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Research Article

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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 Val*^{16}*Ala* 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 ($P_{interaction}$ = 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 *Val*^{16}*Ala* 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: *Val*^{16}*Ala* 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 processes (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 (O_2) and hydrogen peroxide (H_2O_2; 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). *Val*^{16}*Ala* 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 was associated with increased risks of different types of cancer (11). Moreover, *Val*^{16}*Ala* polymorphism

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had an impact on the effect of antioxidant supplementation. A prospective study showed that among men with the *Ala/Ala* genotype, β-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 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*

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

Variable	All subjects *n* = 3,355	Normal, SG, mild CAG* *n* = 1,454	Severe CAG *n* = 133	IM *n* = 1,316	DYS *n* = 452
Mean age in 1994 (SD)	47.2 (9.2)	45.4 (8.4)	44.1 (8.2)	48.2 (9.3)	50.8 (9.8)
P			0.095	<0.001	<0.001
Gender (%)	*n* = 3,355	*n* = 1,454	*n* = 133	*n* = 1,316	*n* = 452
Male	1,684 (50.2)	687 (47.2)	53 (39.8)	666 (50.6)	278 (61.5)
Female	1,671 (49.8)	767 (52.8)	80 (60.2)	650 (49.4)	174 (38.5)
P†			0.102	0.077	<0.001
H. pylori infection (%)	*n* = 3,353	*n* = 1,452	*n* = 133	*n* = 1,316	*n* = 452
Positive	2,289 (68.3)	808 (55.6)	111 (83.5)	1,030 (78.3)	340 (75.2)
Negative	1,064 (31.7)	644 (44.4)	22 (16.5)	286 (21.7)	112 (24.8)
P			<0.001	<0.001	<0.001
Smoking (%)	*n* = 3,313	*n* = 1,439	*n* = 130	*n* = 1,300	*n* = 444
Yes	1,434 (43.3)	568 (39.5)	41 (31.5)	574 (44.2)	251 (56.5)
No	1,879 (56.7)	871 (60.5)	89 (68.5)	726 (55.8)	193 (43.5)
P			0.075	0.013	<0.001
Drinking (%)	*n* = 3,308	*n* = 1,438	*n* = 130	*n* = 1,297	*n* = 443
Yes	1,508 (45.6)	630 (43.8)	50 (38.5)	593 (45.7)	235 (53.0)
No	1,800 (54.4)	808 (56.2)	80 (61.5)	704 (54.3)	208 (47.0)
P			0.239	0.316	0.001

Abbreviations: SG, superficial gastritis; IM, intestinal metaplasia; DYS, dysplasia.
*Normal, superficial gastritis, and mild CAG as a reference group.
†Compared with the reference group.

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′-GTAGCACCAGCACTAGCAGCAT-3′and 5′-GCGTTGATGTGAGGTTCCAG-3′), 0.25 mmol/L of deoxynucleotide triphosphate, and 0.5U 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 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 (0.59-1.49)*
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 (1.96-3.58)*
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 (2.79-5.74)*
Smoking status								
Val/Val	No	651 (45.2)	68 (52.3)	1.00	517 (39.8)	1.00	138 (31.1)	1.00
Val/Ala+Ala/Ala	No	220 (15.3)	21 (16.2)	1.00 (0.59-1.69)†	209 (16.1)	1.30 (1.03-1.64)†	55 (12.4)	1.22 (0.85-1.76)†
Val/Val	Yes	401 (27.9)	29 (22.3)	0.91 (0.47-1.75)†	441 (33.9)	1.31 (1.00-1.72)†	169 (38.0)	1.41 (0.96-2.07)†
Val/Ala+Ala/Ala	Yes	167 (11.6)	12 (9.2)	0.93 0.42-2.05)†	133 (10.2)	1.00 (0.72-1.39)†	82 (18.5)	1.96 (1.27-3.03)†
Drinking status								
Val/Val	No	594 (41.3)	61 (46.9)	1.00	509 (39.2)	1.00	147 (33.2)	1.00
Val/Ala+Ala/Ala	No	214 (14.9)	19 (14.6)	0.97 (0.56-1.68)‡	195 (15.0)	1.17 (0.92-1.49)‡	61 (13.8)	1.25 (0.87-1.78)‡
Val/Val	Yes	457 (31.8)	36 (27.7)	0.91 (0.53-1.58)‡	448 (34.6)	1.00 (0.80-1.27)‡	160 (36.1)	0.88 (0.62-1.24)‡
Val/Ala+Ala/Ala	Yes	173 (12.0)	14 (10.8)	0.97 (0.47-2.00)‡	145 (11.2)	0.90 (0.67-1.22)‡	75 (16.9)	1.20 (0.80-1.82)‡

*Adjusted for age, gender, 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.

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Table 4. Risk of gastric lesions related to the *Val[16]Ala* polymorphism by serum *H. pylori* IgG titer

MnSOD Genotype	IgG titer	Normal, SG, mild CAG	Severe CAG		IM		DYS	
		n (%)	*n* (%)	OR (95% CI)*	*n* (%)	OR (95% CI)	*n* (%)	OR (95% CI)
Val/Val	<1	479 (33.2)	15 (11.3)	1.00	227 (17.3)	1.00	81 (18.0)	1.00
Val/Ala+Ala/Ala	<1	204 (14.2)	8 (6.1)	1.32 (0.55-3.21)	80 (6.1)	0.80 (0.58-1.09)	35 (7.8)	0.96 (0.61-1.50)
Val/Val	1~2.94	289 (20.0)	36 (27.3)	3.96 (2.09-7.51)	361 (27.4)	2.82 (2.25-3.54)	122 (27.1)	3.02 (2.15-4.24)
Val/Ala+Ala/Ala	1~2.94	101 (7.0)	14 (10.6)	4.49 (2.06-9.75)	137 (10.4)	3.30 (2.42-4.51)	46 (10.2)	3.44 (2.19-5.39)
Val/Val	>2.94	289 (20.0)	49 (37.1)	5.37 (2.90-9.94)	381 (29.0)	3.05 (2.43-3.82)	107 (23.8)	2.60 (1.84-3.67)
Val/Ala+Ala/Ala	>2.94	81 (5.6)	10 (7.6)	4.00 (1.71-9.34)	129 (9.8)	3.87 (2.79-5.37)	59 (13.1)	5.59 (3.59-8.68)

*Adjusted for age, gender, smoking status, and drinking 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 χ^2 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

Table 5. *Val[16]Ala* polymorphism and its effects on chemoprevention

	Regression	No regression	OR (95% CI; regression vs no regression)	Progression	No progression	OR (95% CI; progression vs no progression)
	n (%)	*n* (%)		*n* (%)	*n* (%)	
H. pylori treatment						
Val/Val	109 (69.4)	533 (73.3)	1.00	284 (72.3)	358 (72.9)	1.00
Val/Ala+Ala/Ala	48 (30.6)	194 (26.7)	1.06 (0.71-1.58)*	109 (27.7)	133 (27.1)	1.07 (0.79-1.45)*
Garlic supplementation						
Val/Val	170 (71.4)	851 (74.8)	1.00	429 (74.0)	592 (74.5)	1.00
Val/Ala+Ala/Ala	68 (28.6)	286 (25.2)	1.13 (0.81-1.56)†	151 (26.0)	203 (25.5)	1.07 (0.83-1.38)†
Vitamin supplementation						
Val/Val	147 (69.0)	863 (74.1)	1.00	431 (75.1)	579 (72.0)	1.00
Val/Ala+Ala/Ala	66 (31.0)	302 (25.9)	1.28 (0.92-1.07)‡	143 (24.5)	225 (28.0)	0.83 (0.64-1.06)‡

*Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, garlic supplementation, and vitamin supplementation.

†Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, *H. pylori* treatment, and vitamin supplementation.

‡Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, *H. pylori* treatment, and garlic supplementation.

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Table 6. *Val*^{16}*Ala* polymorphism and its effects on chemoprevention by serum *H. pylori* IgG titer

	Regression	No regression	OR (95% CI; regression vs no regression)	Progression	No progression	OR (95% CI; progression vs no progression)
	n (%)	*n* (%)		*n* (%)	*n* (%)	
Serum IgG titer: <1						
Garlic supplement						
Val/Val	72 (71.3)	271 (75.5)	1.00	122 (74.4)	221 (74.7)	1.00
Val/Ala+Ala/Ala	29 (28.7)	88 (24.5)	1.17 (0.69-1.97)*	42 (25.6)	75 (25.3)	1.12 (0.71-1.75)*
Vitamin supplement						
Val/Val	63 (73.3)	253 (70.7)	1.00	108 (72.0)	208 (70.7)	1.00
Val/Ala+Ala/Ala	23 (26.7)	105 (29.3)	0.90 (0.52-1.56)†	42 (28.0)	86 (29.3)	0.90 (0.58-1.41)†
Serum IgG titer: 1-2.94						
H. pylori treatment						
Val/Val	52 (67.5)	255 (73.3)	1.00	127 (69.4)	180 (74.4)	1.00
Val/Ala+Ala/Ala	25 (32.5)	93 (26.7)	1.06 (0.58-1.91)‡	56 (30.6)	62 (25.6)	1.31 (0.84-2.04)‡
Garlic supplement						
Val/Val	52 (74.3)	280 (71.8)	1.00	144 (70.9)	188 (73.2)	1.00
Val/Ala+Ala/Ala	18 (25.7)	110 (28.2)	0.86 (0.46-1.59)*	59 (29.1)	69 (26.8)	1.05 (0.68-1.61)*
Vitamin supplement						
Val/Val	46 (76.7)	287 (74.5)	1.00	152 (73.8)	181 (75.7)	1.00
Val/Ala+Ala/Ala	14 (23.3)	98 (25.5)	0.92 (0.45-1.85)†	54 (26.2)	58 (24.3)	1.04 (0.66-1.65)†
Serum IgG titer: >2.94						
H. pylori treatment						
Val/Val	49 (68.1)	264 (74.4)	1.00	151 (75.5)	162 (71.4)	1.00
Val/Ala+Ala/Ala	23 (31.9)	91 (25.6)	1.30 (0.73-2.31)‡	49 (24.5)	65 (28.6)	0.84 (0.54-1.31)‡
Garlic supplement						
Val/Val	40 (67.8)	289 (77.5)	1.00	156 (76.5)	173 (75.9)	1.00
Val/Ala+Ala/Ala	19 (32.2)	84 (22.5)	1.39 (0.73-2.65)*	48 (23.5)	55 (24.1)	1.12 (0.70-1.80)*
Vitamin supplement						
Val/Val	34 (54.8)	311 (77.0)	1.00	169 (78.6)	176 (70.1)	1.00
Val/Ala+Ala/Ala	28 (45.2)	93 (23.0)	2.45 (1.37-4.38)†	46 (21.4)	75 (29.9)	0.72 (0.46-1.12)†

NOTE: Among 2,758 subjects, 2,707 had serum *H. pylori* IgG titer, and 852 of them were assigned to *H. pylori* treatment, 1,352 to garlic supplements, and 1,355 to vitamin supplements.

*Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, *H. pylori* treatment, and vitamin supplement.

†Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, *H. pylori* treatment, and garlic supplement.

‡Adjusted for age, gender, *H. pylori* infection, smoking status, drinking status, baseline pathology, garlic supplement, and vitamin supplement.

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 *Val*^{16}*Ala* 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 *Val*^{16}*Ala* 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

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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 ($P_{interaction}$ = 0.05) was found, whereas the interaction on the risk of dysplasia failed to reach a statistical significance ($P_{interaction}$ = 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 ($P_{interaction}$ = 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 ($P_{interaction}$ = 0.01).

We also evaluated whether this polymorphism can 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) and 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_2O_2, and the latter is further catalyzed into H_2O 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_2O_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_2O_2 and carcinogenesis, which could further explain the association between *MnSOD* polymorphism and cancer risk. According to these studies, an increased H_2O_2 level is associated with decreased sensitivity to tumor necrosis factor α–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_2O_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

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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 titers and *Ala* allele, which represented a higher activity form, subsequently increased levels of H_2O_2 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 $Val^{16}Ala$ polymorphism on the risk of advanced gastric lesions.

Several studies have found that $Val^{16}Ala$ 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* $Val^{16}Ala$ genotype.

In heavily *H. pylori*–infected *Ala* carriers, H_2O_2 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 $Val^{16}Ala$ polymorphism may modify the effect of exogenous antioxidants.

In summary, our population-based study provided evidence that *MnSOD* $Val^{16}Ala$ 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 $Val^{16}Ala$ 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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