Low Expression Myeloperoxidase Genotype Negatively Associated with Helicobacter pylori Infection

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Our previous study revealed that a polymorphism of the interleukin (IL) 1β gene, encoding the pro-inflammatory cytokine IL-1β, influenced the prevalence of persistent Helicobacter pylori (HP) infection. In this paper, a polymorphism of another inflammation-related enzyme, myeloperoxidase (MPO), was examined with respect to association with the HP infection. The polymorphism is due to a G-to-A transition at −463 in the promoter region of MPO. The G allele is the wild type with normal expression, while the A allele is a low expression allele. The subjects were 241 non-cancer outpatients (118 males and 123 females) aged 39 to 69 who participated in an HP eradication program at Aichi Cancer Center Hospital. High-molecular weight Campylobacter-Associated-Protein (HM-CAP) ELISA (Enteric Products Ins., Westbury, NY) was used for the identification of HP-infected participants. The frequency was 79.7% (192/241) for the GG genotype, 19.5% (47/241) for the GA genotype, and 0.8% (2/241) for the AA genotype. The sex-age-adjusted odds ratio (OR) relative to GG was 0.69 (95% confidence interval (CI), 0.35–1.35) for individuals with the A allele, but among male participants the OR was 0.31 (0.11–0.84). Subgroup analysis revealed significantly reduced ORs with the GA/AA genotypes for current smokers (0.19, 0.04–0.96), and for those who were occasional/no milk drinkers (0.25, 0.09–0.72). These findings are consistent with the results for IL-1B in our earlier study, suggesting that inflammatory responses in the gastric mucosa may influence persistent HP infection, and that smoking and milk intake may be effect-modifiers.

Key words: Helicobacter pylori infection — Myeloperoxidase — Lifestyle factors

Helicobacter pylori (HP) infection is a risk factor for gastric and duodenal ulceration, gastric mucosal atrophy, stomach cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.1–4 The infection rate depends largely on sanitary conditions, especially during childhood.5–7 Lifestyle factors such as salty food intake,8 fruit intake,9 and smoking9–13 have been reported to influence the prevalence, although there are studies reporting no association.8, 12, 14 These environmental factors are generally considered to play important roles in determining the prevalence of infected individuals. However, the susceptibility of the host due to endogenous gene-controlled factors may also be important for persistent HP infection, and it appears that individuals may remain uninfected even after substantial exposure.

We earlier found that a polymorphism (C-31T) in the interleukin-1β gene (IL-1B) located in chromosome 2q13–24 impacts on persistent infection; infection rates measured with an anti-HP antibody test were 45.2% for the CC genotype, 67.7% for the CT genotype, and 63.6% for the TT genotype.15 Interleukin-1β is a pro-inflammatory cytokine,16 induced by HP infection,17 which is also a potent inhibitor of gastric acid secretion.18 The T allele makes a TATA box, suspected of enhancing gene expression. It is not clear whether the inhibitory activity on acid secretion, high inflammatory activity, or both influence the persistent HP infection.

Myeloperoxidase (MPO) is a lysosomal enzyme in polymorphonuclear leukocytes and monocytes; it produces hypochlorous acid, which has microbicidal activity against a wide range of organisms,20 resulting in tissue inflammation. Recently, it was reported that HP water extract can activate neutrophils20 and enhance the secretion of MPO.21 The gene is located in chromosome 17q23.1. Severe deficiency of enzyme activity is associated with Arg569Trp, Val173Cys, or Met251Thr polymorphism of MPO, which causes fatal diseases such as chronic granulomatous disease.22 One polymorphism of MPO, at −463 (G to A), located in a hormone-response-element region, has been reported to influence mRNA expression through loss of a SP1 transcription factor binding site. The G allele shows a 25-fold higher transcription level than the A allele,23 and the polymorphism had been reported to influence cancer development in the lung24–27 and larynx.28 We have also reported a possible association of this polymor-
phism with esophageal cancer. The hypothesized mechanism is that MPO activity generates carcinogens such as benzo[a]pyrene diol epoxide, elevating the risk of cancers, especially smoking-related cancers. Though the reported association was not strong, rather consistent results have accumulated in favor of a role of this MPO polymorphism in elevation of cancer risk. The biological and epidemiological findings suggest that this polymorphism might impact on persistent HP infection. To our knowledge, this is the first study examining this possible association. Interactions with lifestyle factors were also investigated to cast light on the biological background of lifestyle factors related to HP prevalence.

MATERIALS AND METHODS

Study subjects The subjects were the same as reported in our previous paper (15), i.e., participants in an HP eradication program at Aichi Cancer Center Hospital aged 39 to 69 years. Written informed consent was obtained for genotyping. Lifestyle information including data on smoking, alcohol, food intake frequency, physical exercise, and past history of cancer was collected by self-administered questionnaire. In total, 283 outpatients (138 males and 145 females) participated in the study until December 1999. Excluding 42 participants (38 with a history of cancer, 3 hepatitis virus carriers without blood samples, and 1 who refused blood sampling after entry), the remaining 241 outpatients were analyzed. This study was approved by the Ethical Committee of Aichi Cancer Center in 1999 (Approval number 12-23).

HP infection An HP IgG antibody test, High-Molecular-weight Campylobacter-Associated-Protein (HM-CAP) ELISA (“Detaminor H. pylori antibody,” Enteric Products Ins., Westbury, NY) was used for the identification of HP-infected participants. A value of 2.3 or over was regarded as HP infection-positive. The sensitivity of HM-CAP is reportedly 98.7%, with a specificity of 100% in the United States, though the sensitivity was not as high for the present subjects.

Genotyping DNA was extracted from 200 µl of buffy coat preserved at −40°C with a QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA), and a PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method was used to detect the G-to-A polymorphism of MPO at −463. The PCR amplification was conducted using the primers, 5′-CGG TAT AGG CAC ACA ATG GTG AG-3′ and 5′-GCA ATG GTT CAA GCG ATT CTT C-3′. Aliquots of genomic DNA (30 to 100 ng) were assayed in a 25-µl reaction mixture with 0.2 mM dNTPs, 12.5 pmol of each primer, 1 unit of TaKaRa Taq (TaKaRa Shuzo Co., Ltd.), and 2.5 µl of 10× PCR buffer including 15 mM MgCl₂ (TaKaRa Shuzo Co., Ltd.). Amplification conditions were 2 min of initial denaturation at 94°C, followed by 35 cycles of 30 s each at 94, 56, and 72°C, then 7 min at 72°C. The amplified products were incubated with AciI (New England Biolab, Schwalbach, Germany) for 3 h at 37°C. The digested fragments were visualized on a 3% agarose gel with ethidium bromide staining. Genotypes were distinguished as follows; 169, 120, and 61 bp fragments for the G allele, and 289 and 61 bp fragments for the A allele.

Statistical analysis An unconditional logistic model was applied for estimating odds ratios (ORs) and 95% confidence intervals (95% CIs) with the computer program STATA Version 6 (STATA Corp., College Station, TX). Adjustment was conducted for sex and age (as a continuous variable) in the model.

RESULTS

Table I shows anti-HP antibody-positive rates according to age group and genotypes of MPO. Increase with age was apparent, especially in males. The GG genotype was
associated with a higher infection rate than the GA genotype in males, but not in females. Only two participants were encountered with the AA genotype.

Table II shows sex-age-adjusted ORs and 95% CIs according to lifestyle factors. Smoking status (current, former, and never) adjustment provided the same OR (0.25, 0.09–0.70) for the polymorphism in occasional/no milk drinkers. Fruit intake and physical exercise exerted heterogeneous effects on infection in relation to the IL-1B polymorphism, but no variation in risk was observed for the present MPO polymorphism. Salty food is reported to have an association with infection, but no modification was observed in this study. The ORs were similar among subgroups defined by intake frequencies of salty food.
meat, fish, bean curd, raw vegetables, Japanese tea, and coffee.

The heterogeneous effects of smoking and the genotype on infection were examined in more detail. Current smokers harboring the A allele, non-current smokers with the GG genotype, and non-current smokers harboring the A allele were found to have similar low ORs with respect to current smokers having the GG genotype as the reference group (Fig. 1). The antibody-positive rates were 40.0% (4/10), 60.5% (89/147), 59.0% (23/39), and 77.8% (35/45), respectively. The interaction term obtained from the logistic model including age, sex, genotype, smoking habit (current vs. non-current), and the interaction term between the genotype and smoking habit, was 4.57 ($P=0.08$).

**DISCUSSION**

Genetic susceptibility to pathogenic microbes is a very interesting research field. One good example is the role of the CCR5 polymorphism and HIV infection. The identification of a genotype resistant to HIV infection gave rise to the proposal that a search for genetic polymorphisms related to susceptibility would also be informative for other infectious diseases. Concerning HP infection, however, there have been few reports from other research groups, to our knowledge. We earlier found that an IL-1B functional polymorphism is related to the prevalence of HP, and this prompted the present examination of another inflammation-related enzyme.

The GG genotype of MPO in this study accounted for 79.7% of the subjects. Although there are no reports on this genotype frequency for Japanese in Japan from other research groups, this is greater than the published values for Japanese-Americans in Hawaii (70.6%, n=163), Caucasians (48.8%, n=121), 57.3%, n=171, 61.0%, n=459, and 61.1%, n=270), and African-Americans (49.6%, n=244), but similar to that in Hawaiians (78.6%, n=103). Since the GG genotype has the higher risk genotype for persistent HP infection, the high prevalence rate in Japan could be partly explained by this genetic susceptibility. A similar finding was obtained for the IL-1B polymorphism at $−31.15$

Interactions with smoking are very important with respect to prevention. Here, the highest risk was observed for smokers with the GG genotype. Of interest is the finding that their non-current smoker counterparts had a similar OR to the group harboring the A allele. If the effect of this genotype is restricted only to current smokers, this is naturally good news. Our previous study showed that ex-smokers had a reduced risk of persistent HP infection, suggesting that quitting the habit may increase the probability of spontaneous eradication. There are several papers reporting an association with smoking for inhabitants, outpatients, and participants in eradication programs, although the data are not all consistent. The inconsistency should be re-examined in the light of genetic susceptibility. In addition, biological research is required to unravel the entangled associations among HP infection, smoking, and inflammation-related gene polymorphisms.

The smoking habit partly explained the difference in the OR between males and females, but not that between everyday milk-drinkers and occasional/no milk-drinkers. Although the change in gastric acidity caused by drinking milk could modify the effect of the MPO polymorphism, the difference in the OR might be a random effect. Since this result was obtained in subgroup analysis in the first study, care is required in its interpretation.

Since the MPO gene is located on a different chromosome from that for IL-1B, there is no linkage between the two. Indeed, the genotypes were distributed independently among the present subjects. Further analyses of combination effects with the IL-1B polymorphism did not produce meaningful findings, because the GG genotype of MPO was dominant (79.7%), and the sample size was relatively small.

We have already examined polymorphisms of CYP17 (T-34C), G-2 adrenoceptor (Gln27Glu), β-3 adrenoceptor (Trp54Arg), catechol-O-methyltransferase (Val158Met), methylenetetrahydrofolate reductase (C677T, A1298C), methionine synthase (A2756G), aldehyde dehydrogenase 2 (Glu487Lys), p53 (Arg72Pro), XRCC1 (Arg399Gln), and ERCC2 (Lys751Gln), but none of these demonstrated any clear association with HP infection.

The discovery of genetic susceptibility to HP provides useful information for HP eradication in countries with a high HP prevalence. Since our subjects were outpatients of Aichi Cancer Center Hospital, the association might be modified by the reasons why the participants visited the hospital. Accordingly, studies of the general population are now required to generalize the findings observed in this study.

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