RESEARCH ARTICLE

Lack of Association of Common Polymorphisms in *MUC1* Gene with *H. pylori* Infection and Non-cardia Gastric Cancer Risk in a Chinese Population

Bin Zhang[1,2&], Guang-Yu Hao[1&], Fang Gao[1], Jian-Zu Zhang[2], Cheng-Jiang Zhou[1], Li-She Zhou[1], Ying Wang[1], Yan-Bin Jia[1*]

Abstract

Several lines of evidence support the notion that *MUC1* is often aberrantly expressed in gastric cancer, and it is a ligand for *Helicobacter pylori*. Genetic variation in *MUC1* gene may confer susceptibility to *H. pylori* infection and gastric cancer. We assessed the association of common polymorphisms in *MUC1* gene with *H. pylori* infection and non-cardia gastric cancer using an LD-based tag SNP approach in north-western Chinese Han population. A total of four SNPs were successfully genotyped among 288 patients with non-cardia gastric cancer and 281 age- and sex-matched controls. None of the tested SNPs was associated with *H. pylori* infection. SNP rs9426886 was associated with a decreased risk of non-cardia gastric cancer, but lost significance after adjustment for multiple testing. Overall, our data indicated that common genetic variations in *MUC1* gene might not make a major contribution to the risk of *H. pylori* infection and non-cardia gastric cancer in our studied population.

Keywords: Non-cardia gastric cancer - *MUC1* - *H. pylori* infection - polymorphism - risk

Asian Pac J Cancer Prev, **14 (12)**, 7355-7358

Introduction

Gastric cancer, the second most common cause of cancer-related death in the world, is characterized as a multifactorial disease that results from individual genetic predisposition and exposure to environmental factors (Brenner et al., 2009). According to anatomic site, gastric cancer can be classified as cardia and non-cardia subtypes. In non-cardia gastric cancer, Helicobacter pylori (*H. pylori*) infection is an established etiological factor (Peek and Blaser, 2002). However, nearly half of the world's population is infected with *H. pylori*, but only a very small proportion of those infected eventually develop non-cardia gastric cancer (Peek and Blaser, 2002), suggesting that genetic factors of host may play an important role in gastric carcinogenesis.

Upon infection, *H. pylori* primarily resides within the mucus layer, adhering to mucins, high molecular weight glycoproteins and major components of the protective layer across the upper mucous surfaces (Peek and Blaser, 2002; Lindén et al., 2008). Some works have documented the role of *MUC1* in the *H. pylori* infection and gastric cancer risk. Thus, *MUC1* is often aberrantly expressed in stomach cancer, and it is a ligand for *H. pylori* (Ng et al., 2008). Susceptibility to *H. pylori* infection and gastric cancer appears to be associated with *MUC1* allele length (Costa et al., 2008; Vinall et al., 2002). Recently, A functional single nucleotide polymorphism (SNP) rs4072037 in *MUC1* gene has been associated with risk of gastric cancer (Xu et al., 2009; Jia et al., 2010; Zhang et al., 2011) with controversial results (Abnet et al., 2010; He et al., 2013). Because gastric cancer has potential genetic heterogeneity among different populations, the current study was conducted to investigate whether common polymorphisms in *MUC1* gene were associated with *H. pylori* infection, and with non-cardia gastric cancer risk in north-western Chinese Han population.

Materials and Methods

Subjects

This case control study was carried out in Baotou, Inner Mongolian Autonomous Region, north-western China. Patients were consecutively recruited between June 2008 and December 2010 at the cancer hospital of Baotou. All patients with histopathologically confirmed incident non-cardia gastric cancer were enrolled. The exclusion criteria of cases included having previous cancer, any metastasized cancer (carcinomas were not originally from stomach) and having previous radiotherapy or chemotherapy. The controls, frequency-matched to the cases on age (± 5 years) and sex, were cancer-free individuals randomly selected from a community health examination program in Baotou during the same period

[1]*School of Basic Medicine, Baotou Medical College,* [2]*The First Affiliated Hospital of Baotou Medical College, Baotou, China* [&]*Equal contributors* **For correspondence: jyb690318@hotmail.com*

Table 1. Demographic Data of Study Subjects

Variable	Controls n (%)	Cases n (%)	*P*-value
Overall	281 (100)	288 (100)	
Gender			
Male	220 (78.3)	224 (77.8)	0.88
Female	61 (21.7)	64 (22.2)	
Age			
Mean±SD (year)	59.10±11.57	59.48±11.23	0.69

Table 2. Association Between Studied SNPs and *H. pylori* Infection in Controls

SNPs	Genotype	n[a]	*H. pylori* (+)[a]	Percentage	OR[b]	95% CI
rs4072037	AA	177	72	40.7	1	Reference
	AG	92	42	45.7	1.260	0.755-2.105
	GG	12	8	66.7	3.003	0.867-10.401
rs2990245	TT	192	86	44.8	1	Reference
	CT	77	31	40.3	0.833	0.486-1.427
	CC	6	2	33.3	0.586	0.104-3.293
rs9628662	GG	169	73	43.2	1	Reference
	GT	90	37	41.1	0.927	0.551-1.559
	TT	17	8	47.1	1.154	0.423-3.148
rs9426886	TT	157	68	43.3	1	Reference
	AT	65	30	46.2	1.123	0.627-2.011
	AA	10	3	30	0.558	0.139-2.238

[a]Sum of column did not add up to total study subjects because of missing data; [b]Adjusted for age and sex

the patients were recruited. The selection criteria for the controls included no individual history of cancer, and no previous diagnosis of gastric disease or genetic disease. All subjects were unrelated ethnic Han Chinese from Baotou, north-western China, and the minor distinct ethnic groups and migrants from other regions were not included. At recruitment, informed consent was obtained from each subject, and the study was approved by the institutional review board of Baotou Medical College.

Diagnosis of H. pylori infection

The presence of *H. pylori* infection was determined using a commercial IgG ELISA kit (Biohit, Helsinki, Finland) in controls. According to the instruction of the kit, 30 EIU or higher was regarded as seropositive.

Selection of SNPs

We used HapMap Phase 2 information for Chinese Han population (http://www.hapmap.org) to select tagSNPs by Tagger algorithm as implemented in Haploview. The region analyzed included 20 kb upstream of the first exon and 10 kb downstream of the termination of the last exon. Parameters of $r^2>0.8$ and a minor allele frequency (MAF) ≥0.05 in Chinese Han population were used for tagSNP selection. The final selection comprised 4 SNPs. Additionally, SNP rs4072037 determining the major splicing variants of *MUC1* and regulating *MUC1* expression in the gastric epithelium (Ng et al., 2008; Xu et al., 2009) was also selected for this study.

Genotyping

Genomic DNA was isolated by standard proteinase K digestion and phenol-chloroform extraction from the blood sample. Among all subjects, SNP rs4072037 was genotyped by sequence-specific primers-polymerase chain reaction (PCR-SSPs) as described elsewhere (Xu et al., 2009), but with a little modification. Briefly, the forward primer was 5'-CTATGGGCACAGAGAAGGAG-3' (primer 1), the reverse SSPs 5'-AGCTTGCATGACCAGAACCC-3' (primer 2) and 5'-AGCTTGCATGACCAGAACCT-3' (primer 3) were used in combination with the consensus forward primer. The primer 1 and 2 led to the allele G, while the primer 1 and 3 led to the allele A. PCRs were conducted in 25 μl volumes containing 60 ng genomic DNA, 200 μM dNTPs, 0.2 μM each upstream and downstream primer, 1×PCR buffer, and 1 U Taq DNA polymerase. The primers and reagents were purchased from Takara Inc., Japan. The PCR protocol was 95°C for 1 min, 5 cycles with 95°C for 40 sec, 70°C for 45 sec, 72°C for 30 sec, then followed by 21 cycles of 95°C for 40 sec, 63°C for 50 sec, 72°C for 30 sec, and then 8 cycles of 95°C for 50 sec, 55°C for 60 sec, 72°C for 90 sec. The PCR product size was 233 bp. More than 10% of the samples were randomly selected for the repeated assays by different persons, and the results were 100% consistent. Genotyping of other four SNPs were performed at Chinese national human genome center, Beijing, using TaqMan method (Applied Biosystems, Foster City, CA, USA). SNP rs3738808 was excluded because of genotyping failure. The genotyping rates of other three SNPs were all above 90% and failed genotypes were not repeated. For quality control purpose, genotyping was performed with blinding to the subjects' case and control status. In addition, six negative experimental controls (water) and six positive experimental controls with known genotype were included in each reaction plate.

Statistical analysis

The student's t- and χ^2 test were used to evaluate differences in the distributions of age and sex between cases and controls, respectively. Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test. Haplotypes were constructed based on the LD blocks derived from the Haploview 4.0 program. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age and sex for non-cardia gastric cancer risk and *H. pylori* infection in association with genotypes and haplotypes. Statistical significance was assessed by empirical p values derived from the Westfall & Young permutation (n=10,000) to account for type I errors of multiple testing (Westfall and Young, 1993). All statistical analyses were conducted by SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

There were 281 controls and 288 cases in the study. The demographic of subjects are summarized in Table 1. There were no significant differences between the two groups with respect to the age and gender distribution.

The infection rate of *H. pylori* among controls was 43.6%. All studied SNPs followed Hardy-Weinberg equilibrium in controls. None of the four SNPs analyzed

Table 3. Association Between Studied SNPs and Non-cardia Gastric Cancer

SNPs	Genotype	Controls n (%)[a]	Cases n (%)[a]	OR (95% CI)[b]
rs4072037	AA	177 (63.0)	179 (62.6)	Reference
	AG	92 (32.7)	92 (33.2)	1.022 (0.717-1.456)
	GG	12 (4.3)	12 (4.2)	0.990(0.433-2.265)
rs2990245	TT	192 (69.8)	222 (77.6)	Reference
	CT	77 (28.0)	61 (21.4)	0.689 (0.467-1.017)
	CC	6 (2.2)	3 (1.0)	0.433(0.106-1.758)
rs9628662	GG	169 (61.2)	184 (65.2)	Reference
	GT	90 (32.6)	88 (31.3)	0.900 (0.627-1.291)
	TT	17 (6.2)	10 (3.5)	0.543(0.242-1.220)
rs9426886	TT	157 (67.7)	214 (75.4)	Reference
	AT	65 (28.0)	66 (23.2)	0.747 (0.500-1.116)
	AA	10 (4.3)	4 (1.4)	0.290(0.089-0.942)[c]

[a]Sum of column did not add up to total study subjects because of missing data; [b]Adjusted for age and sex; [c]The permutation *p* value >0.05

demonstrated a significant association with *H. pylori* infection (Table 2). Minor allele homozygote of rs9426886 was associated with a decreased risk of non-cardia gastric cancer when compared with its major allele homozygote, but was not significant after adjustment for multiple testing. No significant association was detected between other three SNPs and non-cardia gastric cancer risk (Table 3).

Based on the LD data in our study, four SNPs formed one main block and two singletons. rs2990245 and rs9628662 were in one block, but neither the status of *H. pylori* infection nor the risk of non-cardia gastric cancer was significantly associated with any haplotypes in our population (data not shown).

Discussion

To our best knowledge, this is the first comprehensive study to investigate the association of common polymorphisms in *MUC1* gene with *H. pylori* infection and non-cardia gastric cancer risk by tagSNP approach in Chinese population. None of the four studied SNPs showed a statistically significant association with the status of *H. pylori* infection and the overall risk of non-cardia gastric cancer in our studied population.

Gastric cancer includes non-cardia gastric and cardia gastric carcinoma. Two subtypes might have different clinical, epidemiological, etiological, and molecular features (Kim et al., 2005; Correa, 2004). So we restricted our samples to patients with non-cardia gastric cancer to supply relatively more homogeneous samples and improve the power of association study.

One population-based case-control study have been conducted to investigate the association between polymorphisms in *MUC1* gene and *H. pylori* infection as well as gastric cancer risk by a comprehensive LD-based tagSNP approach in Polish population (Jia et al., 2010). The results indicated that common polymorphisms were not associated with *H. pylori* infection, which was in agreement with ours. On the contrary, the study showed all tested tagSNPs were significantly associated with an increased risk of gastric cancer. The inconsistency of two studies may be due to the different ethnic population with genetic heterogeneity. Further studies are needed to clarify the possible mechanisms.

SNP rs4072037, located at the 5' end of exon 2 in *MUC1* gene, controls the alternative splicing of *MUC1* (Xu et al., 2009). In our study, the frequencies of AA, AG, and GG genotypes in controls were 62.7, 32.4, and 4.9%, respectively, which was similar to the data from other studies in Chinese population (Xu et al., 2009; Zhang et al., 2011) and those from HapMap Chinese data. And our results indicated that SNP rs4072037 was not significantly associated with non-cardia gastric cancer. To date, several reports about the relationship between rs4072037 and gastric cancer have been published. Xu et al reported that the AA genotype of SNP rs4072037 was significantly associated with an increased risk of gastric cancer based on 138 cases and 241 controls from north-eastern Chinese (Xu et al., 2009). But their controls consisted of 131 patients with superficial gastritis and 110 patients with atrophic gastritis. And atrophic gastritis is the well-established precursor lesion of gastric cancer. Moreover, their study did not provide information for association between rs4072037 and non-cardia gastric cancer. Furthermore, the same group failed to identify that rs4072037 was associated with the risk of gastric cancer in another study (He et al., 2013). Zhang et al found that SNP rs4072037 was significantly associated with both non-cardia gastric cancer and cardia gastric cancer in eastern Chinese population using a relatively larger sample size (Zhang et al., 2011). One possible explanation for the discrepancy between their study and ours is that the modulation of gastric cancer risk might depend not only on a single gene/single SNP, but also on a joint effect of multiple polymorphisms within different genes, or on close interaction between polymorphisms and environmental factors (Jiang et al., 2010). Of course, we could not exclude that the inconsistent might be result from our small sample size. More interestingly, on the basis of Chinese population, Abnet et al performed a GWAS in 1625 cases with gastric cancer and 2100 controls and identified that SNP rs4072037 was significantly associated with gastric cancer risk in the initial scanning phase. However, the observed association was not supported in the second phase, as well as in the combined data (Anbet et al., 2010). Moreover, one study reported that SNP 4072037 was not associated with the risk of chronic atrophic gastritis (Frank et al., 2012), the important precursor lesion of gastric cancer. Three studies mentioned above suggested that our results might not be by chance.

Several limitations in our study needed to be addressed. First of all, we recruited cases from one hospital and selected controls from communities, which might not be representative of the general population and resulted in potential selection bias. Secondly, because the data of *H. pylori* infection was not available among cases, it is difficult for us to adjust the potential confounding bias from *H. pylori* infection. However, it is difficult to measure *H. pylori* infection in gastric cancer patients because the loss of *H. pylori* from the stomach and reduced immune response occurs during gastric carcinogenesis (Karnes et

al., 1991; Farinati et al., 1993). Thus, a prospective study should be further conducted for concomitant analysis of the genetic susceptibility and exposure to *H. pylori*. Finally, our sample size was in general small, so we cannot exclude the possibility that we failed to detect a smaller genetic effect of *MUC1* polymorphisms.

In conclusion, our results indicated that common polymorphisms in *MUC1* gene were not associated with *H. pylori* infection and non-cardia gastric cancer risk in our studied population. Additional studies are required to reveal the real relationship between genetic variations of *MUC1* gene and gastric cancer risk.

Acknowledgements

This study is supported by grants from the National Natural Science Foundation of China (No. 30960169); the Inner Mongolian Committee of Science and Technology, China (No. 20110501); the Scientific Research Foundation of the Higher Education Institutions of Inner Mongolian Autonomous Region, China (No. NJzy08138); the Foundation of Medicine and Health of Baotou, China (No. 2008-34); the Baotou Committee of Science and Technology, China (No. 2009S1001-44).

References

Abnet CC, Freedman ND, Hu N, et al (2010). A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet*, **42**, 764-7.

Brenner H, Rothenbacher D, Arndt V (2009). Epidemiology of stomach cancer. *Methods Mol Biol*, **472**, 467-77.

Correa P (2004). The biological model of gastric carcinogenesis. *IARC Sci Publ*, **157**, 301-10.

Costa NR, Mendes N, Marcos NT, et al (2008). Relevance of *MUC1* mucin variable number of tandem repeats polymorphism in H pylori adhesion to gastric epithelial cells. *World J Gastroenterol*, **14**, 1411-4.

Farinati F, Valiante F, Germanà B, et al (1993). Prevalence of Helicobacter pylori infection in patients with precancerous changes and gastric cancer. *Eur J Cancer Prev*, **2**, 321-6.

Frank B, Weck MN, Müller H, et al (2012). Polymorphisms in *MUC1*, MUC2, MUC5B and MUC6 genes are not associated with the risk of chronic atrophic gastritis. *Eur J Cancer*, **48**, 114-20.

He C, Tu H, Sun L, et al (2013). Helicobacter pylori-related host gene polymorphisms associated with susceptibility of gastric carcinogenesis: a two-stage case-control study in Chinese. *Carcinogenesis*, **34**, 1450-7.

Jia Y, Persson C, Hou L, et al (2010). A comprehensive analysis of common genetic variation in *MUC1*, MUC5AC, MUC6 genes and risk of stomach cancer. *Cancer Causes Control*, **21**, 313-21.

Jiang Z, Li C, Xu Y, Cai S (2010). A meta-analysis on XRCC1 and XRCC3 polymorphisms and colorectal cancer risk. Int *J Colorectal Dis*, **25**, 169-80.

Karnes WE Jr, Samloff IM, Siurala M, et al (1991). Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. *Gastroenterology*, **101**, 167-74.

Kim MA, Lee HS, Yang HK, Kim WH (2005). Clinicopathologic and protein expression differences between cardia carcinoma and noncardia carcinoma of the stomach. *Cancer*, **103**, 1439-46.

Lindén SK, Wickström C, Lindell G, Gilshenan K, Carlstedt I (2008). Four modes of adhesion are used during Helicobacter pylori binding to human mucins in the oral and gastric niches. *Helicobacter*, **13**, 81-93.

Ng W, Loh AX, Teixeira AS, Pereira SP, Swallow DM (2008). Genetic regulation of *MUC1* alternative splicing in human tissues. *Br J Cancer*, **99**, 978-85.

Peek RM Jr, Blaser MJ (2002). Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer*, **2**, 28-37.

Vinall LE, King M, Novelli M, et al (2002). Altered expression and allelic association of the hypervariable membrane mucin *MUC1* in Helicobacter pylori gastritis. *Gastroenterology*, **123**, 41-9.

Westfall P, Young S (1993). Resampling-based multiple testing: examples and methods for p-value adjustment. Wiley publisher, USA.

Xu Q, Yuan Y, Sun LP, et al (2009). Risk of gastric cancer is associated with the *MUC1* 568 A/G polymorphism. *Int J Oncol*, **35**, 1313-20.

Zhang H, Jin G, Li H, et al (2011). Genetic variants at 1q22 and 10q23 reproducibly associated with gastric cancer susceptibility in a Chinese population. *Carcinogenesis*, **32**, 848-52.