Host Gene Polymorphisms in Relation to Helicobacter Pylori Infection and Associated Diseases in a Population Based Cohort

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Abstract

Background: This prospective population based cohort study explores possible associations between host gene polymorphisms, blood group and life style factors on the one hand, and Helicobacter pylori infection, peptic ulcer, and the grade of inflammation, atrophy and intestinal metaplasia of the gastric mucosa, on the other hand.

Methods: The study population (472 volunteers) has previously undergone screening with gastroduodenoscopy, biopsy and blood sampling. The host gene polymorphisms of IL1B-31C/T, IFNγR1-56T/C, the IL1RN VNTR in exon 2 and the HLA-DRB1 gene alleles were analyzed using PCR and pyrosequencing.

Results: H. pylori infection was negatively related to HLA DRB1*03 (odds ratio (OR) 95% CI: 0.388 - 0.989) and was more frequent in individuals with blood group O than A (OR 95% CI: 1.121 - 2.677). There was a lower risk of moderate to severe inflammation in the antrum among individuals with IL1B-31 TC compared to CC carriers (OR 95% CI: 0.094 - 0.733). The IL1RN*L2 genotype was associated with higher risk of IM in the antrum than the *LL genotype (OR 95% CI: 1.570 - 15.878). There was a negative relation between the HLA DRB1 alleles *04 (OR 95% CI: 0.234 - 0.831) and *08 (OR 95% CI: 0.013 - 0.915), and IM in the antrum.

Conclusion: The IL1RN VNTR and the IL1β-31 alleles seem to be associated with intestinal metaplasia of the corpus mucosa and the grade of inflammation of the antrum, respectively. However, no unambiguous correlations could be identified between the host polymorphisms and the occurrence of H. pylori infection, peptic ulcer, and the grade of inflammation, atrophy and IM of the gastric mucosa.

Keywords: Atrophy; Gastritis; Peptic ulcer; Pyrosequencing; Stomach

Introduction

Helicobacter pylori (H. pylori) infection is associated with gastroduodenal diseases, such as peptic ulcer (PU), the premalignant condition atrophic gastritis (AG), gastric carcinoma (GC) and gastric mucosa associated lymphoid tissue (MALT) lymphoma [1]. Infection with these Gram-negative, microaerophilic bacteria is often life long, but exactly how H. pylori eludes the immune defence system is still not clear. Previous studies indicate that a combination of host gene polymorphisms, H. pylori virulence genes and environmental factors determines the outcome of the infection [2, 3].

Several host gene variations have been related to H. pylori infection and the development of associated gastroduodenal diseases. Interleukin-1β (IL1β) and interleukin-1 receptor antagonist (IL1RN) gene polymorphisms have been found to be associated with increased risk of AG, GC and duodenal ulcer (DU) [4, 5], but contradictory results have been reported [6, 7].

A recent meta-analysis [7] concluded that the IL1 receptor antagonist gene contains a variable number of tandem repeats (VNTR), where a short variant, containing two 86bp-repeats, is associated with GC, specifically in non-Asian populations. However, the authors could not find an overall correlation between cancer and polymorphisms in the promoter region of the very potent gastric acid secretion inhibitor IL1β, except the finding of a reduced risk of cancer
in IL1β-31C carriers in Asian populations. An earlier meta-
alysis [8] concluded that there is an increased risk of GC
associated with IL1B-511T and IL1RN*2 alleles in Caucas-
sians, but not in Asians, and that there is a trend towards an
association between IL1B-31C and GC in Caucasians.

IFNGR1 is the ligand-binding subunit of the interferon
gamma receptor dimer. By genome wide linkage analysis,
Thye et al (2003) [9] found increased anti-\textit{H. pylori} serum
immunoglobulin G levels among Senegalese siblings, who
were homozygous or heterozygous carriers of the IFNGR1-
56T variant. Since then, several studies have indicated that
there is a more general association between -56C/T SNP
and human pathology related to both bacteria and viruses,
the IFNGR1-56CC genotype being associated with protection
from TB [10] and -56C with spontaneous clearance of
hepatitis B virus [11]. Zhou et al [11] also showed that
the IFNGR-56C variant is associated with higher transcription
level than -56T.

The human leukocyte antigen gene DRB1 encodes the
beta subunit of the HLA class II complex, presenting pep-
tides on the surface of antigen presenting cells [12]. In Ja-
pan, the HLA gene DRB1*0405 allele was associated with
duodenal ulcer [13]. The DRB1*04051 has been shown to be
associated with gastric adenocarcinoma independent of \textit{H. pylori}
infection [14], and the DRB1*1501 was negatively re-
lated to gastric ulcer, duodenal ulcer and \textit{H. pylori}-associat-
ed gastritis when compared to non-infected healthy individu-
als [13]. The DRB1*1601 allele was found to be associated
with increased risk of the diffuse type of stomach cancer in
a Swedish population, especially in \textit{H. pylori} negative indi-
viduals [15].

The aim of this study was to explore whether there are
any associations between the occurrence of IL-1B-31T/C,
IL1RN VNTR alleles, IFNGR1-56C/T, HLA DRB1 alleles,
together with blood group and lifestyle factors on the one
hand, and the occurrence of \textit{H. pylori} infection, peptic ulcer
and the grade of inflammation, atrophy and intestinal meta-
plasia (IM) of the gastric mucosa, on the other hand, in a
prospective population based cohort in Sweden.

Methods

Study population and material

The study was conducted in accordance with the Helsinki
declaration and was approved by the Regional Ethics Com-
mitee of Southeast of Sweden. Informed written consent
was obtained from all participants. The study population is a
cohort of 472 individuals from a larger (n = 506 volunteers)
population study [16, 17]. The study includes all individuals
that underwent screening with gastroduodenoscopy, biopsy
and blood sampling [16] (fasting state), and from whom
DNA of sufficient quality for genotyping analysis could be
isolated. There were 218 females and 254 males included in
the study. Histologic examination of biopsies was performed
as previously described [16]. Gastritis was classified accord-
ting to the Sydney system [16, 18, 19]. The prevalence of
chronic gastritis in the corpus was 9.5% (48.9% males) and
moderate to severe chronic gastritis was observed in 4.0% of
the volunteers (52.6% males). All ulcers diagnosed at gastro-
duodenoscopy were biopsied. No malignant ulcers were en-
countered. According to gastroduodenoscopy and histologic
examination of biopsies, 8.7% of the study population had
benign ulcer (58.5% males) and 3.8% had ulcer exclusively
located in the duodenum (72.2% males).

Blood samples were stored at -80 °C until analysis. \textit{H. pylori}
status was classified as positive when at least two of the
following occurred: \textit{H. pylori} identified by light micro-
scopic examination (Giemsastained sections), positive re-
sult of urease test on fresh biopsy specimen, or elevated level
of \textit{H. pylori} IgG antibodies in serum [16], 187 individuals
fulfilled the criteria for positive \textit{H. pylori} status (54% males).
Serum pepsinogen I (PGI) and II (PGII) concentrations were
analyzed using sandwich enzyme immunoassay (ELISA) as
previously described [17]. The reference interval is 2.0 - 20.0
for the ratio PGI/PGII and values lower than 3.0 are consid-
ered indicative of significant atrophy of the gastric corpus
mucosa.

H, K-ATPase IgG antibodies were analyzed by ELISA
as previously described [20] and results are given as relative
optical density (OD; upper normal limit 15%). \textit{H. pylori} IgG
antibodies against surface antigens were analyzed with the
same method (upper normal limit of OD 5% [17]).

Isolation of DNA and whole genome amplification

DNA was extracted from whole blood at the Department of
Forensic Genetics of the Swedish National Board of Foren-
sic Medicine (Linkoping, Sweden), using the M48 BioRobot
(Qiagen, Hilden, Germany). In cases from whom no whole
blood was available for various reasons, DNA was extracted
from plasma or serum using either the QIAamp DNA mini
isolation kit (Qiagen, Hilden, Germany) following the blood
and body fluid spin protocol, or MagAttract DNA mini M48
kit following the cultured cell protocol. In short, 200 - 400
µL plasma/serum was used and 5 µg dA/dT DNA carrier
probe (GE Healthcare, Uppsala, Sweden) was included in
the manual extraction. For automatic extraction using the
M48 BioRobot (Qiagen, Hilden, Germany) 400 µL plasma/
serum was centrifuged at 3,000 g for 30 min at 4 °C, the pel-
let resuspended in 190 µL buffer G2 and 10 µL proteinase K
was added, the samples were incubated at 37 °C for 0.5 - 1 h
before extraction and 1 µL DNA was used for whole genome
amplification according to the GenomiPhi DNA amplifica-
tion kit (GE Healthcare, Uppsala, Sweden). MDA-DNA con-
centrations were determined using an ND-1000 spectropho-
tometer (Nanodrop Technologies, Wilmington, DE, USA).
Table 1. Summary of Statistically Significant Findings in the Study

| Independent variables | H. pylori infection | Infl. antrum | Infl. corpus | Ulcer | Atrophy of corpus | IM antrum | IM in corpus |
|-----------------------|--------------------|-------------|-------------|-------|------------------|-----------|-------------|
|                       | Overall            | Moderate-Severe | Overall    | Moderate-Severe | Overall | Duodenal       | Overall | Moderate-Severe | Overall |
| H, K-ATPase antibodies| ↑                  | ↑            | ↑           | ↑      | ↑                | ↓         | ↑           |
| H. pylori infection   | ↑                  | ↑            | ↑           | ↑      | ↑                | ↓         | ↑           |
| Gender = Male         | ↓                  |              |             |        |                  |           |             |
| BMI                   | ↓                  | ↓            |             |        |                  |           |             |
| Age                   | ↑                  | ↑            | ↑           | ↑      | ↑                | ↓         | ↑           |
| Smoker                | ↑                  |              |             |        |                  |           |             |
| Alcohol consumption   | ↑                  |              |             |        |                  |           |             |
| (weekly)              |                    |              |             |        |                  |           |             |
| Blood group O comp. to A | ↑                |              |             |        |                  |           |             |
| Blood group AB comp. to A | ↑              |              |             |        |                  |           |             |
| DRB1*01               |                    |              |             |        |                  |           |             |
| DRB1*03               | ↓                  |              |             |        |                  |           |             |
| DRB1*04               |                    |              |             |        |                  |           |             |
| DRB1*08               |                    |              |             |        |                  |           |             |
| IL1RN L2 comp. to 22  |                    |              |             |        |                  |           |             |
| IL1B -31 TC comp. to CC |                  |              |             |        |                  |           |             |

Results for the ratio PGI/PGII are obtained using the GLM analysis and the remaining results are obtained using the logistic regression model. An up arrow (↑) represents a significant positive correlation, and a down arrow (↓) represents a significant negative correlation for each independent variable. No arrow indicates a lack of significant association. Atrophy and inflammation are graded histologically according to the Sydney system.
PCR analysis

The presence of amplified DNA was verified using human 18S primers as previously described [21]. Amplification and pyrosequencing analyses of IL1B SNPs rs1143627 (-31C/T), and IFNGR1 SNP rs2234711 (-56T/C), and amplification and gel electrophoresis analysis of IL1RN VNTR in exon 2 were done as previously described [21]. The HLA-DRB1 gene was analyzed at the Department of Forensic Medicine (Linköping, Sweden) following the recommendation for the HLA-DR low kit (Olerup, Norway).

Statistical analysis

The continuous dependent variable PGI/PGII was analyzed using general linear model (GLM) and Minitab (v. 15). All other dependent variables were dichotomous, and were analyzed using forward stepwise (Wald method) binary logistic regression (BLR) and the SPSS statistical analysis program (v. 17). The Hosmer and Lemeshow goodness of fit test was checked at the last step for verification of each analysis. A P-value of < 0.05 was considered significant.

The genotypes frequencies for all analyzed cytokine polymorphisms in this study are presented in supplementary Table 2.

Table 2. Result From Binary Logistic Regression With of H. Pylori Infection as Dependent Variable

| Dependent variable | No. of positive cases | No. of included cases | Variables in the equation | P-value | 95% CI for OR |
|--------------------|-----------------------|-----------------------|--------------------------|---------|--------------|
| H. pylori infection| 170                   | 442                   | Age                      | 0.000   | 1.027 - 1.066|
|                    |                       |                       | ABO                      | 0.047   |              |
|                    |                       |                       | ABO (B)                  | 0.758   | 0.416 - 1.894|
|                    |                       |                       | ABO (O)                  | 0.013   | 1.121 - 2.677|
|                    |                       |                       | ABO (AB)                 | 0.160   | 0.797 - 3.949|
|                    |                       |                       | DRB1*03                  | 0.045   | 0.388 - 0.989|

Hosmer and Lemeshow goodness of fit test was used as indicator of the validity of the equation at the last step of iterations; #The significance level was set to P < 0.050.
The distribution of IL1B-31 genotype TT/TC/CC was 42.8/42.2/15.0%. For IFNGR1-56 the distribution of genotype TT/TC/CC was 44.1/42.6/13.3%. The IL1RN 86bp VNTR genotypes were classified into allele 2 (2 repeats) or L for three or more repeats [4], and the distribution of genotypes LL/L2/22 was 50.0/39.2/10.8%. The frequency of HLA-DRB1 allele types is shown in supplementary 1 (www.gastrores.org).

Statistical analysis

A summary of all analyses in which the regression coefficient was significantly different from zero is shown in Table 1. The results are grouped according to the dependent variable analyzed: H. pylori status, scores for chronic inflammation of the corpus and antrum mucosa, ulcer overall (duodenal or gastric), duodenal ulcer, any degree of atrophy of the corpus mucosa, moderate to severe atrophy of the corpus mucosa, PGI/PGII as surrogate marker for atrophy of the corpus mucosa, and IM of the corpus and antrum mucosa. The details of each variable analyzed are presented below in five parts: H. pylori infection, inflammation, ulcer, atrophy and IM.

Relations between the analyzed polymorphisms and H. pylori status

Results from binary logistic regression analysis of H. pylori infection (170 positive cases of 442 examined subjects) are presented in Table 2. H. pylori infection was positively related to age (odds ratio (OR) 95% CI: 1.027 - 1.066) and negatively related to presence of the HLA-DRB1*03 allele (OR 95% CI: 0.388 - 0.989). There was an overall significant correlation with H. pylori status.

### Table 3. Result From Binary Logistic Regression Analysis With Chronic Inflammation as Dependent Variable

| Dependent variable                        | No. of positive cases | No. of included cases | Variables in the equation | P-value | 95% CI for OR Lower | 95% CI for OR Upper |
|-------------------------------------------|-----------------------|-----------------------|---------------------------|---------|---------------------|---------------------|
| Inflammation overall grade 1-3 of the corpus | 181                   | 442                   | H, K-ATPase ab            | 0.001   | 1.017               | 1.061               |
|                                            |                       |                       | H. pylori inf.            | 0.000   | 44.090              | 170.14              |
|                                            |                       |                       | Age                       | 0.015   | 1.007               | 1.072               |
| Inflammation grade 2-3 of the corpus      | 42                    | 442                   | H. pylori inf.            | 0.000   | 3.028               | 15.713              |
|                                            |                       |                       | Age                       | 0.007   | 1.013               | 1.088               |
|                                            |                       |                       | ABO (A)                   | 0.049   |                     |                     |
|                                            |                       |                       | ABO (B)                   | 0.857   | 0.291               | 4.408               |
|                                            |                       |                       | ABO (O)                   | 0.949   | 0.448               | 2.123               |
|                                            |                       |                       | ABO (AB)                  | 0.010   | 1.400               | 11.705              |
| Inflammation overall (grade 1-3) of the antrum | 181                   | 442                   | H, K-ATPase ab            | 0.019   | 1.003               | 1.038               |
|                                            |                       |                       | H. pylori inf.            | 0.000   | 241.34              | 2331.7              |
| Inflammation grade 2-3 of the antrum      | 114                   | 442                   | H. pylori inf.            | 0.000   | 55.397              | 509.42              |
|                                            |                       |                       | Alcohol cons. (weekly)    | 0.016   | 1.247               | 8.277               |
|                                            |                       |                       | IL1B-31 (CC)              | 0.026   |                     |                     |
|                                            |                       |                       | IL1B-31 (TC)              | 0.011   | 0.094               | 0.733               |
|                                            |                       |                       | IL1B-31 (TT)              | 0.140   | 0.169               | 1.285               |

*Hosmer and Lemeshow goodness of fit test was used as indicator of the validity of the equation at the last step of iterations; the significance level was set to P < 0.050.
difference between blood groups (P = 0.047), and in comparisons between pairs of blood groups with A as reference category, blood group O showed a higher risk of *H. pylori* infection (OR 95% CI: 1.121 - 2.677).

**Relations between the analyzed polymorphisms and the degree of chronic inflammation of the gastric mucosa**

Results from the binary logistic regression analysis of the grade of chronic inflammation in the corpus and antrum mucosa (overall or moderate to severe) are shown in Table 3. Overall inflammation (grade 1-3) in the corpus (181 cases of 442 examined subjects) was associated with elevated levels of *H. pylori* antibodies (OR 95% CI: 1.003 - 1.038), and *H. pylori* infection (OR 95% CI: 241.34 - 2331.7). Moderate to severe (grade 2-3) inflammation of the corpus mucosa (114/442 subjects) was positively related to *H. pylori* infection (OR 95% CI: 55.397 - 509.42) and alcohol consumption on a weekly basis (OR 95% CI: 1.247 - 8.277). There was a significant difference between the IL1β-31 genotypes (P = 0.026) and the TC genotype showed lower risk of moderate to severe inflammation of the antrum than the CC genotype (OR 95% CI: 0.094 - 0.733).

**Relations between the analyzed polymorphisms and the prevalence of ulcer**

Using the binary logistic regression model, peptic ulcer, location disregarded (38/442 subjects), was significantly positively associated with *H. pylori* infection (OR 95% CI: 4.576 - 28.052) and with decreasing BMI (OR 95% CI: 0.725 - 0.938; Table 4). Duodenal ulcer specifically (18/442 subjects) was positively related to the levels of *H. pylori* antibodies (OR 95% CI: 1.004 - 1.042) and *H. pylori* infection (OR 95% CI: 1.678 - 18.377), and more prevalent in women (OR 95% CI: 1.246 - 11.636). There was a significant difference between ABO blood groups (P = 0.014), and group AB showed higher risk for duodenal ulcer than blood group A (OR 95% CI: 2.284 - 46.953).

### Table 4. Result From Binary Logistic Regression for Peptic Ulcer

| Dependent variable | No. of positive cases | No. of included cases* | Variables in the equation | P-valueb | 95% CI for OR Lower | Upper |
|--------------------|-----------------------|------------------------|---------------------------|----------|---------------------|-------|
| Ulcer overall      | 38                    | 442                    | *H. pylori* inf.          | 0.000    | 4.576               | 28.052|
|                    |                       |                        | BMI                       | 0.003    | 0.725               | 0.938 |
| Duodenal ulcer     | 18                    | 442                    | *H. pylori* inf.          | 0.013    | 1.005               | 1.042 |
|                    |                       |                        | H, K-ATPase ab            | 0.005    | 1.678               | 18.377|
|                    |                       |                        | Gender                    | 0.031    | 0.077               | 0.885 |
|                    |                       |                        | BMI                       | 0.012    | 0.663               | 0.950 |
|                    |                       |                        | Smoker                    | 0.019    | 1.246               | 11.636|
|                    |                       |                        | ABO (A)                   | 0.014    |                     |       |
|                    |                       |                        | ABO (B)                   | 0.826    | 0.061               | 9.362 |
|                    |                       |                        | ABO (O)                   | 0.416    | 0.477               | 6.017 |
|                    |                       |                        | ABO (AB)                  | 0.002    | 2.284               | 46.953|

*In some cases data were not available for all variables; *b* Hosmer and Lemeshow goodness of fit test was used as indicator of the validity of the equation at the last step of iterations; *c* The significance level was set to P < 0.050.
CI: 2.284 - 46.953). There was also a negative relation between BMI (OR 95% CI: 0.663 - 0.950) and the occurrence of duodenal ulcer. Relations between the analyzed polymorphisms and the prevalence of atrophy in the corpus mucosa

Results from the binary logistic regression analysis of atrophy of the corpus mucosa (overall or moderate to severe) are shown in Table 5. Results from the statistical analysis using the serum level of the ratio PGI/PGII as surrogate marker for atrophy of the corpus (as determined histomorphologically) are presented in Table 6. Atrophy of the corpus mucosa (41/442 subjects) was positively related to the levels of H, K-ATPase antibodies (OR 95% CI: 1.016 - 1.042), H. pylori infection (OR 95% CI: 1.772 - 8.520), and negatively related to the presence of HLA-DRB1*01 (OR 95% CI: 0.050 - 0.837). When including only individuals with histologically determined moderate to severe atrophy of the corpus (17/442 subjects), there was a significantly positive association with the H, K-ATPase antibody levels (OR 95% CI: 1.010 - 1.038) and age (OR 95% CI: 1.099 - 1.323).

Using the ratio PGI/PGII as surrogate marker of histologically classified atrophy, GLM analysis revealed a significant negative association between the titer of H, K-ATPase antibodies (95% CI: -0.0648 - -0.0302), H. pylori infection (95% CI: -4.2608 - -2.9303), and age (95% CI: -0.0647 - -0.00397), and the ratio PGI/PGII.

Relations between the analyzed polymorphisms and the presence of IM in the corpus and antrum mucosa

Table 5. Result From Binary Logistic Regression Analysis With Atrophy of the Corpus Mucosa as Dependent Variable

| Dependent variable | No. of positive cases | No. of included cases | Variables in the equation | P-value<sup>b</sup> | 95% CI for OR | Lower | Upper |
|--------------------|-----------------------|-----------------------|---------------------------|--------------------|----------------|-------|-------|
| Atrophy overall (grade 1-3) of the corpus<sup>c</sup> | 41 | 442 | H, K-ATPase ab | 0.000 | 1.016 | 1.042 |
| | | | H. pylori inf. | 0.001 | 1.772 | 8.520 |
| | | | Age | 0.004 | 1.018 | 1.101 |
| | | | DRB1*01 | 0.027 | 0.050 | 0.837 |
| Moderate to severe atrophy of the corpus<sup>d</sup> | 17 | 442 | H, K-ATPase ab | 0.001 | 1.010 | 1.038 |
| | | | Age | 0.000 | 1.099 | 1.323 |

<sup>a</sup>The significance level was set to P < 0.050.

Table 6. Result From GLM Analysis Using the Ratio Pepsinogen I/Pepsinogen II (PGI/PGII) as Surrogate Marker for Atrophy of the Corpus Mucosa

| Dependent variable | Variables in the equation | Coef. | SE Coef. | T | P-value<sup>a</sup> | 95% CI | Lower | Upper |
|--------------------|---------------------------|-------|----------|---|-------------------|-------|-------|-------|
| PGI/PGII | H, K-ATPase ab | -0.047523 | 0.008793 | -5.40 | 0.000 | -0.0648 | -0.03024 |
| | H. pylori inf. | -3.5956 | 0.3385 | -10.62 | 0.000 | -4.2608 | -2.93031 |
| | Age | -0.03434 | 0.01545 | -2.22 | 0.027 | -0.06470 | -0.00397 |
IM in the corpus (22 cases with grade 1-3 out of 442 subjects) was positively associated with the H, K-ATPase antibody titer (OR 95% CI: 1.018 - 1.042) and age (OR 95% CI: 1.070 - 1.220). Only 5 cases of moderate to severe IM in the corpus mucosa were found in this population. The Hosmer and Lemeshow goodness of fit test was significant, indicating a non-valid equation, and the results were excluded from further analysis.

IM in the antrum (89 cases with grade 1-3 out of 442 subjects) was positively associated with *H. pylori* infection (OR 95% CI: 9.675 - 38.480), and decreasing BMI (OR 95% CI: 0.758 - 0.927), increasing age (OR 95% CI: 1.003 - 1.061), and negatively related to carriage of the HLA DRB1 alleles *04 (OR 95% CI: 0.234-0.831) and *08 (OR 95% CI: 0.013 - 0.915) (Table 7). There was an overall significant difference between IL1RN genotypes (P = 0.013), and IL1RN*L2 carriers showed a higher risk of IM in the antrum than genotype *22 carriers (OR 95% CI: 1.570 - 15.878).

Considering moderate to severe IM in the antrum (grade 2-3; 11 cases), there was a significant relation to increasing age (OR 95% CI: 1.027 - 1.188) and smoking (OR 95% CI: 1.155 - 17.446) (Table 7). None of the genotypes was associated with moderate to severe IM.

**Discussion**

The aim of this study was to explore possible associations between IL-1B-31T/C, IL1RN VNTR alleles, IFNGR1-56C/T, the HLA DRB1 alleles, blood group and life style factors and *H. pylori* infection, the occurrence of peptic ulcer, grade of gastric mucosal inflammation, atrophy, and IM in a prospective population based cohort in Sweden. The findings are summarized in Table 1. As stated in our first publication concerning this population [17], there was a modest overrepresentation of digestive symptoms among those volunteers that agreed to participate in the screening study compared to an age and sex matched population, which was not being asked to participate in any examinations.

Several human cytokine genes have been thoroughly studied with respect to their influence on the host-microbial interaction of the *H. pylori* colonization, establishment of in-
fection and the long-term outcome such as duodenal ulcer and the premalignant condition atrophic gastritis. The findings in these studies are not unequivocal and differ in various populations [7]. Two well-studied genes are the IL1B and its receptor antagonist IL1RN. El-Omar et al (2000) [4] showed that mutations in the IL1B gene promoter position -31 (C instead of T) and the short form of the IL1RN VNTR (allele No. 2) were significantly associated with increased risk of developing gastric carcinoma following H. pylori infection. In the present study, using logistic regression analysis, no significant association was found between the IL1B-31T/C and H. pylori infection, peptic ulcer, gastric atrophy, or IM (Table 1). However, a negative correlation between the IL1B-31TC genotype was found for moderate to severe inflammation in the antral mucosa, but not for overall inflammation (grade 1-3 according to the Sydney system) or for any degree of inflammation in the corpus mucosa.

No associations were found between IL1RN VNTR alleles and H. pylori infection, any grade of inflammation, ulcer, or atrophy. However, in pairwise comparison between IL1RN genotypes, the *L2 genotype showed a higher risk for IM (grade 1-3) in the antrum (Table 7) than *22. No relation to the IL1RN VNTR alleles was seen when including only cases with moderate to severe IM (11 cases). When searching for papers reporting studies of IL1RN and IM, we found two studies reporting no association between IL1RN*2 genotype and IM [22, 23], and one study reporting an association between IL1RN*2 and IM in an Italian population [24]. However, they do not report any possible association between the IL1RN*L2 genotype and IM, and our findings cannot be verified.

Discussion of the results from each step is presented below in the following order: 1) H. pylori infection; 2) grade of inflammation; 3) ulcer; 4) atrophy of the corpus mucosa; and 5) IM of the corpus and antrum mucosa.

Beside a positive relation to age, we found a higher risk of H. pylori infection for the ABO blood group O (compared to group A). We also noted a negative relation between the DRB1*03 allele and H. pylori infection. This negative relation was also found by Veneri et al (2005) [25], but their study included only 52 patients with idiopathic thrombocytopenic purpura (of whom 34 had H. pylori infection). Kunstmann et al (2002) [26] found no such relation in a German cohort. The positive association between blood group O and H. pylori infection and gastroduodenal diseases has been described and characterized in other studies [2, 27, 28], but discrepant data regarding this are on record [29, 30].

Overall inflammation (grade 1-3) of the corpus mucosa was related to increased titer of H, K-ATPase antibodies, H. pylori infection and increasing age. If only moderate to severe (grade 2-3) inflammation of the corpus was included in the statistical model, no relation to the titer of anti-H, K-ATPase antibodies was found, but there was, however, a higher risk of inflammation of the corpus for blood group AB than group A. Previously, blood group AB has been found to be negatively associated with H. pylori infection [27], a relation that was not observed in this study. To the best of our knowledge, this is the first time a positive relation between moderate to severe inflammation of the corpus mucosa and blood group AB has been demonstrated.

We found a positive relation between overall inflammation (grade 1-3) in the antrum and H. pylori infection and increasing titer of H, K-ATPase antibodies. However, there was no association between the presence of moderate to severe (grade 2-3) antral inflammation and the titer of anti-H, K-ATPase antibodies. Instead, we noted a relation between weekly alcohol consumption and lower risk of inflammation for the IL1B-31 TC genotype (compared to the CC genotype) (Table 3). To our knowledge, this relation has not been described previously.

For peptic ulcer, location disregarded, a positive relation was found between H. pylori infection and low BMI. Regarding individuals with ulcers exclusively located to the duodenum, we found associations to H, K-ATPase antibodies, H. pylori infection, smoking, blood group AB (compared to group A), female gender and decreasing BMI (Table 1). Sierra et al (2008) [31] also found an association between duodenal ulcer and smoking, but no association between IL1-RN and duodenal ulcer in a Costa Rican dyspeptic population. This is in agreement with our data. The relation to blood group AB is discordant with the findings of previous investigators who have demonstrated an association between blood group O and the prevalence of peptic ulcer [32] (reviewed by Anstee and Clarke et al [28, 33]). However, others found no association between ABO blood groups and ulcer [29].

Regarding atrophy of the corpus mucosa (grade 1-3 according to the Sydney system), a positive relation was found between H, K-ATPase antibodies, H. pylori infection and increasing age, and a negative relation to DRBI*01 carriage. A negative relation between the DRBI*01 allele and atrophic gastritis has also been demonstrated by Lahner et al (2010) [34] with an OR of 0.27 (95% CI 0.08-0.089; P = 0.02). However, we did not find any association between the DRBI*03 or *04 and atrophic gastritis as found by Lahner et al (2010) [34]. When including only moderate to severe cases of atrophy (grade 2-3) of the corpus mucosa, we found associations to atrophy and H, K-ATPase antibodies and increasing age. The PGI/PGII ratio is commonly used as a screening marker for AG [35], where a low-value ratio indicates presence of AG. When using the ratio PGI/PGII as surrogate marker for AG of the corpus, a negative association between H, K-ATPase antibody titer, H. pylori infection, and age and increasing ratio (of PGI/PGII) was noted. This is in accordance with our results for AG overall (grade 1-3 according to the Sydney system), except that no association between PGI/PGII ratio and DRBI*01 allele carriage emerged.

In this study, IM in the corpus mucosa of grade 1-3 (ac-
cording to the Sydney system) was found in 22 cases and this was related to increasing titer of H, K-ATPase antibodies and increasing age (Table 7). Only 5 cases with moderate to severe IM were found in the corpus mucosa of this population. In the statistical analysis, the Hosmer and Lemenshow goodness of fit test was significant, indicating a non-valid equation, and the results were excluded from further analysis.

IM overall (grade 1-3) in the antrum mucosa was found in 89 subjects and was associated with H. pylori infection, decreasing BMI, increasing age, the HLA-DRB1 alleles *04 and *08 and the IL1RN *22 (the latter discussed above). IM of moderate to severe grade (2-3) in the antrum mucosa (11 cases) was only associated with increasing age and smoking. The association between IM and increasing age and smoking is well established [36]. The relation of IM in the antrum mucosa and the two HLA-DRB1 alleles *04 and *08 has to our knowledge not been reported before. The findings could not be confirmed for cases with moderate to severe IM in the antrum mucosa (Table 7).

Concerning IFNGR1-56C/T, we found no significant relation to any of the diseases studied here.

In summary, in this prospective Swedish general population based cohort study, we found positive associations between the presence of blood group O and H. pylori infection. We also found a positive association between blood group AB and moderate to severe inflammation of the corpus mucosa, as well as the occurrence of duodenal ulcer. For the HLA DRB1 alleles, a negative association was found between DRB1*03 and H. pylori infection, and for DRB1*04 and *08 in relation to IM (grade 1-3) of the antrum mucosa. A higher risk of overall IM in the antrum mucosa for heterozygous IL1RN*L2 carriers than *22 carriers, and a lower risk of moderate-severe inflammation of the antrum for IL1β-31 TC carriers than CC carriers, were established.

No other significant correlations were identified between the occurrence of the polymorphisms together with life style factors on the one hand, and the occurrence of H. pylori infection, peptic ulcer, and the grade of inflammation, atrophy and IM of the gastric mucosa, on the other hand.

H. pylori infection and age were clearly associated to most of the gastrointestinal diseases studied here. The IL1RN VNTR and the IL1β-31 alleles seem to be associated with IM of the corpus mucosa and the grade of inflammation of the antrum, respectively. However, no unambiguous correlations could be identified between the host polymorphisms and the occurrence of H. pylori infection, peptic ulcer, and the grade of inflammation, atrophy and IM of the gastric mucosa.

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Conflicts of Interest

The authors disclose no conflicts.

Author Contributions

AR has been involved in the acquisition, analysis and interpretation of data, and statistical analysis. FP and SR has participated in the acquisition, analysis and interpretation of data. OE has been involved in the generation and interpretation of statistical analysis data. KB designed the study and participated in the acquisition of data and analysis and interpretation of data. All authors have participated in the drafting of the manuscript and have approved of the final version.

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