RESEARCH ARTICLE

Common Genetic Variations in the *MUC5AC* Gene are Not Related to *Helicobacter pylori* Serologic Status

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Abstract

Several lines of evidence suggest that *MUC5AC* genetic polymorphisms might confer susceptibility to *H. pylori* infection and therefore gastric cancer risk. We here assessed the association of common polymorphisms in the *MUC5AC* gene with *H. pylori* seroprevalence using an LD-based tagSNP approach in a north-western Chinese Han population. A total of 12 tagSNPs were successfully genotyped among 281 unrelated ethnic Han Chinese who had no cancer history, and no identifiable gastric disease or genetic disease. No significant association between any alleles, genotypes or haplotypes and *H. pylori* seroprevalence was observed. Our results suggest that common genetic variations in *MUC5AC* gene might not make a major contribution to the risk of *H. pylori* infection.

Keywords: *H. pylori - MUC5AC* – tagSNPs - seroprevalence

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic, flagellated bacterium that colonizes the human stomach (Osman et al., 2014). It is the major cause of chronic active gastritis, gastric and duodenal ulcers, atrophic gastritis, gastric cancer, and gastric mucosa-associated lymphoid tissue lymphoma (Lillehoj et al., 2012). Studies have shown that host genetic factors are estimated to contribute 57% of variation in acquisition of *H. pylori* infection (Malaty et al., 1998). And host genetic variations can influence the susceptibility to *H. pylori* infection (Zheng et al., 2009; Mayerle et al., 2013).

Upon infection, *H. pylori* primarily reside within the mucus layer, adhering to mucins, high molecular weight glycoproteins and major components of the protective layer across the upper mucous surfaces (Peek et al., 2002). Normal gastric mucosa shows cell type specific expression of secreted mucin *MUC5AC* in the surface epithelium (Wang et al., 2006; Lindén et al., 2010). Several studies have strongly suggested that *MUC5AC* forms the major receptor for *H. pylori* in the human stomach (Van de Bovenkamp et al., 2003; Lindén et al., 2008), and the infection of *H. pylori* can alter the expression of *MUC5AC* (Kocer et al., 2004).

Our previous study suggested that common polymorphisms in *MUC5AC* gene were associated with the risk of non-cardia gastric cancer risk in north-western Chinese Han population (Zhou et al., 2014). However, whether or not this association was mediated through *H. pylori* infection has not been elucidated. So in this study, we evaluated the associations between *MUC5AC* common polymorphisms and risk of *H. pylori* infection using tagSNP approach in the same population.

Materials and Methods

Study population

A case-control study on non-cardia gastric cancer was conducted in Baotou, inner Mongolian Autonomous Region of north-western China between June 2008 and December 2010, as described previously (Zhou et al., 2014). Cases were patients newly diagnosed with histologically confirmed non-cardia gastric cancer. Controls were randomly selected from a community health examination program and frequency matched to the cases by age (± 5 years) and sex. The subjects of present study were the normal controls from the case-control study. Briefly, a total of 281 unrelated ethnic Han Chinese (220 males and 61 females, mean age: 59.10±11.57 years) were included in this study. All subjects had no cancer history, and no identifiable gastric disease or genetic disease. At recruitment, informed consent was obtained from each subject, and the study was approved by the institutional review board of Baotou Medical College.

Tests for H. pylori serologic status

The serologic status of *H. pylori* was determined by commercial enzyme-linked immunosorbent assay (ELISA) kits (Biohit, Helsinki, Finland). As an indicator for current or previous infection, seroprevalence was defined as an anti-*H. pylori* IgG titer equal to or greater than 30 EIU,

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according to the manufacturer's recommendation.

TagSNP selection and genotyping

The selection and genotyping of tagSNPs were previously described by Zhou et al. (Zhou et al., 2014). Briefly, According to HapMap Phase 2 information for Chinese Han population (http: //hapmap.ncbi.nlm.nih.gov), 14 SNPs with a pairwise r2 ≥0.8 and minor allele frequency (MAF) ≥0.05, were selected using Tagger algorithm as implemented in Haploview. Genomic DNA was extracted from leucocytes of peripheral blood by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. TagSNPs were genotyped by the TaqMan allelic discrimination according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). And the genotyping was performed at Chinese national human genome center, Beijing. Twelve SNPs were successfully genotyped. Data from Zhou's study were used for the present analysis.

Statistical analysis

All statistical analyses were conducted by SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium was tested by a goodness-of-fit $\chi 2$ 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 *H. pylori* seroprevalence with alleles, genotypes and haplotypes.

Table 1. Associations between Alleles of TagSNPs and *H. pylori* Seroprevalence

SNPs	Alleles	*H. pylori* (+) n (%)[a]	*H. pylori* (-) n (%)[a]	OR (95% CI)[b]
rs3793966	C	152 (63.9)	206 (65.6)	1
	T	86 (36.1)	108 (34.4)	1.041 (0.872-1.242)
rs7118568	C	167 (68.4)	217 (69.6)	1
	G	77 (31.6)	95 (30.4)	1.022 (0.852-1.225)
rs868903	T	126 (52.1)	170 (53.8)	1
	C	116 (47.9)	146 (46.2)	1.036 (0.876-1.225)
rs3793964	T	127 (52.5)	172 (55.1)	1
	C	115 (47.5)	140 (44.9)	1.058 (0.894-1.252)
rs3750919	G	170 (70.8)	210 (68.2)	1
	A	170 (29.2)	98 (31.8)	0.941 (0.782-1.132)
rs5743942	T	217 (88.9)	275 (88.7)	1
	C	27 (11.1)	35 (11.3)	0.990 (0.758-1.292)
rs4963062	G	171 (71.2)	227 (72.7)	1
	A	69 (28.8)	85 (27.3)	1.034 (0.857-1.247)
rs885454	C	159 (66.2)	204 (68.0)	1
	T	81 (33.8)	96 (32.0)	1.045 (0.872-1.252)
rs6578810	T	184 (76.7)	231 (76.5)	1
	G	56 (23.3)	71 (23.5)	0.986 (0.806-1.205)
rs11040869	G	164 (68.3)	218 (70.8)	1
	A	76 (31.7)	90 (29.2)	1.062 (0.884-1.277)
rs7118481	C	142 (59.2)	193 (62.7)	1
	G	98 (40.8)	115 (37.3)	1.074 (0.903-1.277)
rs7105198	G	190 (79.8)	248 (79.2)	1
	C	48 (20.2)	64 (20.5)	0.982 (0.796-1.212)

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

Results

The genotype frequencies of all SNPs followed Hardy-Weinberg equilibrium in the subjects. Allele and genotype frequencies as well as ORs and 95%CI for the selected SNPs are shown in Table 1 and Table 2, respectively. No

Table 2. Associations between Genotypes of TagSNPs and *H. pylori* Seroprevalence

SNPs	Genotypes	*H. pylori* (+) n (%)[a]	*H. pylori* (-) n (%)[a]	OR (95% CI)[b]
rs3793966	CC	47 (39.5)	66 (42.1)	1
	CT	58 (48.7)	74 (47.1)	1.122 (0.673-1.870)
	TT	14 (11.8)	17 (10.8)	1.149 (0.516-2.561)
rs7118568	CC	57 (46.7)	76 (48.7)	1
	CG	53 (43.4)	65 (41.7)	1.084 (0.658-1.788)
	GG	12 (9.9)	15 (9.6)	1.040 (0.450-2.401)
rs868903	TT	35 (28.9)	44 (27.8)	1
	CT	56 (46.3)	82 (51.9)	0.858 (0.490-1.502)
	CC	30 (24.8)	32 (20.3)	1.182 (0.606-2.306)
rs3793964	TT	32 (26.4)	47 (30.1)	1
	CT	63 (52.1)	78 (50.0)	1.210 (0.688-2.130)
	CC	26 (21.5)	31 (19.9)	1.246 (0.625-2.485)
rs3750919	GG	60 (50.0)	70 (45.5)	1
	AG	50 (41.7)	70 (45.5)	0.837 (0.506-1.385)
	AA	10 (8.3)	14 (9.0)	0.834 (0.343-2.030)
rs5743942	TT	95 (77.9)	125 (80.6)	1
	CT	27 (22.1)	25 (16.2)	1.441 (0.782-2.655)
	CC	0	5 (3.2)	-
rs4963062	GG	61 (50.8)	82 (52.6)	1
	AG	49 (40.8)	63 (40.4)	1.039 (0.630-1.713)
	AA	10 (8.4)	11 (7.0)	1.201 (0.478-3.015)
rs885454	CC	50 (41.7)	66 (44.0)	1
	CT	59 (49.2)	72 (48.0)	1.092 (0.657-1.816)
	TT	11 (9.1)	12 (8.0)	1.233 (0.502-3.032)
rs6578810	TT	71 (59.2)	87 (57.6)	1
	GT	42 (35.0)	57 (37.8)	0.892 (0.536-1.483)
	GG	7 (5.8)	7 (4.6)	1.163 (0.386-3.506)
rs11040869	GG	56 (46.7)	77 (50.0)	1
	AG	52 (43.3)	64 (41.6)	1.137 (0.685-1.887)
	AA	12 (10.0)	13 (8.4)	1.264 (0.536-2.980)
rs7118481	CC	43 (35.8)	56 (36.4)	1
	CG	56 (46.7)	81 (52.6)	0.898 (0.532-1.517)
	GG	21 (17.5)	17 (11.0)	1.597 (0.749-3.405)
rs7105198	GG	71 (62.2)	102 (65.4)	1
	CG	42 (35.3)	44 (28.2)	1.292 (0.763-2.188)
	CC	3 (2.5)	10 (6.4)	0.415 (0.110-1.565)

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

Table 3. Associations between Haplotypes and *H. pylori* Seroprevalence

Blocks	Haplotypes	*H. pylori* (+) (%)[a]	*H. pylori* (-) (%)[b]	OR (95% CI)[c]
Block 1[a]	CC	34.5	38.1	1
	CG	31.6	30.2	1.174 (0.780-1.767)
	TC	34.0	31.6	1.253 (0.837-1.876)
Block 2[b]	ATGC	32.0	29.3	1
	GTAT	28.9	31.6	1.841 (0.547-1.292)
	GGGT	23.1	23.5	0.906 (0.572-1.437)
	GTGC	15.1	15.3	0.885 (0.525-1.492)

*[a]The SNP order was rs885454-rs7118568; [b]The SNP order was rs11040869-rs6578810-rs3750919-rs3793964; [c]Adjusted for age and sex

significant association between any alleles or genotypes and *H. pylori* seroprevalence was observed.

Based on the LD data in our study, 12 tagSNPs formed 2 blocks and several singletons. Similarly as for single-locus analysis, none of the haplotypes were associated with *H. pylori* seroprevalence (Table 3).

Discussion

Chronic inflammation of the gastric mucosa resulted from *H. pylori* infection is a serious public health problem worldwide. To date, accumulating data has showed that host genetic factors contribute to the susceptibility to *H. pylori* infection. For example, there are differences in *H. pylori* susceptibility between African Americans and US residents of European ancestry after adjusting for socioeconomic status, age and living conditions (Graham et al., 1991). Approximately 5% to 10% of a population is never infected with *H. pylori*, even in the presence of high exposure rates (Bardhan, 1997). Twin study has showed that there is significantly higher concordance for *H. pylori* infection in monozygotic compared with dizygotic twins, with a heritability estimate in twins of 57% (Malaty et al., 1994). A genome-wide association study suggests that host genetic factors confer susceptibility to *H. pylori* infection (Mayerle et al., 2013). Some polymorphisms are associated with the increased risk of *H. pylori* infection (Zhao et al., 2012; Cao et al., 2013).

H. pylori colonization of the stomach is initiated through pathogen binding to cell surface receptors expressing the sialyl-Lewis a (sLea), Lewis b (Leb), and sialyl-Lewis x (sLex) glycoconjugates (Lindén et al., 2008). The corresponding *H. pylori* adhesions, blood group antigen binding adhesion (BabA) and sialic acid binding adhesion (SabA), interact with these host receptors (Lindén et al., 2008). Among the epithelial glycoproteins containing these Lewis antigens are gastric mucins. *MUC5AC* is a mucin core protein of the gastric surface mucous cells and a major mucin core protein of the mucins forming the gastric surface mucous gel layer covering the gastric mucosa (Ho et al., 2004). Either at low pH or under neutral pH conditions, BabA can binds to the *MUC5AC* mucin glycoprotein (Lillehoj et al., 2012). Upon *H. pylori* infection, the expression of *MUC5AC* has altered (Kocer et al., 2004). Furthermore, *MUC5AC* genetic polymorphisms are associated with the risk of gastric cancer, a *H. pylori* related carcinoma (Jia et al., 2010; Zhou et al., 2014). Given the importance and the potential biological mechanism of *MUC5AC*, it is conceivable that the genetic variation in *MUC5AC* gene may potentially play an important role in the development of *H. pylori* infection. So in this study, we investigated the association between *MUC5AC* common polymorphisms and *H. pylori* susceptibility using tagSNP approach. However, our results indicated that no significant association existed between tagSNPs of *MUC5AC* gene and *H. pylori* seroprevalence.

To date, only one population-based case-control study has been conducted to investigate the association between polymorphisms in *MUC5AC* gene and *H. pylori* infection in Polish population, and found no significant association existed (Jia et al., 2010). More interesting, the study also found that common genetic variations had an effect on the risk of non-cardia gastric cancer, which is consistent with our study, suggesting *MUC5AC* polymorphisms might be involved in other processes besides bacterial binding in developing gastric cancer. Further studies are needed to elucidate the underlying mechanisms.

Because the loss of *H. pylori* from the stomach and reduced immune response often occurs during gastric carcinogenesis (Farinati et al., 1993), it is difficult to measure *H. pylori* infection in patients with gastric cancer. Furthermore, gastric cancer cases have probably received long-term *H. pylori* eradication therapy, and significant serological changes would possibly occur over the long term of *H. pylori* eradication therapy. So as other studies (Zheng et al., 2009; Jia et al., 2010), we did not evaluate the correlation between *MUC5AC* polymorphisms and *H. pylori* infection in patients with non-cardia gastric cancer. A prospective study should be further conducted to analyze the relationship between genetic polymorphisms in *MUC5AC* gene and susceptibility to *H. pylori* infection in patients with non-cardia gastric cancer.

In conclusion, this preliminary study suggests there is no significant association between common genetic variations in *MUC5AC* gene and *H. pylori* seroprevalence. This finding requires replication in other larger studies.

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