RESEARCH ARTICLE

Association Analysis of Common Genetic Variations in MUC5AC Gene with the Risk of Non-cardia Gastric Cancer in a Chinese Population

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Abstract

Several lines of evidence suggest that genetic variation in MUC5AC gene might contribute to the risk of gastric cancer. We conducted a case-control study to evaluate the relationship between common genetic variations in MUC5AC gene and non-cardia gastric cancer using an LD-based tagSNP approach in Baotou, north-western China. We genotyped 12 tagSNPs by TaqMan method among 288 cases with non-cardia gastric cancer and 281 normal controls. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for non-cardia gastric cancer risk in association with alleles, genotypes and haplotypes. We observed that the frequencies of rs3793964 C allele and rs11040869 A allele were significantly lower in cases than in controls. Meanwhile, minor allele homozygotes of rs3793964 and rs11040869 were significantly associated with a decreased risk of non-cardia gastric cancer when compared with their major allele homozygotes. Furthermore, a statistically significantly protective effect of rs885454 genotypes on non-cardia gastric cancer was also observed (for CT vs. CC: OR=0.581, 95%CI=0.408-0.829; for CT/TT vs. CC: OR=0.623, 95%CI=0.451-0.884). Our results indicated that some common genetic variations in the MUC5AC gene might have effects on the risk of non-cardia gastric cancer in our studied population.

Keywords: Non-cardia gastric cancer - MUC5AC - tagSNP - risk - Baotou, China

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Introduction

Gastric cancer remains the fourth most common cancer and the second leading cause of cancer-related mortality worldwide (Guggenheim et al., 2013). Although the exact etiology of gastric cancer remains to be identified, accumulating studies have shown that gastric carcinogenesis is a complex, multistep and multifactorial process involving genetic factors and environmental triggers (Pan et al., 2013). According to anatomic site, gastric cancer can be classified as cardia and non-cardia subtypes. It is now generally accepted that *Helicobacter pylori* (*H. pylori*) infection is the single most important risk factor in the development of non-cardia gastric cancer (Peek and Blaser, 2002; Compare et al., 2010). However, most infected individuals do not develop gastric cancer (Abnet et al., 2010), suggesting *H. pylori* infection is insufficient to cause gastric cancer, other cofactors, especially host genetic factors may play a significant 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). Normal gastric mucosa shows cell type specific expression of secreted mucin MUC5AC in the surface epithelium (Babu et al., 2006; Wang and Fang, 2006). Several published investigations 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). Some studies have shown that MUC5AC is aberrantly expressed in gastric cancer (Reis et al., 1997; Xu et al., 2009; İlhan et al., 2010). Given the importance and the potential biological mechanism of MUC5AC, it is conceivable that genetic variation in MUC5AC gene may play an important role in the development and progression of gastric cancer. However, there are few data on the association of polymorphisms in MUC5AC gene with gastric cancer in Chinese population.

To clarify the potential role of genetic variation in MUC5AC gene with respect to gastric cancer risk, we conducted a case-control study to investigate the association between MUC5AC polymorphisms and non-cardia gastric cancer risk using tagSNP approach in north-western Chinese Han population.

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Materials and Methods

Subjects

Details of the study subjects have been described elsewhere (Zhang et al., 2013). Briefly, all patients with histopathologically confirmed incident non-cardia gastric cancer were consecutively recruited between June 2008 and December 2010 at the cancer hospital of Baotou. Patients with metastasized cancer from other organs and having previous radiotherapy or chemotherapy were excluded. 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 the patients were recruited. The selection criteria for the controls included no individual history of cancer, and no identifiable gastric disease or genetic disease. All subjects were unrelated ethnic Han Chinese from Baotou, Inner Mongolian Autonomous Region, north-western China. At recruitment, informed consent was obtained from each subject, and the study was approved by the institutional review board of Baotou Medical College.

Selection of tagSNPs

For the selection of tagSNPs, we used HapMap Phase 2 information for Chinese Han population (http://www.hapmap.org). TagSNPs were selected using 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 used for tagSNP selection were minor allele frequency (MAF) ≥0.05 in Chinese Han population, and r^2>0.8 between each pair of tagged and tagging SNPs (pairwise tagging). The final selection comprised 14 SNPs.

Genotyping

Genomic DNA was isolated and purified from leucocytes of peripheral blood by standard proteinase K digestion and phenol/chloroform extraction. 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. From the 14 SNPs initially selected, 2 were excluded because of assay design or genotyping failure. The genotyping rates of other tested SNPs were all above 96%. For quality control purpose, two negative experimental controls (water) and two positive experimental controls with known genotype were included in each reaction plate. In addition, about 5% of the samples were randomly selected and genotyped twice, and the results were 100% concordant.

Statistical analysis

All statistical analyses were conducted by SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). 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) for non-cardia gastric cancer risk with alleles, genotypes and haplotypes. Results are presented without correction for multiple testing to avoid the loss of valuable information due to the limited number of subjects.

Results

This study consisted of 281 controls and 288 cases. Age and gender distributions were comparable between the two groups. The demographic of subjects are shown in Table 1 (Zhang et al., 2013).

The genotype frequencies of all SNPs followed Hardy-Weinberg equilibrium in controls. Allelic frequencies of all SNPs in both controls and cases are summarized in Table 2. The frequencies of rs3793964 C allele and rs11040869 A allele were significantly lower in cases than in controls (adjusted OR=0.863, 95%CI=0.766-0.971 for rs3793964 C allele; adjusted OR=0.876, 95%CI=0.768-0.998 for rs11040869 A allele).

Of the 12 tested tagSNPs, minor allele homozygotes of rs3793964 and rs11040869 were significantly associated with a decreased risk of non-cardia gastric cancer when compared with their major allele homozygotes, with adjusted OR=0.529, 95%CI=0.320-0.875 for rs3793964 CC genotype, and adjusted OR=0.479, 95%CI=0.239-0.960 for rs11040869 AA genotype respectively. Meanwhile, a statistically significantly protective effect

Table 1. Demographic Data of Study Subjects

Variable	Controlsn (%)	Casesn (%)	*p* value
Overall	281 (100)	288 (100)	
Gender			
Male	220 (78.3)	224 (77.8)	0.88
Female	61 (21.7)	64 (22.2)	
AgeMean±SD (year)	59.10±11.57	59.48±11.23	0.69

Table 2. Associations between Alleles of TagSNPs and Risk of Non-cardia Gastric Cancer

SNP	Allele	Cases n(%)[a]	Controls n(%)[a]	OR (95% CI)[b]
rs3793966	C	388(68.1)	358(64.9)	1
	T	182(31.9)	194(35.1)	0.931(0.822-1.054)
rs7118568	C	383(67.4)	384(69.1)	1
	G	185(32.6)	172(30.9)	1.040(0.917-1.179)
rs868903	T	312(54.4)	296(53.0)	1
	C	262(45.6)	262(47.0)	0.975(0.867-1.095)
rs3793964	T	346(61.1)	299(54.0)	1
	C	220(38.9)	255(46.0)	0.863(0.766-0.971)
rs3750919	G	382(67.3)	380(69.3)	1
	A	186(32.7)	168(30.7)	1.050(0.926-1.191)
rs5743942	T	512(91.4)	492(88.8)	1
	C	48(8.6)	62(11.2)	0.860(0.705-1.048)
rs4963062	G	381(67.6)	398(72.1)	1
	A	183(32.4)	154(27.9)	1.116(0.981-1.268)
rs885454	C	410(72.2)	363(67.2)	1
	T	158(27.8)	177(32.8)	0.888(0.781-1.010)
rs6578810	T	405(72.6)	415(76.6)	1
	G	153(27.4)	127(23.4)	1.112(0.970-1.274)
rs11040869	G	429(75.0)	382(69.7)	1
	A	143(25.0)	166(30.3)	0.876(0.768-0.998)
rs7118481	C	349(61.9)	335(61.1)	1
	G	215(38.1)	213(38.9)	0.984(0.872-1.110)
rs7105198	G	434(75.6)	438(79.6)	1
	C	140(24.4)	112(20.4)	1.125(0.977-1.296)

*[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 Genotypes of TagSNPs and Risk of Non-cardia Gastric Cancer

SNP	Genotype	Cases n (%)[a]	Controls n (%)[a]	OR (95% CI)[b]
rs3793966	CC	127(44.6)	113(40.9)	1
	CT	134(47.0)	132(47.9)	0.901(0.634-1.278)
	TT	24(8.4)	31(11.2)	0.695(0.385-1.255)
	CT/TT	158(55.4)	163(59.1)	0.861(0.616-1.205)
rs7118568	CC	134(47.2)	133(47.8)	1
	CG	115(40.5)	118(42.5)	0.971(0.683-1.381)
	GG	35(12.3)	27(9.7)	1.293(0.741-2.256)
	CG/GG	150(52.8)	145(52.2)	1.031(0.740-1.437)
rs868903	TT	86(30.0)	79(28.3)	1
	CT	140(48.8)	138(49.5)	0.937(0.637-1.379)
	CC	61(21.2)	62(22.2)	0.905(0.567-1.444)
	CT/CC	201(70.0)	200(71.7)	0.927(0.645-1.333)
rs3793964	TT	102(36.0)	79(28.5)	1
	CT	142(50.2)	141(50.9)	0.772(0.530-1.126)
	CC	39(13.8)	57(20.6)	0.529(0.320-0.875)
	CT/CC	181(64.0)	198(71.5)	0.702(0.491-1.003)
rs3750919	GG	132(46.5)	130(47.4)	1
	AG	118(41.5)	120(43.8)	0.970(0.683-1.379)
	AA	34(12.0)	24(8.8)	1.398(0.786-2.487)
	AG/AA	152(53.5)	144(52.6)	1.041(0.747-1.453)
rs5743942	TT	235(83.9)	220(79.4)	1
	CT	42(15.0)	52(18.8)	0.747(0.477-1.170)
	CC	3(1.1)	5(1.8)	0.568(0.134-2.409)
	CT/CC	45(16.1)	57(20.6)	0.731(0.474-1.128)
rs4963062	GG	136(48.2)	143(51.8)	1
	AG	109(38.7)	112(40.6)	1.025(0.720-1.458)
	AA	37(13.1)	21(7.6)	1.868(1.040-3.355)
	AG/AA	146(51.8)	133(48.2)	1.157(0.830-1.613)
rs885454	CC	154(54.2)	116(43.0)	1
	CT	102(35.9)	131(48.5)	0.581(0.408-0.829)
	TT	28(9.9)	23(8.5)	0.917(0.502-1.674)
	CT/TT	130(45.8)	154(57.0)	0.632(0.451-0.884)
rs6578810	TT	148(53.0)	158(58.3)	1
	GT	109(39.1)	99(36.5)	1.180(0.829-1.680)
	GG	22(7.9)	14(5.2)	1.672(0.825-3.391)
	GT/GG	131(47.0)	113(41.7)	1.242(0.886-1.740)
rs11040869	GG	157(54.9)	133(48.5)	1
	AG	115(40.2)	116(42.3)	0.834(0.590-1.181)
	AA	14(4.9)	25(9.2)	0.479(0.239-0.960)
	AG/AA	129(45.1)	141(51.5)	0.771(0.553-1.075)
rs7118481	CC	111(39.4)	99(36.1)	1
	CG	127(45.0)	137(50.0)	0.825(0.574-1.186)
	GG	44(15.6)	38(13.9)	1.032(0.619-1.722)
	CG/GG	171(60.6)	175(63.9)	0.870(0.617-1.226)
rs7105198	GG	163(56.8)	176(64.0)	1
	CG	108(37.6)	86(31.3)	1.369(0.959-1.953)
	CC	16(5.6)	13(4.7)	1.322(0.615-2.842)
	CG/CC	124(43.2)	99(36.0)	1.363(0.970-1.915)

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

Table 4. Associations between Haplotypes and Risk of Non-cardia Gastric Cancer

Block		Cases (%)	Controls (%)	OR (95% CI)[d]
Block 1[a]	CC	0.393	0.368	1
	CG	0.327	0.306	0.993(0.751-1.313)
	TC	0.28	0.326	0.805(0.606-1.069)
Block 2[b]	GTAT	0.328	0.305	1
	ATGC	0.248	0.305	0.776(0.572-1.053)
	GGGT	0.28	0.234	1.126(0.825-1.536)
	GTGC	0.144	0.156	0.890(0.618-1.281)
Block 3[c]	TG	0.585	0.607	1
	TA	0.328	0.28	1.226((0.946-1.588)
	CG	0.087	0.113	0.816(0.529-1.258)

*[a]The SNP order was rs885454, rs7118568; [b]The SNP order was rs11040869, rs6578810, rs3750919, rs3793964; [c]The SNP order was rs5743942, rs4963062; [d]Adjusted for age and sex

of rs885454 genotypes on non-cardia gastric cancer was observed (for CT *vs* CC: OR=0.581, 95%CI=0.408-0.829; for CT/TT *vs* CC: OR=0.623, 95%CI=0.451-0.884) (Table 3).

Based on the LD data in our study, 12 tagSNPs formed 3 blocks and several singletons. No significant association between any haplotypes and non-cardia gastric cancer risk was observed (Table 4).

Discussion

Mucins, high molecular weight glycoproteins, are major components of the mucous viscous gel, covering and protecting surface epithelial tissues (Babu et al., 2006). High levels of secreted mucin MUC5AC have been detected in the surface epithelium of normal gastric mucosa (Babu et al., 2006; Wang and Fang, 2006). MUC5AC has long been implicated in the pathogenesis of gastric carcinogenesis. Thus, decreased expression of MUC5AC is a common feature in gastric cancer (İlhan et al., 2010). MUC5AC gene is located on chromosome 11p11.5 (Pigny et al., 1996), a region frequently exhibiting loss of heterozygosity in gastric cancer (Baffa et al., 1996; Moskaluk and Rumpel, 1998). SNPs in MUC5AC gene are associated with the risk of gastric cancer in Caucasian (Jia et al., 2010). However, whether or not MUC5AC polymorphisms affect the susceptibility to gastric cancer in Chinese population has not been reported. So in this study, we utilized available HapMap data and employed an LD-based tagSNP approach to survey common variations in MUC5AC gene, and to analyze their relationship with the non-cardia gastric cancer risk. The major novel findings are that several SNPs in MUC5AC gene might be protective regarding the risk of non-cardia gastric cancer. To our best knowledge, this is the first case-control study to investigate the relationship between common genetic variations in MUC5AC gene and non-cardia gastric cancer risk in Chinese population.

To date, one population-based case-control study has been conducted to investigate the association between polymorphisms in MUC5AC gene and gastric cancer risk by a comprehensive LD-based tagSNP approach in Polish population. The results indicated that minor allele homozygotes of rs2014486, rs2735733, and rs868903 were significantly associated with an increased risk of gastric cancer as well as non-cardia gastric cancer (Jia et al., 2010), which is contrary with our results. In our study, except for SNP rs7105198, the allele frequencies of other SNPs in controls were all similar to the data from HapMap Chinese Han population. And we found that minor allele homozygotes of rs3793964 and rs11040869, as well as heterozygote of rs885454 had a protective effect on non-cardia gastric cancer risk. SNPs associated with the risk of non-cardia gastric cancer in both studies are all located in 3' flanking region of MUC5AC gene, the discrepancies may be due to differences in variant frequencies between different races. And other study also showed genetic polymorphisms could have different risk associations with gastric cancer according to ethnicity (Loh et al., 2009). Similarly, since we used a tagSNP genotyping strategy, our results do not inform on the nature of functional

variants responsible for the observed associations. It is likely that the causative variants are in strong LD with the genotyped and significant ones. The exact nature of the functional alterations associated with each tagSNP will require further exploration.

Gastric cancer includes non-cardia gastric and cardia gastric carcinoma. Two subtypes have differences in the etiology, pathology, carcinogenesis, and prognosis (Shi et al., 2011). So we restricted our cases to patients with non-cardia gastric cancer to supply relatively more homogeneous samples and improve the power of association study.

Some limitations in our study need to be noted. First, the cases in our study were selected from one hospital and controls were recruited from communities, which may not be representative of the general population and have selection bias. Second, failure in the genotyping of two of the selected tagSNPs has reduced the coverage of the genetic variability, limiting our ability to test gene-wide associations. Third, *H. pylori* infection is a well-known cause of non-cardia gastric cancer and has been suggested to influence the expression of MUC5AC (Kocer et al., 2004). 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). Lack of available information on *H. pylori* infection status in our study limited us to adjust the potential confounding bias of this risk factor. Finally, we thought it was important to show all possible results for future studies, and therefore did not correct for multiple testing. So it cannot be ruled out that several of our results were false positives.

In conclusion, our data indicated that common genetic variations in MUC5AC gene might be associated with a decreased risk of non-cardia gastric cancer in north-western Chinese Han population. Our finding further highlights the importance of MUC5AC gene in gastric cancer risk. Further studies are needed to clarify the mechanisms underlying the observed associations.

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