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Association between genetic polymorphisms of DNA base excision repair genes and evolution of precancerous gastric lesions in a Chinese population

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Base excision repair pathway may play an important role in repairing DNA damage related to *Helicobacter pylori*-induced inflammatory process. To evaluate the association between genetic polymorphisms of X-ray repair cross-complementing group 1 (*XRCC1*, *Arg194Trp* and *Arg399Gln*), adenosine diphosphate ribosyl transferase (*ADPRT*, *Val762Ala*), 8-oxoguanine DNA glycosylase (*OGG1*, *Ser326Cys*) and apurinic/apyrimidinic endonuclease 1 (*APE1*, *Asp148Glu*) and evolution of *H.pylori*-associated precancerous gastric lesions, a population-based cohort study was conducted in Linqu County, a high-risk area of gastric cancer in China. Genotypes were determined by polymerase chain reaction (PCR)-based denaturing high-performance liquid chromatography and PCR–restriction fragment length polymorphism analysis in 1281 *H.pylori*-infected subjects. We found that subjects carrying the combined *XRCC1-194Arg/Trp+Trp/Trp* genotype had an elevated chance of regression of gastric lesions [adjusted odds ratio (OR) = 1.44; 95% confidence interval (CI) = 1.06–1.96], whereas subjects carrying the *XRCC1-399Arg/Gln+Gln/Gln* genotype had a decreased chance of regression (OR = 0.68; 95% CI = 0.49–0.92). Stratified analysis indicated that an increased risk of progression was observed in subjects carrying the *XRCC1-399Arg/Gln+Gln/Gln* genotype (OR = 1.60; 95% CI = 1.09–2.36) or *OGG1-326Ser/Cys+Cys/Cys* genotype (OR = 1.95; 95% CI = 1.03–3.71) with intestinal metaplasia or dysplasia at baseline or carrying the *XRCC1-399Arg/Gln+Gln/Gln* genotype and smoking (OR = 1.58; 95% CI = 1.02–2.45). Furthermore, a significantly increased risk of progression was observed in subjects carrying one or two hazard genotypes of *XRCC1-399* or *OGG1-326*, the OR was 2.83 (95% CI = 1.32–6.08), 2.22 (95% CI = 1.24–3.98) or 2.27 (95% CI = 1.26–4.10), respectively. These findings suggest that genetic polymorphisms in XRCC1-*Arg194Trp*, *XRCC1-Arg399Gln* and *OGG1-Ser326Cys* may play important roles in the evolution of *H.pylori*-associated gastric lesions in this high-risk population.

Introduction

Gastric cancer (GC) is the second most common cause of cancer death worldwide, including China (1). Infection with *Helicobacter pylori* causes chronic atrophic gastritis (CAG) and is considered a risk factor in the development of GC (2,3). Linqu County, a rural area in Shandong Province of northeast China, has one of the highest mortality rates of GC in the world (age-adjusted rate exceeding 70 deaths per 100 000 males) (4). The prevalence of precancerous gastric lesions is very high and *H.pylori*-seropositive rate >70% (5). Our 4.5-year gastroscopy-based cohort study in Linqu provides evidence that GC is the end point of a multistep progression of precancerous gastric lesions, and the presence of *H.pylori* at baseline is associated with an increased risk of progression to dysplasia (DYS) and GC (6). Furthermore, a randomized intervention trial revealed that eradication of *H.pylori* had a significant decrease of 40% in the prevalence of severe CAG, intestinal metaplasia (IM) and DYS, as well as favorable effects on GC (7).

Although *H.pylori* infection is the major contributor to the etiology of GC in Linqu, the rates of progression of gastric lesions were different among individuals with a spectrum of precancerous gastric lesions at baseline, and only a small proportion of infected patients developed GC (6). Genetic polymorphism may play important roles in *H.pylori*-associated processes of inflammation and carcinogenesis.

Helicobacter pylori infection-induced chronic inflammation could cause DNA damage to the adjacent epithelial cells by producing reactive oxygen species and nitrogen species (8–11). Base excision repair (BER) pathway plays a key role in repairing DNA damage due to cellular metabolism. Multiple proteins are involved in the BER pathway, in which X-ray repair cross-complementing group 1 (XRCC1), adenosine diphosphate ribosyl transferase (ADPRT), apurinic/apyrimidinic endonuclease 1 (APE1) and 8-oxoguanine DNA glycosylase (OGG1) are major proteins (12,13).

Functional variants of *XRCC1*, *ADPRT*, *APE1* and *OGG1* can alter BER functions and may play an important role in the evolution of gastric lesions. *XRCC1-Arg194Trp*, *XRCC1-Arg399Gln*, *ADPRT-Val762Ala*, *OGG1-Ser326Cys* and *APE1-Asp148Glu* are five common candidate single-nucleotide polymorphisms that cause an amino acid change. *XRCC1-Arg194Trp* is located in an evolutionary conserved linker region, suggesting its functional significance (14–16). Individuals with *Arg/Arg* genotype exhibited highly increased chromosomal breaks (17). *XRCC1-Arg399Gln* occurs in the poly(ADP-ribose) polymerase-binding domain, which may alter the efficiency of the repair process (17,18). The *ADPRT-Val762Ala* polymorphism is located in the sixth helix of catalytic regulatory domain, which may reduce the ability of ADPRT to recruit XRCC1 and other proteins (19,20). *OGG1-Ser326Cys* may lower the OGG1 substrate specificity and capacity to excise 8-oxoguanine due to remodeling of its phosphorylation status and cellular localization (21–24). For *APE1-Asp148Glu* polymorphism, study found that *Glu/Glu* carriers were more sensitive to ionizing radiation than *Asp* carriers (25).

In the present study, we hypothesized that the genetic variants of *XRCC1-194*, *XRCC1-399*, *ADPRT-762*, *OGG1-326* and *APE1-148* were associated with the evolution of *H.pylori*-associated gastric lesions. We tested this hypothesis in a cohort of 1281 *H.pylori*-infected subjects and evaluated the gene–environment and gene–gene joint effects on the risk of progression of gastric lesions. No study on DNA repair gene polymorphisms and evolution of gastric lesions in a high-risk population has yet been reported.

Materials and methods

Study subjects

A 4.5-year follow-up study of gastric lesions and a subsequent intervention study in a population in Linqu were described previously (6,7). Briefly, an endoscopic screening survey was launched in 1989 among 3399 residents aged 35–64 years, and the subjects without GC diagnosis were subsequently followed with a repeat of endoscopic examination conducted in 1994. All subjects provided blood for serology to detect *H.pylori* infection at baseline in 1989. In 1995, a randomized, placebo-controlled intervention trial was conducted in

Abbreviations: ADPRT, adenosine diphosphate ribosyl transferase; APE1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; CAG, chronic atrophic gastritis; CI, confidence interval; DHPLC, denaturing high-performance liquid chromatography; DYS, dysplasia; GC, gastric cancer; IM, intestinal metaplasia; OGG1, 8-oxoguanine DNA glycosylase; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SG, superficial gastritis; XRCC1, X-ray repair cross-complementing group 1.

Linqu, and 2456 of these residents agreed to participate in the subsequent intervention trial.

For the current study, a total of 1281 *H.pylori*-seropositive subjects completing the 4.5-year follow-up and intervention study were selected to evaluate the association between genetic polymorphisms of BER genes and evolution of *H.pylori*-associated gastric lesions. The study was approved by the Institutional Review Board of Peking University School of Oncology, and all subjects gave written informed consent.

Histopathology

Details of the gastroscopy procedures and histopathologic criteria have been described previously (4,6). Briefly, for each subject, biopsy samples were taken at seven standard sites in the stomach mucosa in 1989 and 1994 and given its corresponding histopathologic diagnosis by three senior pathologists independently. Each biopsy was classified according to the presence or absence of superficial gastritis (SG), CAG (mild or severe), IM (superficial or deep), DYS (mild, moderate or severe) or GC. Each biopsy was given a diagnosis based on the most severe histology, and each subject was assigned a 'global' diagnosis based upon the most severe diagnosis among any of the biopsies.

Helicobacter pylori antibody assays

Helicobacter pylori antibody assays were used for determination of *H.pylori* infection status in 1989. Details of serologic assay were described previously (5). In brief, serum levels of anti-*H.pylori* IgG and IgA were measured separately in duplicate with enzyme-linked immunosorbent assay procedures. Quality control samples were assayed at Vanderbilt University, Nashville, TN. An individual was determined to be positive for *H.pylori* infection if the mean optical density for either the IgG or the IgA was >1.0.

Blood sample collection and DNA preparation

A 5 ml blood sample was collected from each subject and allowed to clot for 30–40 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 freezer at −70°C within 2 or 3 days after collection. High molecular weight genomic DNA was isolated by standard proteinase K digestion and phenol–chloroform extraction from the blood sample.

Genotyping

Genotyping was analyzed by polymerase chain reaction (PCR)-based denaturing high-performance liquid chromatography (DHPLC) or PCR–restriction fragment length polymorphism (RFLP) analysis. PCR for each sample was accomplished with a 20 μl reaction mixture containing 100 ng of genomic DNA, 0.5 mM of each primer, 0.2 mM of diethylnitrophyenyl thiophosphate, 2.0 mM of $MgCl_2$ and 0.5 U GoTaq® DNA polymerase in 5× reaction buffer (Promega, Madison, WI). The genotypes revealed by DHPLC or RFLP analysis were further confirmed by DNA sequencing with ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA). The primer sequences, PCR annealing temperatures and genotyping methods are listed in Table I.

DHPLC was performed on a Transgenomic WAVE System (Transgenomic, Omaha, NE) for analysis of *XRCC1-Arg399Gln* and *APE1-Asp148Glu* polymorphisms. Detailed genotyping of DHPLC process was described previously (26). PCR products were denatured for 1 min at 94°C and then gradually reannealed by decreasing the sample temperature from 94 to 45°C over a period of 30 min to form homo and/or hetero duplexes. The PCR products were then applied to the DHPLC column at an optimal oven temperature and eluted with a linear acetonitrile gradient at a flow rate of 0.9 ml/min.

For PCR-based RFLP analysis, the amplified fragments were digested with appropriate restriction endonucleases and then resolved on 2% agarose gels containing ethidium bromide and visualized under UV light. For *XRCC1-Arg194Trp* polymorphism genotyping, a 8.5 μl aliquot was digested with 2.5 U PvuII restriction enzyme (New England Biolabs, Beverly, MA) at 37°C for 4 h. The three possible genotypes were revealed by three distinct patterns of bands seen on the gel: *Arg/Arg* (485 bp), *Arg/Trp* (485, 396 and 89 bp) and *Trp/Trp* (396 and 89 bp). For *ADPRT-Val762Ala*, a 7.5 μl aliquot was digested with 0.6 U EciI restriction enzyme (New England Biolabs) at 37°C for 1 h. The three possible genotypes were revealed by three distinct patterns of bands seen on the gel: *Val/Val* (425 bp), *Val/Ala* (425, 282 and 143 bp) and *Ala/Ala* (282 and 143 bp). For *OGG1-Ser326Cys*, a 8.75 μl aliquot was digested with 1.25 U Fnu4HI restriction enzyme (New England Biolabs) at 37°C for 4 h. The three possible genotypes were revealed by three distinct patterns of bands seen on the gel: *Ser/Ser* (338 bp), *Ser/Cys* (338, 247 and 91 bp) and *Cys/Cys* (247 and 91 bp).

Quality control procedures

Rigorous quality control procedures were applied throughout the genotyping process. To avoid PCR contamination, reagents for PCR were carefully aliquoted and each aliquot was used no more than three times. For each assay, a negative control (no DNA template) was added to monitor PCR contamination. Approximately 10–15% of the samples in each genotype group were randomly selected for repeated assays by PCR following by DHPLC or RFLP.

Statistical methods

Each subject was assigned a global severity score at baseline (A) and end point (B) according to its global histopathologic diagnosis in the Chinese system: 0 for normal, 1 for SG, 2 for mild CAG, 3 for severe CAG, 4 for superficial IM, 5 for deep IM, 6 for mild DYS, 7 for moderate DYS, 8 for severe DYS and 9 for GC. We used (B–A) to determine the evolution status of gastric lesions for each subject. If (B–A) was >0, $=0$ or <0, then this subject was classified into progression group, no change group or regression group, respectively.

Each case was interviewed using a questionnaire to obtain details on basic information such as age, gender, cigarette smoking and alcohol drinking before endoscope examination. Participants reporting smoking or drinking up to the date of the interview were identified as current smokers or drinkers. For baseline pathology, the subjects were divided into four groups: SG or mild CAG, severe CAG, IM and DYS. The Pearson's χ^2 test was used to examine the overall differences among different evolution groups in gender, smoking, drinking and baseline pathology and one-way analysis of variance for the age variable. Association of genetic polymorphism with evolution status was measured by risk of the progression and chance of the regression. Risk of the progression or chance of the regression was obtained by comparing the difference in genotype distribution between progression and no progression (including regression and no change) group or regression and no regression (including progression and no change) group. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association of polymorphisms with evolution status were computed by unconditional logistic regression, adjusting for age, gender, smoking, drinking and baseline lesions.

For gene–smoking or gene–drinking joint effect analysis, different gene–smoking or gene–drinking combination categories were transformed into dummy variables and then except the category as reference, all the other categories entered the unconditional logistic regression model to calculate the adjusted ORs and 95% CIs for each category as compared with the reference. We tested the null hypotheses of multiplicativity and evaluated the departures from multiplicative interaction models. Departures from these multiplicative models were assessed by including main effect variables and their product terms by using the likelihood-ratio test approach to compare logistic regression models with and without product terms. Multifactor

Table I. Primer sequences, PCR and genotyping methods for detection of gene polymorphisms

Polymorphisms	Primer sequence 5′–3′	PCR annealing temperature (°C)	PCR product size (bp)	Genotyping methods	DHPLC oven temperature (°C)
XRCC1-Arg194Trp	F: 5′-GCCAGGGCCCCTCCTTCAA-3′ R: 5′-TACCCTCAGACCCACGAGT-3′	65.2	485	PCR–RFLP	—
XRCC1-Arg399Gln	F: 5′-GCCCCTCAGATCACACCTAA-3′ R: 5′-ATTGCCCAGCACAGGATAAG-3′	60.5	213	PCR–DHPLC	64.0
ADPRT-Val762Ala	F: 5′-CAGGAGGGTTTGCCATT-3′ R: 5′-TCTCAAAGGACCACCAG-3′	60.1	425	PCR–RFLP	—
OGG1-Ser326Cys	F: 5′-AGTCTCACCAGCCCTGAC-3′ R: 5′-CTGTCTCCCTCAATATCCC-3′	59.2	338	PCR–RFLP	—
APE1-Asp148Glu	F: 5′-CTCTGCCCCACCTCTTGATTGC-3′ R: 5′-TTGCGAAAGG CTTCATCCCA-3′	65.8	213	PCR–DHPLC	59.0

dimensionality reduction method was applied for screening possible gene–gene interactions by comparison of cross-validation consistency and sign test *P* value (27). Then the subsequent methods for analysis of gene–gene joint effect were similar to what applied in gene–smoking and gene–drinking analysis. All statistical analyses were carried out using Statistical Analysis System software (version 9.0; SAS Institute, Cary, NC).

Results

A total of 1281 subjects were enrolled in this study. The mean age of the subjects at baseline was 44.9 ± 8.2 years. Among those, 601 were male and 680 were female. Information on smoking and drinking status was available for 1170 subjects. The distribution of gastric lesions was 31.2% for SG/mild CAG, 10.3% for severe CAG, 35.8% for IM and 22.7% for DYS among the subjects at baseline in 1989 versus 31.5, 4.1, 47.9 and 16.2% among the subjects in 1994, respectively.

From baseline in 1989–1994, 408 subjects had decreased histopathologic severity score (indicating regression), whereas 374 subjects had increased severity score (indicating progression) and 499 subjects stayed the same (no change). Table II shows the frequency distribution of age, gender, smoking and drinking status and baseline pathology in different evolution groups. The distributions of age, gender, smoking and drinking status were similar in different groups. However, the percentages of baseline pathology in regression, progression and no change groups were significantly different.

To evaluate the association between genetic variants and evolution of gastric lesions, we compared the frequency distribution of five polymorphisms in the regression and no regression or progression and no progression group. As shown in Table III, there were no significant differences between different groups in the frequencies of the variant genotypes of *ADPRT-Val762Ala*, *OGG1-Ser326Cys* and *APE1-Asp148Glu*. However, the frequencies of *XRCC1-Arg194Trp* and *XRCC1-Arg399Gln* genotypes in the regression group were different from those in the no regression group. Multivariate analysis adjusted for age, gender, smoking, drinking and baseline pathology showed that subjects carrying the *XRCC1-194Arg/Trp* genotype (OR = 1.42; 95% CI = 1.02–1.97) or the combined *Arg/Trp+Trp/Trp* genotype (OR = 1.44; 95% CI = 1.06–1.96) had an elevated chance of regression. However, a significantly decreased chance of regression was associated with either the *XRCC1-399Arg/Gln* genotype (OR = 0.68; 95% CI = 0.49–0.93) or the combined *Arg/Gln+Gln/Gln* genotype (OR = 0.68; 95% CI = 0.49–0.92).

The risks of progression or chances of regression related to these polymorphisms were further examined with stratification by baseline pathology. Since the subjects with severe CAG or DYS were a few in our study, we combined severe CAG with SG or mild CAG and DYS with IM. No significant associations were found between genotypes of any loci and evolution of gastric lesions in subjects with SG or CAG (data not shown). However, in subjects with IM or DYS (Table IV), a significantly elevated chance for regression was associated with the combined *XRCC1-194Arg/Trp+Trp/Trp* genotype (OR = 1.38; 95% CI = 1.02–1.89), and an increased risk for progression was associated with the combined *XRCC1-399Arg/Gln+Gln/Gln* genotype (OR = 1.60; 95% CI = 1.09–2.36) or *OGG1-326Ser/Cys+Cys/Cys* genotype (OR = 1.95; 95% CI = 1.03–3.71).

We also evaluated the association between genetic polymorphisms and evolution of gastric lesions by smoking and drinking status. As shown in Table V, we found that risk of the progression of gastric lesions was more pronounced in subjects carrying the combined *XRCC1-399Arg/Gln+Gln/Gln* genotype and smoking (OR = 1.58; 95% CI = 1.02–2.45), and similar result was observed among subjects carrying the *ADPRT-762Val/Ala+Ala/Ala* genotype and smoking (OR = 1.59; 95% CI = 1.03–2.45). However, the risks of progression were significantly elevated for subjects in each combined category of smoking status and *OGG1-326* genotypes. Compared with subjects carrying *326Ser/Ser* genotype and non-smoking, >1-fold increased progression risks were found in subjects who carried *326Ser/Cys+Cys/Cys* genotype and non-smoked (OR = 2.07; 95% CI = 1.12–3.84), *326Ser/Ser* genotype and smoked (OR = 2.60; 95% CI = 1.18–5.72) and *326Ser/Cys+Cys/Cys* genotype and smoked (OR = 2.51; 95% CI = 1.32–4.79). There were no statistically significant associations of these genetic variants with evolution of gastric lesions by drinking status (data not shown).

To testify the potential gene–gene joint effect in the same BER pathway, we further evaluated the association between the combined genotypes and risk of progression. A significantly increased risk of progression was observed in combined *XRCC1-399* and *OGG1-326* genotypes. As shown in Table VI, when the combined homogeneous wild genotype of *XRCC1/OGG1* was used as the reference, a significantly increased risk of progression was observed in subjects carrying one or two hazard genotypes of *XRCC1* or *OGG1*. The OR was 2.83 (95% CI = 1.32–6.08), 2.22 (95% CI = 1.24–3.98) or 2.27 (95% CI = 1.26–4.10), respectively. No significant association was found with other combined genotypes (data not shown).

Discussion

In a high-risk population of GC, we investigated a panel of five BER gene polymorphisms and their association with the evolution of *H.pylori*-associated precancerous gastric lesions. To our best knowledge, this is the first study exploring the impacts of genetic polymorphisms of BER genes on evolution of gastric lesions. We found an elevated chance of regression of gastric lesions among *XRCC1-194Trp* carriers. Furthermore, we observed a significant association

Table II. Selected characteristics and risk factors in subjects of different evolution groups

Variable	Total, *n* = 1281	Regression, *n* = 408	No change, *n* = 499	Progression, *n* = 374	*P*
Mean age in years (SD)	44.9 ± 8.2	44.6 ± 8.2	45.3 ± 8.3	44.7 ± 8.1	0.339
Gender (%)					0.052
Male	601 (46.9)	204 (50.0)	213 (42.7)	184 (49.2)	
Female	680 (53.1)	204 (50.0)	286 (57.3)	190 (50.8)	
Smoking (%)					0.438
Yes	551 (43.0)	163 (40.0)	213 (42.7)	175 (46.8)	
No	619 (48.3)	208 (51.0)	242 (48.5)	169 (45.2)	
Missing	111 (8.7)	37 (9.0)	44 (8.8)	30 (8.0)	
Drinking (%)					0.982
Yes	510 (39.8)	161 (39.5)	201 (40.3)	148 (39.6)	
No	660 (51.5)	210 (51.5)	254 (50.9)	196 (52.4)	
Missing	111 (8.7)	37 (9.0)	44 (8.8)	30 (8.0)	
Baseline pathology (%)					<0.001
SG/mild CAG	400 (31.2)	10 (2.4)	220 (44.1)	170 (45.5)	
Severe CAG	132 (10.3)	61 (15.0)	11 (2.2)	60 (16.0)	
IM	458 (35.8)	120 (29.4)	199 (39.9)	139 (37.2)	
DYS	291 (22.7)	217 (53.2)	69 (13.8)	5 (1.3)	

Table III. Genotype frequencies of five polymorphisms in different evolution groups

	Regression, *n* (%)	No regression, *n* (%)	OR (95% CI)[a] (regression versus no regression)	Progression, *n* (%)	No progression, *n* (%)	OR (95% CI)[a] (progression versus no progression)
XRCC1-Arg194Trp						
Arg/Arg	167 (41.0)	428 (49.0)	1.00	177 (47.3)	418 (46.1)	1.00
Arg/Trp	188 (46.2)	360 (41.2)	1.42 (1.02–1.97)	163 (43.6)	385 (42.5)	1.05 (0.79–1.40)
Trp/Trp	52 (12.8)	85 (9.8)	1.53 (0.92–2.55)	34 (9.1)	103 (11.4)	0.83 (0.51–1.34)
Arg/Trp+Trp/Trp	240 (59.0)	445 (51.0)	1.44 (1.06–1.96)	197 (52.7)	488 (53.9)	1.00 (0.76–1.32)
XRCC1-Arg399Gln						
Arg/Arg	247 (60.8)	447 (51.2)	1.00	189 (50.5)	505 (55.8)	1.00
Arg/Gln	148 (36.5)	394 (45.1)	0.68 (0.49–0.93)	173 (46.3)	369 (40.8)	1.19 (0.90–1.57)
Gln/Gln	11 (2.7)	32 (3.7)	0.63 (0.25–1.58)	12 (3.2)	31 (3.4)	0.95 (0.42–2.15)
Arg/Gln+Gln/Gln	159 (39.2)	426 (48.8)	0.68 (0.49–0.92)	185 (49.5)	400 (44.2)	1.17 (0.89–1.54)
ADPRT-Val762Ala						
Val/Val	212 (52.2)	421 (48.2)	1.00	182 (48.7)	451 (49.8)	1.00
Val/Ala	162 (39.9)	339 (38.8)	0.82 (0.59–1.13)	156 (41.7)	345 (38.1)	1.30 (0.97–1.75)
Ala/Ala	32 (7.9)	113 (13.0)	0.72 (0.42–1.24)	36 (9.6)	109 (12.1)	0.79 (0.50–1.24)
Val/Ala+Ala/Ala	194 (47.8)	452 (51.8)	0.80 (0.59–1.08)	192 (51.3)	454 (50.2)	1.16 (0.88–1.52)
OGG1-Ser326Cys						
Ser/Ser	64 (15.7)	126 (14.4)	1.00	46 (12.3)	144 (15.9)	1.00
Ser/Cys	198 (48.7)	409 (46.9)	0.95 (0.60–1.49)	180 (48.1)	427 (47.1)	1.37 (0.89–2.08)
Cys/Cys	145 (35.6)	338 (38.7)	0.91 (0.57–1.46)	148 (39.6)	335 (37.0)	1.34 (0.87–2.05)
Ser/Cys+Cys/Cys	343 (84.3)	747 (85.6)	0.93 (0.60–1.43)	328 (87.7)	762 (84.1)	1.35 (0.91–2.02)
APE1-Asp148Glu						
Asp/Asp	138 (33.9)	280 (32.1)	1.00	119 (31.8)	299 (33.0)	1.00
Asp/Glu	202 (49.6)	429 (49.1)	1.09 (0.77–1.53)	186 (49.7)	445 (49.1)	1.02 (0.75–1.39)
Glu/Glu	67 (16.5)	164 (18.8)	0.92 (0.59–1.45)	69 (18.5)	162 (17.9)	0.99 (0.66–1.47)
Asp/Glu+Glu/Glu	269 (66.1)	593 (67.9)	1.04 (0.76–1.44)	255 (68.2)	607 (67.0)	1.01 (0.75–1.35)

[a]Adjusted for age, gender, drinking, smoking and baseline pathology.

Table IV. Genotype frequencies of polymorphisms in different evolution groups among subjects with IM or DYS at baseline

	Regression, *n* (%)	No regression, *n* (%)	OR (95% CI)[a] (regression versus no regression)	Progression, *n* (%)	No progression, *n* (%)	OR (95% CI)[a] (progression versus no progression)
XRCC1-Arg194Trp						
Arg/Arg	133 (39.5)	199 (48.3)	1.00	66 (45.8)	266 (44.0)	1.00
Arg/Trp+Trp/Trp	204 (60.5)	213 (51.7)	1.38 (1.02–1.89)	78 (54.2)	339 (56.0)	0.93 (0.63–1.37)
XRCC1-Arg399Gln						
Arg/Arg	208 (61.9)	215 (52.2)	1.00	71 (49.3)	352 (58.3)	1.00
Arg/Gln+Gln/Gln	128 (38.1)	197 (47.8)	0.64 (0.47–0.88)	73 (50.7)	252 (41.7)	1.60 (1.09–2.36)
ADPRT-Val762Ala						
Val/Val	174 (51.8)	191 (46.4)	1.00	68 (47.2)	297 (49.2)	1.00
Val/Ala+Ala/Ala	162 (48.2)	221 (53.6)	0.79 (0.58–1.07)	76 (52.8)	307 (50.8)	1.16 (0.79–1.71)
OGG1-Ser326Cys						
Ser/Ser	50 (14.8)	60 (14.6)	1.00	13 (9.0)	97 (16.0)	1.00
Ser/Cys+Cys/Cys	287 (85.2)	352 (85.4)	0.91 (0.59–1.40)	131 (91.0)	508 (84.0)	1.95 (1.03–3.71)
APE1-Asp148Glu						
Asp/Asp	115 (34.1)	136 (33.0)	1.00	44 (30.6)	207 (34.2)	1.00
Asp/Glu+Glu/Glu	222 (65.9)	276 (67.0)	0.92 (0.67–1.27)	100 (69.4)	398 (65.8)	1.25 (0.83–1.90)

[a]Adjusted for age, gender, drinking and smoking status.

between the *XRCC1-399* or *OGG1-326* polymorphism and risk of progression in subjects with IM or DYS at baseline, suggesting that these genetic variants could play important roles in the transition of *H.pylori*-related gastric lesions.

Although the molecular mechanisms underlying *H.pylori*-induced processes of inflammation and carcinogenesis are unknown, two principal mechanisms have been proposed for these processes: hyperproliferation of gastric epithelial cells and oxidative damage of stomach mucosa (28). *Helicobacter pylori* is able to induce polymorphonuclear and mononuclear cell accumulation, which produce reactive oxygen species or reactive nitrogen species that cause DNA damage to the adjacent epithelial cells (8–11). It has been demonstrated that *H.pylori*-related gastritis is accompanied by an increased oxygen free radical formation and peroxidative damage (29,30). The BER pathway is one of the important mechanisms responsible for the repair of DNA damage, which can specifically remove alterations of a single base that has been methylated or oxidized or rectify single-strand interruptions in DNA (12,13). XRCC1 or ADPRT can temporarily bind to single-strand interruptions in DNA and act to recruit repair proteins (19,31–33). OGG1 is an enzyme for removing 8-oxoguanine from DNA, which is a major base damage produced by reactive oxygen species (21,22). APE1, a multifunctional enzyme, is responsible for the repair of apurinic/apyrimidinic sites in DNA and also functions as a redox factor facilitating the DNA-binding capability of activator protein-1 (25,32,33). Functional polymorphisms of these genes are candidates that may contribute to the susceptibility to cancer, as well as evolution of precancerous lesions.

Accumulating evidence suggests that reduced DNA repair capacity caused by genetic polymorphism of BER genes is associated with increased risk of lung, breast, skin or GC (14,18,20,31,32,34–36); however, little has been published on such association with evolution of *H.pylori*-associated gastric lesions. Previous studies have identified that *XRCC1-194Trp* allele was a protective allele against various types

Table V. Association between genetic polymorphisms and evolution of gastric lesions by smoking

	Regression versus no regression				Progression versus no progression			
	Non-smoker	OR (95% CI)[a]	Smoker	OR (95% CI)[a]	Non-smoker	OR (95% CI)[a]	Smoker	OR (95% CI)[a]
XRCC1-Arg194Trp								
Arg/Arg	85/209	1.00	66/180	0.88 (0.53–1.47)	89/205	1.00	76/170	1.11 (0.72–1.71)
Arg/Trp+Trp/Trp	122/202	1.48 (0.97–2.26)	97/208	1.21 (0.73–1.99)	80/244	0.84 (0.58–1.24)	99/206	1.34 (0.88–2.05)
XRCC1-Arg399Gln								
Arg/Arg	125/198	1.00	105/217	0.72 (0.46–1.15)	82/241	1.00	93/229	1.37 (0.90–2.07)
Arg/Gln+Gln/Gln	81/213	0.58 (0.38–0.88)	58/171	0.57 (0.34–0.95)	87/207	1.20 (0.82–1.77)	82/147	1.58 (1.02–2.45)
ADPRT-Val762Ala								
Val/Val	110/204	1.00	90/191	1.00 (0.62–1.61)	84/230	1.00	84/197	1.15 (0.75–1.74)
Val/Ala+Ala/Ala	96/207	0.93 (0.62–1.41)	73/197	0.64 (0.39–1.05)	85/218	0.99 (0.68–1.45)	91/179	1.59 (1.03–2.45)
OGG1-Ser326Cys								
Ser/Ser	32/56	1.00	26/58	0.88 (0.38–2.00)	15/73	1.00	27/57	2.60 (1.18–5.72)
Ser/Cys+Cys/Cys	175/355	0.94 (0.52–1.68)	137/330	0.77 (0.41–1.46)	154/376	2.07 (1.12–3.84)	148/319	2.51 (1.32–4.79)
APE1-Asp148Glu								
Asp/Asp	72/140	1.00	56/114	0.82 (0.47–1.42)	63/149	1.00	47/123	0.91 (0.55–1.51)
Asp/Glu+Glu/Glu	135/271	1.02 (0.66–1.57)	107/274	0.86 (0.53–1.40)	106/300	0.77 (0.52–1.16)	128/253	1.26 (0.82–1.93)

[a]Adjusted for age, gender, drinking and baseline pathology.

Table VI. Possible joint effect of *XRCC1-399* and *OGG1-326* polymorphisms associated with the evolution of gastric lesions

Combined genotypes		Regression versus no regression		Progression versus no progression	
		n/n	OR (95% CI)[a]	*n/n*	OR (95% CI)[a]
XRCC1-399	*OGG1-326*				
Arg/Arg	*Ser/Ser*	46/65	1.00	17/94	1.00
Arg/Arg	Ser/Cys+Cys/Cys	201/382	0.78 (0.45–1.34)	172/411	2.22 (1.24–3.98)
Arg/Gln+Gln/Gln	Ser/Ser	17/61	0.41 (0.17–0.97)	29/49	2.83 (1.32–6.08)
Arg/Gln+Gln/Gln	Ser/Cys+Cys/Cys	142/365	0.56 (0.32–0.98)	156/351	2.27 (1.26–4.10)

[a]Adjusted for age, gender, smoking, drinking and baseline pathology.

of cancer including GC (14,35,37,38). In our study, we found an inverse association between *XRCC1-194Arg/Trp+Trp/Trp* genotype and the risk of progression of gastric lesions, suggesting that *194Trp* allele may play a protective effect in this population. Several studies have shown that *XRCC1-399Gln* allele was associated with an increased risk of breast cancer and tobacco-related cancers, whereas *OGG1-326Cys* allele with increased risk of lung, esophageal and prostate cancers (24,38,39), whereas the results drawn from GC were inconsistent (35,36,40). Our study suggested that the *XRCC1-399Gln* carriers had an elevated risk of progression of gastric lesions, consisting with the results of previous studies (38,39). Moreover, the *OGG1-326Ser/Cys+Cys/Cys* genotype was associated with an increased risk of progression in subjects with IM or DYS at baseline, indicating that the *326Cys* allele was a hazard allele in this high-risk population. However, we could not find an association between *ADPRT-Val762Ala* or *APE1-Asp148Glu* variants and risk of progression of gastric lesions.

Our previous study in Linqu County indicated that rates of the progression increased significantly with the severity of gastric lesions (6), suggesting that baseline pathology was a major risk factor impacting the transitions of gastric lesions. Therefore, we controlled for baseline pathology by stratified analysis as well as for age, gender, smoking and drinking status in our study. We found no significant associations between any genotypes from these loci and evolution of gastric lesions in subjects with SG or CAG. However, in subjects with IM or DYS, a significant association was observed between the combined *XRCC1-399Arg/Gln+Gln/Gln* genotype or *OGG1-326Ser/Cys+Cys/Cys* genotype and risk of progression, suggesting that these variants affected transitions primarily in advanced gastric lesions.

Numerous investigations have shown that SG or CAG is a relatively common *H.pylori*-associated inflammatory condition and may be reversible. Indeed, nearly half of the subjects with severe CAG at baseline had less advanced lesions, 4.5 years later in our previous cohort study (6). There may be many factors contributing to the inverse inflammatory process (2,3), and the possible main roles of other factors and pathways could reduce the power to detect the effects of genetic variants of BER genes. However, in IM or DYS, cells replicate excessively with abnormal differentiation and disorganization and an accumulation of DNA damage, as a consequence, GC risk was increased in advanced gastric lesions (41,42). BER is a protective mechanism against DNA damage (12,13); therefore, the genetic polymorphisms of BER genes could play an important role in the transitions of advanced gastric lesions with extensive and severe DNA damage.

Cigarette smoking is an established risk factor for GC (43), and our previous follow-up study in Linqu also indicated that smoking is a risk factor for progression to DYS or GC (44,45). In the present study, although the interaction between the genotypes of *XRCC1-399* or *ADPRT-762* and smoking failed to reach a statistical significance, we found that subjects carrying the *XRCC1-399* or *ADPRT-762* risk genotype and smoking significantly elevated the risk of progression. These results suggested that there may be possible gene–environment joint effects between the BER gene polymorphisms and smoking. We also found that *OGG1-326* polymorphism or smoking might be an independent risk factor for progression of gastric lesions.

Considering potential joint effects among variants involved in the same BER pathway, we also evaluated the gene–gene joint effect in the present study. A significantly increased risk of progression was observed in subjects carrying one or two hazard genotypes of *XRCC1-399* or *OGG1-326*. However, there was no statistical evidence for gene–gene interaction in multiplicative interaction models. It is possible that this study had a limited study power to detect such an interaction. Moreover, since our study sample size was not large enough, we could not test the possible three-way gene–gene–environment joint effects in our study.

Our study has some limitations. Potential selection bias may have occurred because all subjects selected in our study were *H.pylori* positive. However, the design of this study was to evaluate the association between genetic variants and evolution of *H.pylori*-associated gastric lesions, and such association would be detected by this design. In addition, our relatively large sample was selected from a well-defined population over a 4.5-year follow-up, and the gastroscopy-based study allowed us to investigate the genetic factor associated with the risk of gastric lesions. Another potential drawback is that we only analyzed five candidate loci of BER gene polymorphisms, and studies on the relationship between genetic variations of other genes in BER pathway and even in other pathways and transition of gastric lesions are required in the future.

In conclusion, our population-based study provided evidence that genetic polymorphisms of *XRCC1-Arg194Trp*, *XRCC1-Arg399Gln* and *OGG1-Ser326Cys* were associated with the evolution of *H.pylori*-associated gastric lesions, suggesting that genetic variants of BER genes could play important roles in the evolution of precancerous gastric lesions.

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