Published in final edited form as:
Cancer Causes Control. 2010 February ; 21(2): 313–321. doi:10.1007/s10552-009-9463-3.

A comprehensive analysis of common genetic variation in *MUC1, MUC5AC, MUC6* genes and risk of stomach cancer

Yanbin Jia[1,2], **Christina Persson**[1], **Lifang Hou**[3,4], **Zongli Zheng**[1], **Meredith Yeager**[5], **Jolanta Lissowska**[6], **Stephen J. Chanock**[5], **Wong-Ho Chow**[3], and **Weimin Ye**[1,*]

[1] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden [2] Departmentof Basic Medicine, Baotou Medical College, Baotou, China [3] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA [5] Core Genotyping Facility, Advanced Technology Center, National Cancer Institute, Gaithersburg, MD, USA [6] Department of Cancer Epidemiology and Prevention, The M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Abstract

Objective—MUC1, MUC5AC, and MUC6 are main constituents of the mucus barrier in the stomach, which protects the underlying epithelium from acid, proteases, mechanical trauma, and pathogenic microorganisms. Accumulating evidence implicates potential roles of *MUC1*, *MUC5AC*, and *MUC6* genetic variation in the development of stomach cancer.

Methods—We evaluated the relationship between common genetic variations in these genes and stomach cancer risk, using a LD-based tagSNP approach in a population-based case-control study conducted in Warsaw, Poland, during 1994–1996. We genotyped 6, 8, and 14 tagSNPs in *MUC1*, *MUC5AC* and *MUC6* genes, respectively, among 273 cases newly diagnosed with stomach cancer and 377 controls.

Results—Each of the six tagSNPs tested across the *MUC1* region showed statistically significant associations with an increased risk of stomach cancer. Carriers of the haplotype ACTAA rare alleles of rs4971052, rs4276913, rs4971088, rs4971092 and rs4072037 had a nearly doubled risk (OR =1.93, 95% CI =1.49–2.48) compared to the referent haplotype GTAAG. Out of the 8 tagSNPs across *MUC5AC* region, only minor allele of rs868903 was significantly associated with an increased risk of stomach cancer (OR = 1.80, 95% CI = 1.22–2.63).

Conclusions—Overall, our data provide evidence that some common variations in *MUC1* and *MUC5AC* genes contribute to an elevated risk of stomach cancer. Further studies are needed to confirm these novel findings.

Keywords

MUC1; *MUC5AC*; *MUC6*; tagSNPs; stomach cancer; risk

[*]Correspondence to: Weimin Ye, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Box 281, SE-171 77 Stockholm, Sweden, Tel: +46-8-52486184, Fax: +46-8-314975, weimin.ye@ki.se.
[4]Current address: Northwestern University Feinberg School of Medicine, Chicago, IL

Conflict of interest statement
None declared.

Introduction

Although decreasing in most countries, stomach cancer remains the fourth in cancer incidence and the second leading cause of cancer-related death in the world (1). *Helicobacter pylori* (*H. pylori*) is a well-established risk factor for stomach cancer and has been classified as a definite human carcinogen by the IARC based on epidemiological and animal studies (2). Nearly half of the world's population is infected with *H. pylori*. However, only a minority of those infected eventually developed stomach cancer even in high-risk areas, such as Japan (3), suggesting that host genetic and environmental factors are important.

Within the human body, *H. pylori* reside primarily in the gastric mucous layer (4). Mucins are heavily glycosylated glycoproteins that constitute the major components of the mucous protective layer across the upper mucous surfaces (5). To date, many distinct mucin genes have been identified. *In situ* hybridization and immunohistochemical studies have shown that these mucins are differentially expressed in epithelia with cell type specificity (6–8). The normal gastric mucosa shows cell type specific expression of MUC1, MUC5AC, and MUC6, with the first two mucins found in the superficial epithelium and the MUC6 in the deep glands (6,9–13). Studies have suggested that MUC5AC forms the major receptor for *H. pylori* in the human stomach (14,15) and that the infection can alter the expression of *MUC1*, *MUC5AC*, and *MUC6* genes. During the process of gastric carcinogenesis the expression of these genes are altered (9–11,16–18). High levels of MUC1 mRNA have been detected in gastric carcinomas, whereas decreased levels have been reported for MUC5AC and MUC6 (19). The cumulative evidence suggests a possible role for the *MUC1*, *MUC5AC*, and *MUC6* genetic variation in the development and progression of stomach cancer.

Some studies have examined the relationship between a variable number tandem repeat (VNTR) polymorphism of *MUC1* gene and the risk of stomach cancer, where the smaller size *MUC1* VNTR alleles are suggested to be associated with *H. pylori* infection (16). In the Portuguese population, which has a relatively high stomach cancer incidence in Europe, the smaller size *MUC1* VNTR alleles have been shown to be associated with an increased risk for gastric carcinoma (20), as well as chronic atrophic gastritis and incomplete intestinal metaplasia (21). In addition, the smaller size VNTR alleles of *MUC6* have been associated with *H. pylori* infection (22) and an excess risk of stomach cancer (23), suggesting that the *MUC6* VNTR polymorphism is involved in the stomach cancer development. To date, the role of *MUC5AC* gene polymorphisms on the risk of stomach cancer has not been reported.

To better understand the roles of genetic variation in the *MUC1*, *MUC5AC*, and *MUC6* genes in the development of stomach cancer, we explored the associations between genetic variation in these genes and gastric cancer risk by a comprehensive LD-based tagSNP approach in a population-based case-control study in Warsaw, Poland, a high-risk area for stomach cancer among Caucasians.

Materials and methods

Subjects

Details of the study design and method have been published elsewhere (23). Briefly, Warsaw residents aged 21–79 years, all Caucasian, who were newly diagnosed with gastric cancer between March 1994 and April 1996, were eligible as cases. All cases were pathologically confirmed as gastric adenocarcinoma after identification through collaborating physicians in 22 hospitals and 8 private endoscopic units serving the study area and by regular reviews of the Cancer Registry to ensure completeness of case

ascertainment. Controls were randomly selected among Warsaw residents using a computerized registry of all legal residents in Poland, updated monthly with nearly 100% registration completeness. Controls were frequency-matched to cases by gender and age in 5-year strata.

Information was collected through interview on demographic background and lifestyle factors. Among eligible cases (n = 515) and controls (n = 549), successful interviews were conducted for 464 cases (90%); 324 direct interviews and 140 next-of-kin interviews for deceased cases and 480 (87%) controls. Blood samples were obtained from 305 (66%) and 427 (89%) of the participating cases and controls, respectively. Genomic DNA was available in 273 cases and 377 controls. Each participant gave informed consent, and the study was approved by Institutional Review Boards at the US National Cancer Institute, the Cancer Center and Institute of Oncology of Health in Warsaw, Poland and the Regional Ethics Committee of Karolinska Institutet in Sweden.

Selection of tagSNPs

For the selection of tagSNPs, we used information from HapMap Phase 2 data (http://www.hapmap.org). TagSNPs were chosen using the LD-based method by Carlson *et al* (24). The region analyzed included 20 kb upstream of the first exon and 10 kb downstream of the termination of the last exon. A LD threshold of $r^2 > 0.8$ and a minor allele frequency (MAF) > 5% were used for tagSNP selection.

The HapMap2 database contains 26, 18, and 57 SNPs with MAF > 5% in the *MUC1*, *MUC5AC*, and *MUC6* genes and their adjacent regions, respectively. TagSNP selection yielded 10 bins for *MUC1*, 12 bins for *MUC5AC*, as well as 19 bins for *MUC6* gene region.

Genotyping

Genomic DNA was extracted from buffy coats by standard methods. Genotyping was performed by SNPlex method at Core Genotyping Facility of National Cancer Institute, MD. SNPlex reactions were analyzed on an Applied Biosystems 3730 DNA Sequencer. Details about assays, primers, probes, and procedures are available at the National Cancer Institute's SNP500 website (http://snp500cancer.nci.nih.gov). Among a minimum set of 10, 12, and 19 tagSNPs selected in the *MUC1, MUC5AC,* and *MUC6* genes, respectively, 13 SNPs were excluded because of assay design or genotyping failure. Finally, 6, 8, and 14 tagSNPs in *MUC1*, *MUC5AC*, and *MUC6* gene, respectively, were genotyped, which were further described in Appendix A. Genotyping data for each tested SNP were successfully obtained for more than 98% of the subjects. Laboratory staff was blinded to case/control status. For quality control purpose, eight subjects chosen randomly were genotyped twice for all tagSNPs, and the results were 100% concordant.

We successfully genotyped 28 tagSNPs spanning a region of 39.3 kb, 27.1 kb, and 110.5 kb across *MUC1*, *MUC5AC*, and *MUC6* gene, respectively, in 273 cases with gastric cancer and 377 controls. Of the 28 tagSNPs, three (rs6427184 in *MUC1* gene, rs11602663 and rs7119740 in *MUC6* gene) did not conform to fitness for Hardy-Weinberg proportion among controls, based on the Pearson's χ^2 test. Hence, these three SNPs were excluded from haplotype analyses. The deviation from Hardy-Weinberg equilibrium could be due to systematic errors in genotyping or population stratification. We believe though, due to the success of genotyping of other tagSNPs, that such systematic error unlikely occurred in this setting. However, genotyping errors due to poor quality of the matching probes, could explain the deviation from Hardy-Weinberg equilibrium especially when competing with other probes in a multiplex setting. The concern of differences in geographic distribution or population stratification also is unlikely as all our study subjects are Caucasians living in

Warsaw. We could not exclude the possibility that some mucin gene variants are truly not in Hardy-Weinberg equilibrium. Further data are needed to conform this observation.

Statistical analysis

Unconditional logistic regression model was used to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) adjusted for age and gender. For all SNPs, the homozygote of the common allele was used as the referent. To account for type I errors of multiple testing, statistical significance was assessed by empirical P values derived from the Westfall & Young permutation (n=10000) with minimum *P* values and step-down method (25). Haplotypes were imputed by Expectation-Maximization method using all 5 tagSNPs for *MUC1* and 8 tagSNPs for *MUC5AC*. Because *MUC6* has a large number of tagSNPs, its haplotypes were constructed based on the LD blocks derived from the HaploView 4.0 program (26). Rare haplotypes with frequency less than 2% among the controls were combined as one group. The probabilities of having certain haplotypes for each individual, inferred from the Expectation-Maximization algorithm, were used as weights in weighted logistic regression models with Sandwich covariance (27). Global P values derived from the regression models were used to assess the difference in overall haplotype profiles between comparison groups. All statistical analyses were performed using SAS 9.2 (SAS institute, Cary, NC).

Results

There was no significant difference in the distribution of age, gender, smoking, *H. pylori* status, cancer phenotype, or alcohol use between the study subjects who were included in the genetic analysis from those who were not (data not shown). Characteristics of participants in the present analysis are summarized in Table 1. Cases and controls were similar with respect to the distribution of age and gender, as these factors were frequency-matched. Cases were more likely than controls to report a history of smoking and a family history of stomach cancer. Compared to controls, there were more former drinkers and less current drinkers among the cases. The majority of the cases were of the intestinal histologic type (66%) and non-cardia subsite of origin (72%).

MUC1 polymorphism and stomach cancer risk

Each of the six tagSNPs tested across the *MUC1* region showed statistically significant associations with an increased risk of stomach cancer, and the associations remained statistically significant after adjusting for multiple tests (Table 2). Furthermore, a gene-dosage effect was observed for all tagSNPs, with significantly higher ORs associated with increasing number of minor allele, except for rs4971092 and rs6427184. For rs4971092, homozygotes of minor allele were rare (only 5 cases and 5 controls), and rs6427184 had no minor allele homozygotes.

Haplotype analysis revealed 5 common haplotypes with frequency ≥ 5%, which had a cumulative frequency of 97% in controls (Table 3). A statistically significant global association of haplotypes with stomach cancer risk was observed ($P_{global} < 0.0001$). Carriers of the haplotype ACTAA containing the rare alleles of rs4971052, rs4276913, rs4971088, rs4971092 and rs4072037, with a frequency of 34.4% in controls, displayed a significantly increased risk of stomach cancer (OR =1.93, 95% CI =1.49–2.48) compared to the referent haplotype GTAAG. The other two less common haplotypes (around 5% in controls), GTAGG and GTAGA, were also associated with significantly increased risks of the malignancy.

MUC5AC polymorphism and stomach cancer risk

Of the 8 tagSNPs across *MUC5AC* region, minor allele of rs868903 was significantly associated with an increased risk of stomach cancer (OR = 1.80, 95% CI = 1.22–2.63). There was also a tendency of gene-dosage effect (P_{trend} = 0.0055), with ORs increasing from 1.74 for heterozygotes to 1.92 for homozygote carriers of the minor allele when compared to the common allele homozygotes. In addition, minor allele homozygotes of rs2014486 and rs2735733 were associated with an increased risk of stomach cancer when compared with their respective referent group, but both were not significant after adjustment for multiple testing (Table 4).

Ten haplotypes with a frequency ranging from 2% to 30% accounted for 93.4% of the observed haplotypes in controls (Table 5). An overall association with stomach cancer risk was observed (P_{global} = 0.026). Haplotype GACACCTA containing the minor allele of rs868903 had a frequency of 14.5% in controls and was associated with an increased risk of stomach cancer (OR=1.60, 95% CI=1.19–2.15). Haplotype AGCGTCTA, with a frequency of 6.6% in controls, was also significantly associated with an increased risk (OR = 1.86, 95% CI = 1.20–2.89) (Table 5).

MUC6 polymorphism and stomach cancer risk

Fourteen tagSNPs across the *MUC6* region were examined in relation to stomach cancer risk, and there was little evidence supporting any main effects of these SNPs, which were further described in Appendix B. To minimize uncertainty in haplotype inference due to inclusion of a large number of tagSNPs, we estimated haplotype effects separately in LD blocks. Based on the HapMap data, these 12 SNPs formed two main blocks and several singletons. SNPs rs1128413, rs4077293, rs7483870, rs7943115, and rs11605303 were in block 1, and SNPs rs11601642 and rs10902076 were in block 2. We did not observe any significant association with any haplotypes in block 1 or block 2 (data not shown).

When we restricted our analyses to non-cardia stomach cancer cases, and subjects with *H. pylori* infection defined by positivity in anti-*H. pylori* or/and anti-CagA assays, the results did not change materially (data not shown). Among controls, no significant association was observed between the tagSNPs examined, either by single SNP approach or by haplotype approach, and the status of *H. pylori* infection (data not shown).

Discussion

In the present study, we investigated the relationship between common genetic polymorphisms in *MUC1, MUC5AC*, and *MUC6* genes and stomach cancer risk using a tagSNP-based approach. The results revealed that multiple SNPs in *MUC1* and *MUC5AC* gene, as well as their haplotypes, might be associated with an increased risk of stomach cancer.

MUC1 is a highly polymorphic mucin type glycoprotein expressed on the surface of many epithelia, including gastric mucosa. SNP rs4072037, located in exon 2 of *MUC1* gene, controls alternative splicing at the boundary between exon 1 and exon 2; specifically the G allele results in the MUC1/A splice product while the A allele results in the MUC1/B variant (28,29). The MUC1/A splice variant encodes MUC1 protein with an additional 9 amino acids on the amino terminal side of the tandem repeat region and also is predicted to have an altered amino terminus because of a change of signal peptidase cleavage (28). This additional sequence could alter intracellular trafficking and/or subsequent MUC1 processing and, therefore, be relevant to MUC1 function. Interestingly, the presence of splice variant A corresponds remarkably with the expression of mRNAs of the larger size class, whereas the presence of variants B corresponds with the expression of smaller ones (28–30). Therefore,

SNP rs4072037 G allele is correlated with larger size VNTRs and A allele is associated with smaller size. Further, our study showed that homozygotes of smaller size VNTR alleles had an excess risk of stomach cancer. Smaller size VNTRs previously have been associated with an increased risk of *H. pylori* infection (16). However, our results suggested that the effect of SNP rs4072037 on stomach cancer risk might not be mediated through *H. pylori* infection. Further studies are needed to elucidate the mechanisms underlying the observed association. The other five tagSNPs are all located in 3′ flanking region of *MUC1* gene. Additional work is required to characterize the functional aspects of these SNPs and also to determine whether they are themselves the high risk alleles or in LD with a causal variant.

MUC5AC gene is located on chromosome 11p15.5 (31), a region frequently exhibiting loss of heterozygosity in stomach cancer (32,33). The expression of MUC5AC is reduced in carcinomas compared to normal tissue independently on gender, age, staging or tumor grading. The reduction of the MUC5AC reactivity has been correlated with worse survival of gastric cancer (34). To our knowledge, no previous study has investigated the relationship between genetic variation in *MUC5AC* gene and risk of stomach cancer. In the present study, of the eight *MUC5AC* tagSNPs, three (rs2014486, rs2735733, and rs868903) showed statistically significant association with stomach cancer risk (although the latter two did not reach significance level after adjustment for multiple testing), and an overall haplotype association with stomach cancer was also observed. The three SNPs are all located in 3′ flanking region of *MUC5AC* gene. Because the selection of SNPs in this study was based on a tagging approach rather than on putative function, we are unable to comment in great detail on the possible functional significance of these findings. The exact nature of the functional alterations associated with each tagSNP will require further exploration. Although two studies showed that MUC5AC forms the major receptor for *H. pylori* in the human stomach (14,15), our results suggested processes other than bacterial binding might also be involved. In the present study, we found no significant difference in association by *H. pylori* status; one possible reason may be the small sample size, given that 84% of the cases and controls tested positive for *H. pylori* infection.

MUC6 gene is also located on chromosome 11p15.5 (31). Like *MUC1*, *MUC6* shows extensive VNTR variations (35). One study showed that small VNTR alleles of *MUC6* were associated with an increased risk of *H. pylori* infection, but did not investigate the relation to gastric cancer (22). Another study found that small *MUC6* alleles were more frequent in stomach cancer cases than in healthy blood donors (23). In the present study, we have not genotyped this VNTR polymorphism. This polymorphism has a large number of alleles, which results in fractioning the study population into a large number of classes. Even with a large sample size, this makes statistical analysis very difficult. Alternatively, we utilized gene-wide tagging approach to investigate the relationship between common variants of *MUC6* gene and stomach cancer risk, and found no evidence for any association. Conflicting results might be explained by the differences in allele frequency due to differences in population structure. An alternative explanation is the limited statistical power. For most of the allele frequencies we studied (≥15%), our sample size had an 80% power to detect an OR of 1.76 or higher. Therefore we cannot exclude the possibility that we failed to detect a smaller genetic effect. Furthermore, we cannot exclude the existence of rare causal or protective variants which were not monitored by our approach. Future studies are needed to clarify the role of variations of *MUC6* gene in the development of stomach cancer.

The strengths of our study included its population-based nature and the use of tagSNP approach. The recent availability of comprehensive SNP frequency data through the HapMap consortium allows for more robust assessment of genomic regions of interest rather than simply genotyping SNPs of theoretical a priori significance. Evidence is also accumulating that tagSNPs selected using HapMap data efficiently tag for common variants

in European population (36,37). However, we were unable to interrogate all tagSNP selected in these genes for technical reasons (assay design or genotyping failure), limiting our ability to test gene-wide associations. Further, 140 cases died before they could be reached, thus their DNA samples were not available; if genetic variants in the mucin genes were associated with survival, then it could lead to survival bias. We compared the characteristics of cases who were interviewed in-person and cases whose information was provided by next of kin. We found no meaningful differences in distributions by gender, age, education, and smoking status, although deceased cases tended to have more advanced tumor stage at diagnosis (38). We also examined the distribution of the *MUC1* and *MUC5AC* genetic variants across tumor stages, and did not find any pattern of association (data not shown). To our knowledge, no studies to date have linked the mucin genetic variations with stomach cancer prognosis. Notwithstanding, our results need to be replicated preferably in large cohort studies.

In conclusion, this is the first comprehensive analysis of common polymorphisms in *MUC1, MUC5AC,* and *MUC6* genes in relation to the risk of stomach cancer, using the tagSNP approach based on the HapMap data. Our findings provide evidence for association of an increased risk of stomach cancer with some common genetic variations in *MUC1* and *MUC5AC* genes. Further evaluation of the functional relevance of identified variants may eventually lead to a better understanding of gastric carcinogenesis.

Acknowledgments

This study was partially supported by the Intramural Research Program of NIH, Division of Cancer Epidemiology and Genetics and the grant from the Swedish Research Council (K2009-69X-15372-05-2)

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2): 74–108. [PubMed: 15761078]
2. Schistosomes, liver flukes and Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans; Lyon. 7–14 June 1994; 1994. p. 1-241.
3. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001;345(11): 784–9. [PubMed: 11556297]
4. Peek RM Jr, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002;2(1):28–37. [PubMed: 11902583]
5. Kim YS, Gum J Jr, Brockhausen I. Mucin glycoproteins in neoplasia. Glycoconj J 1996;13(5):693–707. [PubMed: 8909996]
6. De Bolos C, Garrido M, Real FX. MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology 1995;109(3): 723–34. [PubMed: 7657100]
7. Gendler SJ, Spicer AP. Epithelial mucin genes. Annu Rev Physiol 1995;57:607–34. [PubMed: 7778880]
8. Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP. Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. J Histochem Cytochem 1993;41(10):1479–85. [PubMed: 8245407]
9. Babu SD, Jayanthi V, Devaraj N, Reis CA, Devaraj H. Expression profile of mucins (MUC2, MUC5AC and MUC6) in Helicobacter pylori infected pre-neoplastic and neoplastic human gastric epithelium. Mol Cancer 2006;5:10. [PubMed: 16545139]
10. Wang RQ, Fang DC. Effects of Helicobacter pylori infection on mucin expression in gastric carcinoma and pericancerous tissues. J Gastroenterol Hepatol 2006;21(2):425–31. [PubMed: 16509869]

11. Reis CA, David L, Correa P, Carneiro F, de Bolos C, Garcia E, et al. Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. Cancer Res 1999;59(5):1003–7. [PubMed: 10070955]
12. Reis CA, David L, Nielsen PA, Clausen H, Mirgorodskaya K, Roepstorff P, et al. Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. Int J Cancer 1997;74(1):112–21. [PubMed: 9036879]
13. Carrato C, Balague C, de Bolos C, Gonzalez E, Gambus G, Planas J, et al. Differential apomucin expression in normal and neoplastic human gastrointestinal tissues. Gastroenterology 1994;107(1): 160–72. [PubMed: 8020658]
14. Kocer B, Ulas M, Ustundag Y, Erdogan S, Karabeyoglu M, Yldrm O, et al. A confirmatory report for the close interaction of Helicobacter pylori with gastric epithelial MUC5AC expression. J Clin Gastroenterol 2004;38(6):496–502. [PubMed: 15220684]
15. Van de Bovenkamp JH, Mahdavi J, Korteland-Van Male AM, Buller HA, Einerhand AW, Boren T, et al. The MUC5AC glycoprotein is the primary receptor for Helicobacter pylori in the human stomach. Helicobacter 2003;8(5):521–32. [PubMed: 14535999]
16. Vinall LE, King M, Novelli M, Green CA, Daniels G, Hilkens J, et al. Altered expression and allelic association of the hypervariable membrane mucin MUC1 in Helicobacter pylori gastritis. Gastroenterology 2002;123(1):41–9. [PubMed: 12105832]
17. Reis CA, David L, Carvalho F, Mandel U, de Bolos C, Mirgorodskaya E, et al. Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. J Histochem Cytochem 2000;48(3):377–88. [PubMed: 10681391]
18. Ho SB, Shekels LL, Toribara NW, Kim YS, Lyftogt C, Cherwitz DL, et al. Mucin gene expression in normal, preneoplastic, and neoplastic human gastric epithelium. Cancer Res 1995;55(12):2681–90. [PubMed: 7780985]
19. Baldus SE, Hanisch FG. Biochemistry and pathological importance of mucin-associated antigens in gastrointestinal neoplasia. Adv Cancer Res 2000;79:201–48. [PubMed: 10818682]
20. Carvalho F, Seruca R, David L, Amorim A, Seixas M, Bennett E, et al. MUC1 gene polymorphism and gastric cancer--an epidemiological study. Glycoconj J 1997;14(1):107–11. [PubMed: 9076520]
21. Silva F, Carvalho F, Peixoto A, Seixas M, Almeida R, Carneiro F, et al. MUC1 gene polymorphism in the gastric carcinogenesis pathway. Eur J Hum Genet 2001;9(7):548–52. [PubMed: 11464247]
22. Nguyen TV, Janssen M Jr, Gritters P, te Morsche RH, Drenth JP, van Asten H, et al. Short mucin 6 alleles are associated with H pylori infection. World J Gastroenterol 2006;12(37):6021–5. [PubMed: 17009402]
23. Garcia E, Carvalho F, Amorim A, David L. MUC6 gene polymorphism in healthy individuals and in gastric cancer patients from northern Portugal. Cancer Epidemiol Biomarkers Prev 1997;6(12): 1071–4. [PubMed: 9419405]
24. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74(1):106–20. [PubMed: 14681826]
25. Westfall, P.; Young, S. Examples and methods for p-value adjustment. John Wiley & Sons; 1993. Resampling-based multiple testing.
26. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263–5. [PubMed: 15297300]
27. French B, Lumley T, Monks SA, Rice KM, Hindorff LA, Reiner AP, et al. Simple estimates of haplotype relative risks in case-control data. Genet Epidemiol 2006;30(6):485–94. [PubMed: 16755519]
28. Ligtenberg MJ, Gennissen AM, Vos HL, Hilkens J. A single nucleotide polymorphism in an exon dictates allele dependent differential splicing of episialin mRNA. Nucleic Acids Res 1991;19(2): 297–301. [PubMed: 2014168]

29. Imbert Y, Darling DS, Jumblatt MM, Foulks GN, Couzin EG, Steele PS, et al. MUC1 splice variants in human ocular surface tissues: possible differences between dry eye patients and normal controls. Exp Eye Res 2006;83(3):493–501. [PubMed: 16631167]

30. Ng W, Loh AX, Teixeira AS, Pereira SP, Swallow DM. Genetic regulation of MUC1 alternative splicing in human tissues. Br J Cancer 2008;99(6):978–85. [PubMed: 19238635]

31. Pigny P, Guyonnet-Duperat V, Hill AS, Pratt WS, Galiegue-Zouitina S, d'Hooge MC, et al. Human mucin genes assigned to 11p15.5: identification and organization of a cluster of genes. Genomics 1996;38(3):340–52. [PubMed: 8975711]

32. Baffa R, Negrini M, Mandes B, Rugge M, Ranzani GN, Hirohashi S, et al. Loss of heterozygosity for chromosome 11 in adenocarcinoma of the stomach. Cancer Res 1996;56(2):268–72. [PubMed: 8542579]

33. Moskaluk CA, Rumpel CA. Allelic deletion in 11p15 is a common occurrence in esophageal and gastric adenocarcinoma. Cancer 1998;83(2):232–9. [PubMed: 9669804]

34. Baldus SE, Monig SP, Arkenau V, Hanisch FG, Schneider PM, Thiele J, et al. Correlation of MUC5AC immunoreactivity with histopathological subtypes and prognosis of gastric carcinoma. Ann Surg Oncol 2002;9(9):887–93. [PubMed: 12417511]

35. Lancaster CA, Peat N, Duhig T, Wilson D, Taylor-Papadimitriou J, Gendler SJ. Structure and expression of the human polymorphic epithelial mucin gene: an expressed VNTR unit. Biochem Biophys Res Commun 1990;173(3):1019–29. [PubMed: 2268309]

36. Montpetit A, Nelis M, Laflamme P, Magi R, Ke X, Remm M, et al. An evaluation of the performance of tag SNPs derived from HapMap in a Caucasian population. PLoS Genet 2006;2(3):e27. [PubMed: 16532062]

37. Lundmark PE, Liljedahl U, Boomsma DI, Mannila H, Martin NG, Palotie A, et al. Evaluation of HapMap data in six populations of European descent. Eur J Hum Genet 2008;16(9):1142–50. [PubMed: 18398430]

38. Lan Q, Chow WH, Lissowska J, Hein DW, Buetow K, Engel LS, et al. Glutathione S-transferase genotypes and stomach cancer in a population-based case-control study in Warsaw, Poland. Pharmacogenetics 2001;11(8):655–61. [PubMed: 11692073]

Table 1

Characteristics of study subjects enrolled in a case-control study on stomach cancer conducted in Warsaw, Poland, 1994–1996

	Cases *n* (%), total 273	Controls *n* (%), total 377
Men	185 (67.8)	245 (65.0)
Age		
0–49	32 (11.7)	46 (12.2)
50–59	48 (17.6)	63 (16.7)
60–64	55 (20.1)	62 (16.5)
65–69	55 (20.1)	87 (23.0)
70–74	54 (19.9)	69 (18.3)
75–79	29 (10.6)	50 (13.3)
Smoking		
Non-smoker	78 (28.6)	156 (41.4)
1–19 pack-years	46 (16.8)	68 (18.0)
20–39 pack-years	65 (23.8)	95 (25.2)
≥ 40 pack-years	81 (29.7)	57 (15.1)
Unknown	3 (1.1)	1 (0.3)
Drinking		
Non-drinker	96 (35.2)	123 (32.6)
Former drinker	88 (32.2)	52 (13.8)
Current drinker	83 (30.4)	202 (53.6)
Unknown	6 (2.2)	0
H. pylori positive*	230 (84.2)	320 (84.9)
Family history of stomach cancer	34 (12.5)	16 (4.2)
By histology type		
Intestinal	181 (66.4)	
Diffuse	46 (16.8)	
Mix or missing	46 (16.8)	
By anatomic site		
Cardia	34 (12.4)	
Non Cardia	197 (72.2)	
Mix or unclassified	42 (15.4)	

* Presence of IgG antibodies against surface antigens of *H. pylori* and/or CagA

Table 2

Association between *MUC1* tagSNPs and risk of stomach cancer

SNP		Controls[a]	Cases[a]	
	Genotype	***n***	***n***	**OR (95% CI)** [b]
rs6427184[c]	TT	299	191	Reference
	CT	76	80	**1.69 (1.17–2.44)***
	CC	0	0	--
	CT/CC	76	80	**1.69 (1.17–2.44)***
rs4971052	GG	151	80	Reference
	AG	178	126	1.33 (0.93–1.90)
	AA	45	66	**2.76 (1.72–4.42)****
	AG/AA	223	192	**1.62 (1.16–2.26)***
rs4276913	TT	152	81	Reference
	CT	177	125	1.32 (0.93–1.89)
	CC	46	66	**2.68 (1.68–4.28)****
	CT/CC	223	191	**1.60 (1.15–2.23)***
rs4971088	AA	122	70	Reference
	AT	190	120	1.10 (0.76–1.61)
	TT	60	79	**2.28 (1.45–3.57)****
	AT/TT	250	199	1.39 (0.98–1.97)
rs4971092	AA	304	192	Reference
	AG	67	74	**1.80 (1.23–2.63)***
	GG	5	5	1.61 (0.45–5.71)
	AG/GG	72	79	**1.79 (1.23–2.59)***
rs4072037	GG	103	56	Reference
	AG	194	121	1.14 (0.77–1.70)
	AA	79	95	**2.20 (1.41–3.44)****
	AG/AA	273	216	**1.45 (1.00–2.10)**

[a] Sum of column did not add up to total study subjects because of missing data.

[b] Odds ratio (95% confidence interval), adjusted for age and sex.

[c] The *P* value of fitness for Hardy–Weinberg proportion in controls is 0.029.

* The permutation *P* value < 0.05.

** The permutation *P* value < 0.01.

Table 3

Association between haplotypes of *MUC1* gene and risk of stomach cancer

Haplotypes [a]	Controls (%)	Cases (%)	OR (95% CI) [b, c]
GTAAG	46.2	31.4	Reference
ACTAA	34.4	45.2	**1.93 (1.49–2.48)**
GTTAA	6.3	4.0	0.92 (0.53–1.59)
GTAGG	5.3	8.8	**2.46 (1.59–3.81)**
GTAGA	4.8	6.6	**2.06 (1.25–3.40)**
Others	3.0	4.1	**2.02 (1.13–3.61)**

[a] The SNP order was rs4971052, rs4276913, rs4971088, rs4971092 and rs4072037.

[b] Odds ratio (95% confidence interval), adjusted for age and sex.

[c] P_{global} value < 0.0001.

Table 4

Association between *MUC5AC* tagSNPs and risk of stomach cancer

SNP	Genotype	Controls[a]	Cases[a]	OR (95% CI) [b]
rs1541314	GG	311	216	Reference
	AG	65	54	1.23 (0.82–1.84)
	AA	0	3	--
	AG/AA	65	57	1.30 (0.87–1.94)
rs2014486	AA	123	72	Reference
	AG	175	123	1.22 (0.84–1.77)
	GG	79	77	**1.66 (1.08–2.55)**
	AG/GG	254	200	1.35 (0.96–1.92)
rs2075859	CC	192	123	Reference
	CT	148	110	1.16 (0.83–1.63)
	TT	36	37	1.57 (0.94–2.62)
	CT/TT	184	147	1.24 (0.91–1.70)
rs2672785	AA	236	158	Reference
	AG	130	102	1.19 (0.85–1.65)
	GG	10	13	1.94 (0.82–4.58)
	AG/GG	140	115	1.24 (0.90–1.71)
rs2735733	CC	146	92	Reference
	CT	165	112	1.09 (0.76–1.56)
	TT	60	65	**1.73 (1.11–2.68)**
	CT/TT	225	177	1.26 (0.91–1.75)
rs7118568	CC	335	235	Reference
	CG	39	35	1.27 (0.78–2.08)
	GG	1	2	2.60 (0.23–29.25)
	CG/GG	40	37	1.31 (0.81–2.11)
rs868903	CC	106	50	Reference
	CT	183	147	**1.74 (1.16–2.60)**
	TT	84	74	**1.92 (1.21–3.05)**
	CT/TT	267	221	**1.80 (1.22–2.63)***
rs4963049	AA	305	231	Reference
	AG	67	40	0.76 (0.50–1.17)
	GG	3	1	0.43 (0.04–4.21)
	AG/GG	70	41	0.75 (0.49–1.15)

[a] Sum of column did not add up to total study subjects because of missing data.

[b] Odds ratio (95% confidence interval), adjusted for age and sex.

* The permutation P value < 0.05.

Table 5

Association between haplotypes of *MUC5AC* gene and risk of stomach cancer

Haplotypes [a]	Controls (%)	Cases(%)	OR (95% CI) [b,c]
GACACCCA	30.4	24.1	Reference
GACACCTA	14.5	17.8	**1.60 (1.19–2.15)**
GGTATCTA	13.1	14.7	1.43 (1.00–2.05)
GGTATCCA	9.4	10.1	1.37 (0.96–1.96)
AGCGTCTA	6.6	9.2	**1.86 (1.20–2.89)**
GACACCCG	6.4	4.1	0.79 (0.47–1.30)
GGTGTGTA	4.9	6.2	1.54 (0.93–2.54)
GGCGCCTA	4.1	2.4	0.71 (0.38–1.33)
GACACCTG	2.1	1.7	1.02 (0.51–2.03)
AGCGTCCA	2.0	1.9	1.24 (0.63–2.42)
Others	6.6	7.8	1.50 (0.82–2.77)

[a] The SNP order was rs1541314, rs2014486, rs2075859, rs2672785, rs2735733, rs7118568, rs868903 and rs4963049.

[b] Odds ratio (95% confidence interval), adjusted for age and sex.

[c] $P_{global} = 0.026$.

Appendix A

ummary of the SNPs evaluated in the study

Gene	Chromosome	SNP	Alleles [a]
MUC1	1	rs6427184	C/T
		rs4971052	A/G
		rs4276913	C/T
		rs4971088	T/A
		rs4971092	G/A
		rs4072037	G/A
MUC5AC	11	rs1541314	A/G
		rs2735733	T/C
		rs2014486	A/G
		rs2075859	T/C
		rs2672785	G/A
		rs7118568	G/C
		rs868903	T/C
		rs4963049	G/A
MUC6	11	rs7943115	T/C
		rs7483870	A/G
		rs4077293	T/C
		rs1128413	C/T
		rs11602663	A/G
		rs11605303	T/C
		rs10794359	T/C
		rs7112267	A/G
		rs12574439	C/G
		rs7119740	G/C
		rs11601642	C/A
		rs10902076	C/G
		rs2071174	G/A
		rs11245936	T/C

[a]Minor allele was listed at first followed by major allele.

Appendix B

Association between *MUC6* tagSNPs and risk of stomach cancer

SNP	Genotype	Controls[a]	Cases[a]	OR (95% CI) [b]
rs1128413	TT	106	71	Reference
	CT	187	135	1.08 (0.74–1.57)
	CC	82	67	1.24 (0.80–1.94)
	CT/CC	269	202	1.13 (0.79–1.61)
rs4077293	CC	154	120	Reference
	CT	182	125	0.88 (0.63–1.23)
	TT	39	24	0.79 (0.45–1.39)
	CT/TT	221	149	0.87 (0.63–1.19)
rs7483870	GG	227	173	Reference
	AG	133	87	0.86 (0.62–1.21)
	AA	14	11	1.03 (0.45–2.33)
	AG/AA	147	98	0.88 (0.64–1.21)
rs7943115	CC	132	93	Reference
	CT	188	128	0.95 (0.67–1.34)
	TT	54	51	1.37 (0.86–2.20)
	CT/TT	242	179	1.04 (0.75–1.45)
rs11602663 [c]	GG	246	184	Reference
	AG	125	79	0.83 (0.59–1.17)
	AA	5	9	2.38 (0.78–7.26)
	AG/AA	130	88	0.89 (0.64–1.24)
rs11605303	CC	329	233	Reference
	CT	47	39	1.19 (0.75–1.89)
	TT	1	1	1.44 (0.09–23.42)
	CT/TT	48	40	1.20 (0.76–1.89)
rs10902076	GG	151	94	Reference
	CG	160	133	1.36 (0.96–1.93)
	CC	64	44	1.13 (0.71–1.81)
	CG/CC	224	177	1.30 (0.94–1.80)
rs2071174	AA	166	135	Reference
	AG	175	119	0.84 (0.60–1.16)
	GG	36	19	0.62 (0.34–1.14)
	AG/GG	211	138	0.80 (0.58–1.09)
rs11245936	CC	322	232	Reference
	CT	50	40	1.12 (0.71–1.76)
	TT	0	1	-
	CT/TT	50	41	1.15 (0.74–1.80)
rs10794359	CC	118	84	Reference
	CT	189	148	1.10 (0.77–1.56)
	TT	70	39	0.77 (0.48–1.25)

SNP	Genotype	Controls[a]	Cases[a]	OR (95% CI) [b]
	CT/TT	259	187	1.01 (0.72–1.42)
rs7112267	GG	286	201	Reference
	AG	81	63	1.09 (0.75–1.59)
	AA	6	5	1.22 (0.36–4.07)
	AG/AA	87	68	1.10 (0.76–1.59)
rs12574439	GG	282	207	Reference
	CG	90	61	0.93 (0.64–1.36)
	CC	4	5	1.77 (0.46–6.72)
	CG/CC	94	66	0.97 (0.67–1.39)
rs7119740[c]	GG	331	238	Reference
	CG	38	34	1.25 (0.76–2.05)
	CC	4	1	0.37 (0.04–3.31)
	CG/CC	42	35	1.17 (0.72–1.89)
rs11601642	AA	123	81	Reference
	AC	175	139	1.23 (0.85–1.76)
	CC	77	52	1.05 (0.66–1.65)
	AC/CC	252	191	1.17 (0.83–1.65)

[a] Sum of column did not add up to total study subjects because of missing data.

[b] Odds ratio (95% confidence interval), adjusted for age and sex.

[c] The *P* value of fitness for Hardy–Weinberg proportion in controls is 0.013, 0.021 for rs11602663 and rs7119740, respectively.