intracellular receptor to stimulate chemokine production and neutrophil recruitment in vivo. J Exp Med 2006, 203:203-213.
18. Fukazawa A, Alonso C, Kurachi K, Gupta S, Lesser CF, McCormick BA, Reinecker HCF. GEF-H1 mediated control of NOD1 dependent NF-kappaB activation by Shigella effectors. PLoS Pathog 2008, 4:e1000228.
19. Viala J, Chaput C, Bonica IA, Cardona A, Girardin SE, Moran AP, Athrnan R, Ménez S, Huene MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philipot DJ, Ferrerio RL, Nod1 responds to peptidoglycan delivered by Helicobacter pylori cag pathogenicity island. Nat Immunol 2004, 5:1166-1174.
20. Hofner P, Gulya Z, Kiss ZF, Tisza A, Tiszlavicz L, Toth G, Szoke D, Molnar B, Lonovics J, Tulassy Z, Mándi Y. Genetic polymorphisms of NOD1 and IL-8, but not polymorphisms of TLR4 genes, are associated with Helicobacter pylori-induced duodenal ulcer and gastritis. Helicobacter 2007, 12:124-131.
21. Kara B, Akkiz H, Doran F, Bayram S, Erken E, Gurnurdullu Y, Sandıkçı M. The significance of E266K polymorphism in the NOD1 gene on Helicobacter pylori infection: an effective force on pathogenesis? Clin Exp Med 2010, 10:107-112.
22. Maeda S, Akanuma M, Mitsuno Y, Hirsta Y, Ogura K, Yoshida H, Shiratori Y, Omata M. Distinct mechanism of Helicobacter pylori-mediated NF-kappaB activation between gastric cancer cells and monocyctic cells. J Biol Chem 2001, 276:44856-44864.
23. Schmausser B, Andriuls M, Endrich S, Lee SK, Joeschans C, Müller-Hermelink HK, Eck M. Expression and subcellular distribution of toll-like receptors TRL4, TRL5 and TRL9 on the gastric epithelium in Helicobacter pylori infection. Clin Exp Immunol 2004, 136:521-526.
24. El-Omair EM, Ng MT, Hold GL. Polymorphisms in Toll-like receptor genes and risk of cancer. Oncogene 2008, 27:244-252.
25. Fukushima T, Ohara N, Hara Y, Yoshimura A, Yoshida K. Evidence for association between a Toll-like receptor 4 gene polymorphism and moderate/severe periodontitis in the Japanese population. J Periodontal Res 2007, 42:541-545.
26. Hishida A, Matsuoka K, Goto Y, Mitsuda Y, Hinaki A, Naito M, Wakai K, Tajima K, Hamajima N. Toll-like receptor 4 +3’725 G/C polymorphism, Helicobacter pylori seropositivity, and the risk of gastric atrophy and gastric cancer in Japanese. Helicobacter 2009, 14:47-53.
27. Zöning M, Hueber A, Baum W, Evan G. Apoptosis regulators and their role in tumorigenesis. Biochim Biophys Acta 2003, 1551:1-17.
28. Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 1995, 75:1169-1178.
29. Takahama Y, Yamada Y, Emoto K, Fujimoto H, Takayama T, Ueno M, Uchida H, Hirao S, Mizuno T, Nakajima Y. The prognostic significance of overexpression of the decay receptor for Fas ligand (DcR3) in patients with gastric carcinomas. Gastric Cancer 2002, 5:61-68.
30. Kanemitsu S, Ibara K, Safiddin A, Ohtuka T, Takeuchi T, Nagayama J, Kuwano M, Hara T. A functional polymorphism in fas (CD95/APO-1) gene promoter associated with systemic lupus erythematosus. J Rheumatol 2002, 29:1183-1188.
31. Sibley K, Rollinson S, Allan JM, Smith AG, Law GR, Roddam PL, Skibola CF, Smith MT, Morgan GJ. Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. Cancer Res 2003, 63:4327-4330.
32. Liu L, Wu C, Wang Y, Zhong R, Wang F, Zhang X, Duan S, Lou J, Yu D, Tan W, Yuan J, Wu T, Nie S, Miao X, Lin D. Association of candidate genetic variations with gastric cardia adenocarcinoma in Chinese population: a multiple interaction analysis. Carcinogenesis 2011, 32:336-342.
33. Wang M, Wu D, Tan M, Gong W, Xue H, Shen H, Zhang Z. FAS and FAS ligand polymorphisms in the promoter regions and risk of gastric cancer in Southern China. Biochem Genet 2009, 47:559-568.
34. Zhou RM, Wang N, Chen ZF, Duan YN, Sun DL, Li Y. Polymorphisms in promoter region of FAS and FASL gene and risk of cardia gastric adenocarcinoma. J Gastroenterol Hepatol 2010, 25:555-561.
35. Hsu PI, Lu PJ, Wang EM, Ger LP, Lo GH, Tsay FW, Chen TA, Yang HB, Chen HC, Lin WS, Lai KH. Polymorphisms of death pathway genes FAS and FASL and risk of premalignant gastric lesions. Anticancer Res 2008, 28:97-103.
36. Wex T, Ebert MP, Kropf S, Dierkes J, Schüttler K, Röcken C, Höcker M, Naumann M, Roessner A, Malfertheiner P. Interleukin 1 beta (IL1B) gene polymorphisms are not associated with gastric carcinogenesis in Germany. Anticancer Res 2010, 30:505-511.
37. Kupcinskas L, Wex T, Kupcinskas J, Leja M, Ivanauskas A, Jonaitis LV, Janciauskas D, Kudelius G, Funka K, Sudraba A, Chiu HM, Lin JT, Malfertheiner P. Interleukin-1B and interleukin-1 receptor antagonist gene polymorphisms are not associated with premalignant gastric conditions: a combined haplotype analysis. Eur J Gastroenterol Hepatol 2010, 22:1189-1195.
38. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994.
39. Wex T, Leodolter A, Bornschein J, Kuester D, Kähne T, Kropf S, Albrecht C, Naumann M, Roessner A, Malfertheiner P. Lack of association between gene polymorphisms of Angiostatin converting enzyme, Nox-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of Helicobacter pylori-induced premalignant gastric lesions and gastric cancer in Caucasians. BMC Medical Genetics 2011 12:112.