Manganese Superoxide Dismutase Polymorphism and Risk of Gastric Lesions, and Its Effects on Chemoprevention in a Chinese Population

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Abstract

Background: Manganese superoxide dismutase is the primary antioxidant enzyme in the mitochondria and is involved in carcinogenesis. To investigate the association between MnSOD Val16Ala polymorphism and risk of advanced gastric lesions, and its effects on chemoprevention, a population-based study was conducted in Linqu, a high-risk area of gastric cancer in China.

Methods: Genotypes were determined by PCR-RFLP analysis in 3,355 subjects with the baseline histopathologic diagnosis in 1994, and 2,758 of these subjects received subsequent three interventions including vitamin supplementation for 7.3 years. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression model.

Results: We found an increased risk of dysplasia in subjects with the Val/Ala+Ala/Ala genotype (OR, 1.31; 95% CI, 1.02-1.68) compared with the Val/Val genotype. Stratified analysis indicated that a significantly elevated risk of intestinal metaplasia (OR, 3.40; 95% CI, 2.64-4.38) or dysplasia (OR, 4.01; 95% CI, 2.79-5.74) was found in subjects carrying the Val/Ala+Ala/Ala genotype and Helicobacter pylori infection, and an interaction between this genotype and a high serum H. pylori IgG titer (>2.94) on the risk of dysplasia was observed (Pinteraction = 0.01). Furthermore, an elevated chance for regression of gastric lesions was observed in subjects with the Val/Ala+Ala/Ala genotype and high IgG titer in an intervention trial with vitamin supplementation (OR, 2.45; 95% CI, 1.37-4.38).

Conclusions: These findings suggest that Val16Ala polymorphism may play an important role in development of advanced gastric lesions and modify the effect of vitamin supplementation on the evolution of gastric lesions.

Impact: Val16Ala polymorphism is related to gastric cancer development. Cancer Epidemiol Biomarkers Prev; 19(4); 1089–97. ©2010 AACR.

Introduction

Oxidative stress caused by the generation of reactive oxygen species (ROS) is involved in carcinogenesis (1). This stressful condition may appear due to antioxidant depletion, exposure to toxic agents, or pathologic pro- cesses (2), and plays an important role in cancer development by enhancing DNA damage (3). Manganese superoxide dismutase (MnSOD), the primary antioxidant enzyme in the mitochondria, is believed to be a first-line defense against ROS by converting ROS to oxygen (O2) and hydrogen peroxide (H2O2; ref. 4).

A single nucleotide polymorphism in the mitochondrial targeting sequence of MnSOD (rs4880) can result in the change of codon 16 from valine to alanine (5). Val16Ala polymorphism of MnSOD is predicted to alter the secondary structure of MnSOD, which may affect the efficiency of mitochondrial transport of MnSOD (5). A previous study showed that the Ala form of MnSOD allows more efficient MnSOD transport into the mitochondria matrix and generates more active MnSOD compared with the Val form (6). Therefore, the Val form is likely to be associated with higher levels of ROS and a greater risk of cancer. However, several studies have revealed inconsistent results (7-10). A meta-analysis showed that the Ala form of MnSOD allows more efficient MnSOD transport into the mitochondria matrix and generates more active MnSOD compared with the Val form (6). Therefore, the Val form is likely to be associated with higher levels of ROS and a greater risk of cancer. However, several studies have revealed inconsistent results (7-10). A meta-analysis showed that the Ala form was associated with increased risks of different types of cancer (11). Moreover, Val16Ala polymorphism...
had an impact on the effect of antioxidant supplementation. A prospective study showed that among men with the Ala/Ala genotype, \( \beta \)-carotene supplementation can reduce the incidence of prostate cancer, but no significant association was observed in men with the Val/Val or Val/Ala genotype (12).

Linqu County, a rural area in Shandong Province, has one of the highest mortality rates of gastric cancer in the world (age-adjusted rate exceeding 70 deaths per 100,000 males; ref. 13). The prevalence of precancerous gastric lesions is very high (14), and Helicobacter pylori infection is common (15). Reasons for the high rates of gastric cancer and its precursors in this region are still unclear, but \( H. \) pylori infection, cigarette smoking, alcohol drinking, and low consumption of ascorbic acid were identified as risk factors (16-19). Based on the above evidence, we conducted a randomized double-blind factorial trial of one-time antibiotic treatment for \( H. \) pylori infection and 7.3 years of vitamin (including vitamin C, E, and selenium) or garlic supplementation to evaluate the effects on the prevalence of advanced gastric lesions (20).

Because exposure to \( H. \) pylori and other risk factors results in an inflammatory reaction and DNA damage of gastric epithelial cells with the generation of ROS (21-26), and MnSOD is the primary antioxidant enzyme in the mitochondria against ROS, we selected MnSOD as a candidate gene for this study. We investigated the relationship between \( MnSOD \) Val\(^{16}\)Ala polymorphism and the risk of advanced gastric lesions, and the possible interaction between this polymorphism and environmental risk factors. Moreover, because Val\(^{16}\)Ala polymorphism could modify the effect of exogenous antioxidants, we were also interested in assessing whether the effect of interventions including vitamin C, E, and selenium on the transition of gastric lesions was dependent on \( MnSOD \) genotype.

Materials and Methods

Study population

Details of the study population have been described elsewhere (27). Briefly, in 1994, 4,010 residents age 35 to 64 y were identified by a census of 13 randomly selected villages in four towns of Linqu County. A total of 3,599 (89.8%) subjects participated in an endoscopic screening survey and provided blood for serology to detect \( H. \) pylori infection. In 1995, a randomized, double-blind, placebo-controlled, factorial intervention trial was conducted, and 3,365 eligible subjects were randomly assigned to three interventions or placebos, including one-time antibiotic treatment for \( H. \) pylori infection and 7.3 y of vitamin supplementation (including vitamin C, E, and selenium) or garlic supplementation. Details of the study population, design, and randomization of the intervention trial have been previously described (20). Information on age, gender, cigarette smoking, and alcohol drinking was obtained by questionnaires.

For the current study, a total of 3,355 subjects with a baseline histopathologic diagnosis in 1994 were selected to evaluate the association between MnSOD Val\(^{16}\)Ala polymorphism and risks of advanced gastric lesions. Among them, 3,310 subjects participated in the subsequent intervention trial. To assess whether this polymorphism can modify the effect of interventions on the evolution of gastric lesions, 2,758 (83.3%) of 3,310 subjects who completed the intervention trial and had gastric histopathologic diagnosis both in 1994 and 2003 were selected. We examined the differences in age, gender, \( H. \) pylori infection, smoking and drinking status, and baseline pathologic diagnosis between the 3,310 randomized subjects and 2,758 completing trial subjects. The mean age was significantly higher in randomized subjects and other variables were similar (Supplement Table S1). Among 2,758 subjects, 1,887 \( H. \) pylori-positive subjects were randomly assigned to three interventions [antibiotics (884) and/or garlic supplements (933) and/or vitamin supplements (944)] or their placebos (1,003, 954, and 943, respectively), and \( H. \) pylori-negative subjects (871) were randomly assigned to garlic supplements (442) and/or vitamin supplements (434) or their placebos (429 and 437; Supplement Table S2). The study was approved by the Institutional Review Boards of the Beijing Institute for Cancer Research, and all participants provided written informed consent.

Histopathology

Gastroscopy procedures and histopathologic diagnosis criteria were reported previously (13, 28). Biopsies were taken from seven standard sites in the stomach: four from the antrum, one from the angulus, and one each from the lesser and greater curvatures of the body. The biopsies were classified into 10 categories based on histopathologic diagnosis in the Chinese system as follows: normal, superficial gastritis, mild chronic atrophic gastritis (CAG), severe CAG, superficial intestinal metaplasia, deep intestinal metaplasia, mild dysplasia, moderate dysplasia, severe dysplasia, and gastric cancer. Each subject was given a diagnosis that was defined as the severest gastric lesion found in the seven biopsies. To assess the agreement between readers, quality control studies were conducted later by Dr. J.Y. Li and two advisors in America and Europe.

Blood sample collection and DNA preparation

A 5-mL blood sample was collected from each fasting subject. The blood sample was allowed to clot for 30 to 40 min at room temperature and then centrifuged at 965 g for 15 min. The resulting serum was separated into vials. The clot and serum were stored immediately at −20°C and then moved into a −70°C freezer at the Beijing Institute for Cancer Research. High-molecular-weight genomic DNA was isolated by standard proteinase-K digestion and phenol-chloroform extraction from the blood samples.

\( H. \) pylori antibody assays

An antigenic preparation for serology was provided by \( H. \) pylori strains cultured from gastric biopsies of two patients in Linqu County. Serum levels of anti-\( H. \) pylori
IgG and IgA were measured separately in duplicate with ELISA. An individual was determined to be positive for *H. pylori* infection if the mean absorbance for either the IgG or the IgA was ≥1.0, a cutoff value from the examination of a group of *H. pylori*-negative persons and reference sera. Quality control samples were assayed at Vanderbilt University, Nashville, Tennessee.

### Genotyping

*MnSOD* genotyping was done using PCR-RFLP analysis. Genomic DNA was amplified in a 10-μL reaction mixture, containing 50 ng of template, 0.125 μmol/L of each primer (5′-GTAGCACCAGCAGTACTGA-3′ and 5′-GCCGTGATGTCAGGTTMCCAG-3′), 0.25 mmol/L of deoxynucleotide triphosphate, and 0.5 U LA Taq DNA polymerase in 2 × GC BufferII (TaKaRa). PCR was accomplished by an initial denaturing temperature of 95°C for 3 min and subsequent 35 cycles of denaturing (94°C, 1 min), annealing (59.6°C, 45 s), extension (72°C, 1 min), with the last cycle followed by a 10-min extension. PCR products were then digested with *Bsa* WI (60°C, 4 h; New England BioLabs). The digested products were visualized using the Ultra Violet gel imaging system on a 2% agarose gel that contained 0.5 μg/mL ethidium bromide. *MnSOD Val*16*Ala* genotypes were determined as follows: two fragments (343 and 89 bp) for the *Val/Val* genotype, three fragments (432, 343, and 89 bp) for the *Val/Ala* genotype, and one fragment (432 bp) for the *Ala/Ala* genotype.

### Quality control procedures

Rigorous quality control procedures were used throughout the genotyping process. To avoid PCR contamination, reagents of PCR were carefully aliquoted and each aliquot was used no more than thrice. A negative control (no DNA template) was added in each assay to monitor PCR contamination. A pilot study (50 samples) was conducted to optimize the restriction digestion conditions. The gel was read by one or two trained technicians blinded to the diagnosis of each subject and the independent triplicate experiments were done for the dubious samples. After genotyping, approximately 10% to 15% of the samples in each genotype group were selected for repeated assays by PCR-RFLP or PCR-DNA sequencing and the concordance rate was >98%.

### Statistical analysis

Because of limited subjects with normal gastric mucosa (*n* = 5) and superficial gastritis (*n* = 81), they were combined with subjects with mild CAG as the reference group. There were four groups in the present study: normal, superficial gastritis, and mild CAG (reference group; *n* = 1,454); severe CAG (*n* = 133); intestinal metaplasia (*n* = 1,316); and dysplasia (*n* = 452). Serum *H. pylori* IgG titer, which was adopted to indicate the intensity of *H. pylori* infection, was divided into three categories, low (<1.0), middle (1.0-2.94), and high (>2.94), according to its distribution in the study population.

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**Table 1. Selected characteristics and risk factors in different precancerous gastric lesion groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All subjects <em>n</em> = 3,355</th>
<th>Normal, SG, mild CAG* <em>n</em> = 1,454</th>
<th>Severe CAG <em>n</em> = 133</th>
<th>IM <em>n</em> = 1,316</th>
<th>DYS <em>n</em> = 452</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in 1994 (SD)</td>
<td>47.2 (9.2)</td>
<td>45.4 (8.4)</td>
<td>44.1 (8.2)</td>
<td>48.2 (9.3)</td>
<td>50.8 (9.8)</td>
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<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1,684 (50.2)</td>
<td>687 (47.2)</td>
<td>53 (39.8)</td>
<td>660 (50.6)</td>
<td>278 (61.5)</td>
</tr>
<tr>
<td>Female</td>
<td>1,671 (49.8)</td>
<td>767 (52.8)</td>
<td>80 (60.2)</td>
<td>650 (49.4)</td>
<td>174 (38.5)</td>
</tr>
<tr>
<td>H. pylori infection (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2,289 (68.3)</td>
<td>808 (55.6)</td>
<td>111 (83.5)</td>
<td>1,030 (78.3)</td>
<td>340 (75.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>1,064 (31.7)</td>
<td>644 (44.4)</td>
<td>22 (16.5)</td>
<td>286 (21.7)</td>
<td>112 (24.8)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,434 (43.3)</td>
<td>568 (39.5)</td>
<td>41 (31.5)</td>
<td>574 (44.2)</td>
<td>251 (56.5)</td>
</tr>
<tr>
<td>No</td>
<td>1,879 (56.7)</td>
<td>871 (60.5)</td>
<td>89 (68.5)</td>
<td>726 (55.8)</td>
<td>193 (43.5)</td>
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<tr>
<td>Drinking (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,508 (45.6)</td>
<td>630 (43.8)</td>
<td>50 (38.5)</td>
<td>593 (45.7)</td>
<td>235 (53.0)</td>
</tr>
<tr>
<td>No</td>
<td>1,800 (54.4)</td>
<td>808 (56.2)</td>
<td>80 (61.5)</td>
<td>704 (54.3)</td>
<td>208 (47.0)</td>
</tr>
<tr>
<td>P</td>
<td>0.239</td>
<td>0.316</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: SG, superficial gastritis; IM, intestinal metaplasia; DYS, dysplasia.

*Normal, superficial gastritis, and mild CAG as a reference group.

†Compared with the reference group.
To assess whether this polymorphism can modify the effect of interventions on the evolution of gastric lesions, each subject was assigned a global severity score at baseline (A) and end point (B) according to the global histopathologic diagnosis in the Chinese system: 0 for normal, 1 for superficial gastritis, 2 for mild CAG, 3 for severe CAG, 4 for superficial intestinal metaplasia, 5 for deep intestinal metaplasia, 6 for mild dysplasia, 7 for moderate dysplasia, 8 for severe dysplasia, and 9 for gastric cancer. We subtracted score A from score B to determine the evolution status of gastric lesions for each subject. If the difference between score B and A was >0, 0, or <0, then the subject was classified into the progression group, no change group, or regression group, respectively. The effect

| Table 2. Genotype frequencies of the Val^{16}Ala polymorphism in different groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Subjects        | Normal, SG, mild CAG | Severe CAG | IM | DYS |
|                 | n (%) | n (%) | OR (95% CI)* | n (%) | OR (95% CI)* | n (%) | OR (95% CI)* |
| Val/Val         | 1,065 (73.2) | 100 (75.2) | 1.00 | 970 (73.7) | 1.00 | 312 (69.0) | 1.00 |
| Val/Ala         | 364 (25.1) | 31 (23.3) | 1.00 (0.65-1.54) | 320 (24.3) | 1.02 (0.85-1.23) | 127 (28.1) | 1.27 (0.98-1.64) |
| Ala/Ala         | 25 (1.7) | 2 (1.5) | 1.12 (0.25-4.93) | 26 (2.0) | 1.33 (0.74-2.38) | 13 (2.9) | 1.90 (0.90-4.02) |
| Val/Ala+Ala/Ala | 389 (26.8) | 33 (24.8) | 1.01 (0.66-1.53) | 346 (26.3) | 1.04 (0.87-1.24) | 140 (31.0) | 1.31 (1.02-1.68) |

*Adjusted for age, gender, H. pylori infection, smoking status, and drinking status.

To assess whether this polymorphism can modify the effect of interventions on the evolution of gastric lesions, each subject was assigned a global severity score at baseline (A) and end point (B) according to the global histopathologic diagnosis in the Chinese system: 0 for normal, 1 for superficial gastritis, 2 for mild CAG, 3 for severe CAG, 4 for superficial intestinal metaplasia, 5 for deep intestinal metaplasia, 6 for mild dysplasia, 7 for moderate dysplasia, 8 for severe dysplasia, and 9 for gastric cancer. We subtracted score A from score B to determine the evolution status of gastric lesions for each subject. If the difference between score B and A was >0, 0, or <0, then the subject was classified into the progression group, no change group, or regression group, respectively. The effect

| Table 3. Risk of gastric lesions related to the Val^{16}Ala polymorphism by H. pylori infection, smoking and drinking |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| MnSOD genotype | Environmental factors | Normal, SG, mild CAG | Severe CAG | IM | DYS |
|                 |                  | n (%) | n (%) | OR (95% CI)* | n (%) | OR (95% CI)* | n (%) | OR (95% CI)* |
| H. pylori infection |
| Val/Val         | Negative  454 (31.3) | 13 (9.8) | 1.00 | 212 (16.1) | 1.00 | 79 (17.5) | 1.00 |
| Val/Ala+Ala/Ala | Negative  190 (13.1) | 9 (6.8) | 1.78 (0.74-4.30)* | 74 (5.6) | 0.80 (0.58-1.10)* | 33 (7.3) | 0.94 |
| Val/Val         | Positive  610 (42.0) | 87 (65.4) | 5.01 (2.70-9.32)* | 758 (57.6) | 2.89 (2.36-3.53)* | 233 (51.5) | 2.64 |
| Val/Ala+Ala/Ala | Positive  198 (13.6) | 24 (18.0) | 4.31 (2.11-8.83)* | 272 (20.7) | 3.40 (2.64-4.38)* | 107 (23.7) | 4.01 |

*Adjusted for age, gender, H. pylori infection, smoking status, and drinking status.

†Adjusted for age, gender, H. pylori infection, and drinking status.

‡Adjusted for age, gender, H. pylori infection, and smoking status.
of this polymorphism on chemoprevention was obtained by comparing the difference in genotype distribution between regression and no regression (including no change and progression) group or progression and no progression (including no change and regression) group.

The t-test was used to evaluate the difference in mean age of different groups. The Pearson’s χ² test was used to examine the differences in distribution among different groups in gender, H. pylori infection, smoking status, and drinking status. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by the unconditional logistic regression model, adjusted for age, gender, smoking status, drinking status, and H. pylori infection; baseline pathology and three treatments also entered the model when calculating the effects of polymorphism on chemoprevention. Potential interactions between this polymorphism and other gastric cancer risk factors were tested on a multiplicative scale by adding an interaction item into the unconditional logistic regression model. All analyses were done in SPSS (version 15.0; SPSS). P value of <0.05 was considered significant and all statistical tests were two sided.

Results

A total of 3,355 subjects were enrolled in our study, and the mean age was 47.2 ± 9.2 years, with 1,684 males and 1,671 females. The information on H. pylori infection, smoking, and drinking was available for 3,353, 3,313, and 3,308 subjects, respectively. The distribution of age, gender, H. pylori infection, smoking status, and drinking status in subjects with different gastric lesions is presented in Table 1. The mean age was significantly...
higher in subjects with intestinal metaplasia or dysplasia than those with normal/superficial gastritis/mild CAG (reference group), and the percentages of the other variables in dysplasia were significantly different from those in the reference group ($P < 0.001$).

The frequencies of Val allele and Ala allele were 85.5% and 14.5%, whereas the frequency of the Val/Val, Val/Ala, and Ala/Ala genotypes was 72.9%, 25.1%, and 2.0% in the study population, respectively. The distribution of the three genotypes fitted the Hardy-Weinberg equilibrium law ($P = 0.51$).

We compared the distribution of three genotypes in different groups. Because the Ala/Ala homozygote was rare in this population, it was combined with the Val/Ala genotype for subsequent analysis. As shown in Table 2, the frequency of the combined Val/Ala+Ala/Ala genotype in subjects with dysplasia was different from those with normal/superficial gastritis/mild CAG. Multivariate analysis adjusted for age, gender, $H. pylori$ infection, smoking, and drinking revealed a weak overall association between Val16Ala polymorphism and dysplasia risk (OR, 1.31; 95% CI, 1.02-1.68) for subjects carrying the Val/Ala+Ala/Ala genotype.

We also evaluated the association between Val16Ala polymorphism and the risk of gastric lesions by $H. pylori$ infection, smoking status, and drinking status. As shown in Table 3, compared with subjects carrying the Val/Val genotype and are $H. pylori$-negative, a significantly elevated risk of intestinal metaplasia or dysplasia was observed in...
subjects carrying the combined Val/Ala+Ala/Ala genotype and *H. pylori* infection, the OR was 3.40 (95% CI, 2.64-4.38) for intestinal metaplasia and 4.01 (95% CI, 2.80-5.75) for dysplasia. A borderline significant interaction between this genotype and *H. pylori* infection on the risk of intestinal metaplasia ($P_{\text{interaction}} = 0.05$) was found, whereas the interaction on the risk of dysplasia failed to reach a statistical significance ($P_{\text{interaction}} = 0.09$). Compared with nonsmokers carrying the Val/Val genotype, a significantly increased risk of dysplasia was observed in subjects carrying the combined Val/Ala+Ala/Ala genotype and smoking (OR, 1.96; 95% CI, 1.27-3.03), but no evidence of interaction between this genotype and smoking on the risk of dysplasia was found ($P_{\text{interaction}} = 0.61$).

We further evaluated the possible interaction between Val$^{16}$Ala polymorphism and *H. pylori* density, which was indicated by levels of serum *H. pylori* IgG titer in current study, and found a significantly increased risk of advanced gastric lesions in subjects with the combined Val/Ala+Ala/Ala genotype and high serum *H. pylori* IgG titer ($>2.94$). As shown in Table 4, the OR was 5.59 (95% CI, 3.59-8.68) for dysplasia and 3.87 (95% CI, 2.79-5.37) for intestinal metaplasia. There was a strong multiplicative interaction between this polymorphism and high serum *H. pylori* IgG titer for dysplasia ($P_{\text{interaction}} = 0.01$).

We also evaluated whether this polymorphism could modify the effect of each of the three interventions on the evolution of gastric lesions. Among 2,758 subjects completing intervention trial, from baseline in 1994 to 2003, 464 subjects had decreased histopathologic severity score (indicating regression), whereas 1,172 subjects had increased severity score (indicating progression) and 1,122 subjects stayed the same (no change). Information on age, gender, *H. pylori* infection, smoking status and drinking status, and baseline pathologic diagnosis in different evolution groups was presented in Supplement Table S3.

Table 5 shows the distribution of genotypes among 2,758 subjects receiving three interventions in the regression or no regression (including no change and progression) or progression or no progression (including no change and regression) groups. No statistically significant effect was found between any genotype and evolution of gastric lesions in subjects receiving *H. pylori* treatment, garlic, or vitamin supplementation. However, stratified analysis by serum anti-*H. pylori* IgG titer indicated that in subjects with high IgG titer ($>2.94$) and who received the vitamin supplementation, a significantly elevated chance for regression was associated with the Val/Ala+Ala/Ala genotype (OR, 2.45; 95% CI, 1.37-4.38; Table 6).

**Discussion**

In an area of high-risk gastric cancer, we examined the relationship between MnSOD Val$^{16}$Ala polymorphism and the risk of advanced gastric lesions, and its effect on chemoprevention. To our best knowledge, this is the first study to evaluate such association. We found that Ala carriers had an increased risk of dysplasia, especially for those with severe *H. pylori* infection, and there was a significant interaction between this polymorphism and *H. pylori* seropositivity. Furthermore, we found that vitamin supplementation had a favorable effect on the evolution of precancerous gastric lesions for Ala carriers with high-grade *H. pylori* infection. Our results suggest that Val$^{16}$Ala polymorphism may play an important role in the development of advanced gastric lesions and may modify the effect of vitamin supplementation on the evolution of gastric lesions.

MnSOD, one of the important antioxidant enzymes in mitochondria, is believed to be a safeguard against oxidative damage. It has been suggested that genetic polymorphisms of MnSOD may have effects on tumor development (4, 29), whereas few of those polymorphisms may influence the function of MnSOD. Studies showed that Val$^{16}$Ala, Ile$^{58}$Thr, or -102C>T polymorphism has a functional relevance (6, 30, 31), and the mechanism of Val$^{16}$Ala polymorphism is relatively clearer. A study showed that Val$^{16}$Ala polymorphism could affect the efficiency of the mitochondrial transport of MnSOD, and the Ala form was 30% to 40% more efficient than the Val form (6). Moreover, it has been proven that Val$^{16}$Ala polymorphism could interact with exogenous antioxidants (2, 12, 32), which makes Val$^{16}$Ala polymorphism a candidate to evaluate the effect on chemoprevention. However, epidemiologic studies yielded mixed results about the relationship between this polymorphism and risks of cancers. Our study is consistent with major studies on cancers (2, 10, 11, 32-34), and provides evidence that Val$^{16}$Ala polymorphism is associated with the increased risks of advanced gastric lesions and can modify the effect of vitamin supplementation.

A balance between ROS generation and oxidative defense exists in cells in normal condition. MnSOD can convert superoxide anion (O$_2^-$), the primary form of ROS (35), to O$_2$ and H$_2$O$_2$, and the latter is further catalyzed into H$_2$O by catalase or glutathione peroxidase (GPx). Hence, high levels of MnSOD (as in Ala form) may lead to enzyme imbalance between the activity of MnSOD and GPx or catalase; thus, H$_2$O$_2$ will accumulate and can potentially react to yield more toxic hydroxyl radical (OH’), which is highly reactive to DNA (11). Recent studies established a relation between the levels of H$_2$O$_2$ and carcinogenesis, which could further explain the association between MnSOD polymorphism and cancer risk. According to these studies, an increased H$_2$O$_2$ level is associated with decreased sensitivity to tumor necrosis factor $\alpha$-mediated apoptosis (36) and can mediate a higher rate of cell proliferation by activating a specific mitogen-activated protein kinase pathway, suggesting the carcinogenic role of H$_2$O$_2$ (37).

*H. pylori* infection is the major contributor to the development of gastric cancer and precancerous gastric lesions in Linqu (15, 20, 38). *H. pylori* infection–induced chronic inflammation could cause oxidative stress by producing ROS, including O$_2^-$ (22-25). It has been shown that *H. pylori*-related gastritis is accompanied by an increased oxygen free radical formation and peroxidative damage (22, 39). Our data also indicated that an elevated risk of dysplasia was
observed among Ala carriers with H. pylori infection, particularly for those with high serum H. pylori IgG titer. Higher levels of serum H. pylori IgG are associated with higher H. pylori density (40, 41) and more severe gastric inflammation (41-43), which is associated with severer stressful condition (44). Thus, in those subjects with high H. pylori IgG titer and Ala allele, which represented a higher activity form, subsequently increased levels of H2O2 could promote the progression of precancerous gastric lesions.

Cigarette smoking is an established risk factor for oxidative stress and a study of male heavy smokers in Finland suggested that subjects carrying the Ala/Ala genotype had a 70% increase in risk for prostate cancer (45). We observed an elevated risk for dysplasia in Ala carriers who smoked, but the interaction failed to reach a statistical significance. Moreover, a mixed result on the risk of intestinal metaplasia suggested that further studies are needed to elucidate whether smoking could modify the effect of Val16Ala polymorphism on the risk of advanced gastric lesions.

Several studies have found that Val16Ala polymorphism of MnSOD interacted with exogenous antioxidants. A previous study showed that breast cancer risk was elevated most pronouncedly among premenopausal women with the Ala/Ala genotype who had low consumption of dietary antioxidants (2). Another study reported a similar result that the Ala/Ala genotype was associated with an increased risk of prostate cancer in subjects with low levels of antioxidants (32). Moreover, a study found that among men with the Ala/Ala genotype, β-carotene supplementation can reduce the incidence of prostate cancer (12).

Although our intervention trial indicated that vitamin supplementation had no significant favorable effects on the evolution of precancerous gastric lesions (20), in the current study, we found that vitamin supplementation had a beneficial effect on the evolution of precancerous gastric lesions for Ala carriers with heavy H. pylori infection. Our findings are consistent with previous studies (2, 12, 32) and suggest that the effect of vitamin supplementation on the evolution of gastric lesions was partially dependent on MnSOD Val16Ala genotype.

In heavily H. pylori–infected Ala carriers, H2O2 accumulates in cells, which may lead to enzyme imbalance and induce toxicity if GPx activity is low or vitamin intake is inadequate. Vitamin C and vitamin E are important antioxidants, and selenium is a crucial component of GPx. Thus, in our intervention trial, vitamin supplementation (including vitamin C, E, and selenium) might ameliorate the oxidative stress in heavily H. pylori–infected Ala carriers and subsequently promoted the regression of gastric lesions. Our finding also suggests that the further vitamin supplementation should be specific to those genetically predisposed to lower activity of antioxidant defense system.

This study has several strengths. First, a large sample size, prospective design, and long-term follow-up allowed us to assess the association between MnSOD polymorphism and risks of advanced gastric lesions as well as its effects on chemoprevention. Second, detailed information, such as H. pylori infection and smoking or drinking status, allowed us to control for those factors and to investigate the possible gene-environment interaction.

Our study also has some potential limitations. Because limited gastric cancer cases (n = 28) were identified at baseline, we could not evaluate the relationship between this polymorphism and gastric cancer risk. Another drawback is that we only analyzed single gene polymorphism and the risk of gastric lesions and its effects on chemoprevention. However, cumulated evidence shows MnSOD is an important antioxidant enzyme involved in H. pylori–associated carcinogenesis and Val16Ala polymorphism may modify the effect of exogenous antioxidants.

In summary, our population-based study provided evidence that MnSOD Val16Ala polymorphism was associated with an increased risk of dysplasia, especially for those with severe H. pylori infection. An interaction between this polymorphism and H. pylori seropositivity suggested that heavy H. pylori infection could affect the effect of MnSOD polymorphism on the risk of advanced gastric lesions. Our study further indicated that Val16Ala polymorphism could modify the relationship between vitamin supplementation and the evolution of gastric lesions, suggesting that the effect of vitamin supplementation on the transition of gastric lesions may be partially dependent on MnSOD genotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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