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Polymorphisms in *MUC1*, *MUC2*, *MUC5B* and *MUC6* genes are not associated with the risk of chronic atrophic gastritis

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ABSTRACT

Mucins represent major components of the mucous layer in the stomach, protecting the underlying epithelium from acid, mechanical trauma, proteases and pathogenic bacteria. Previous studies have shown an association of neoplastic transformation in the stomach with aberrant mucin levels, suggesting a potential role of genetic variation in mucin genes in the development of gastric cancer (GC). We assessed the association of genetic variation in candidate single nucleotide polymorphisms (SNPs) in mucin genes with the risk of chronic atrophic gastritis (CAG), a well-established precursor of GC in the German population-based ESTHER study. We genotyped MUC1 T31T, MUC2 L58P, MUC2 V116M, MUC5B E34G, MUC5B R51W, *MUC5B* rs2014486 (intronic) and MUC6 V619M for 533 serologically defined CAG cases and 1054 age- and sex-matched controls. None of the analysed SNPs was associated with CAG. However, large studies are needed to disclose or exclude potential weak associations of these SNPs with CAG risk.

1. Introduction

Chronic atrophic gastritis (CAG) is a well-established precursor lesion in the development of intestinal gastric cancer (GC), the most common type of GC.[1–3] In contrast to the diffuse type of gastric carcinoma, a recent, notorious decline in incidence has been observed for the intestinal type.[1,4] A number of changes could be identified as precursor to the intestinal type of gastric carcinoma, representing sequential steps in the precancerous process: superficial gastritis, CAG (gland loss), small intestinal metaplasia, colonic metaplasia and dysplasia.[1,3] This progression usually takes decades, providing excellent options for timely detection and intervention at precancerous stages.[1,3,5]

With more than one million new cases in 2008, GC is the fourth in cancer incidence and, after lung cancer, the second leading cause of cancer death in both sexes worldwide.[4] In the most recent period, incidence of GC located to the cardia has increased, while the incidence of distal, non-cardia GC has decreased.[6] The latter arises from precancerous lesions, such as CAG and hypochlorhydria upon infection by *Helicobacter pylori* (*H. pylori*), a gram-negative bacterium that specifically colonises the gastric epithelium.[7–9] Hence, the fall in distal cancer incidence may be the result of improved dietary patterns, cooling techniques and reduced infection rates with *H. pylori*.,[6] the strongest risk factor for malignancies that arise within the stomach. The attributable risk for GC conferred by *H. pylori* has been estimated to be 75%.[7,10] Colonisation by *H. pylori* always causes persistent mucosal inflammation whose distribution and severity varies and impairs degree of risk and clinical outcome.[7,9,11–13] Yet, only a minor fraction of affected individuals develop neoplasia, the risk being dependent on

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strain-specific bacterial factors and/or host genetic traits, and environmental determinants.[7,9,14,15]

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,[9,16] and exerting detrimental effects on the mucosa as well as on the surface cells of the gastric epithelium.[11] The infection leads to the alteration of mucin glycosylation which facilitates bacterial attachment and a collapse of the mucous barrier, assuring the survival of *H. pylori*.[11,17]

Thus, neoplastic transformation in the stomach was shown to be associated with decreased levels of MUC1, MUC5 and MUC6 proteins along with an additional expression of *MUC2*, *MUC3* and *MUC4*.[11,18] These findings suggest a potential role of genetic variation in mucin genes in gastric carcinogenesis. Whereas some studies have shown associations of small size *MUC1* variable tandem repeat (VNTR) alleles with an increased risk of CAG, incomplete intestinal metaplasia and GC,[19–21] others have reported the single nucleotide polymorphism (SNP) rs4072037 in exon 2 of *MUC1* to control alternative splicing and revealed its association with cancer, including breast, ovarian and gastric cancers.[22–25] Moreover, MUC2 showed a significant association with Crohn's disease (CD), which may be attributable to the significant reduction of *MUC2* mRNA expression due to the V116M change.[26] Recent studies provided further evidence for potential contributions of mucin gene family members to GC development, showing associations with *MUC5* and *MUC6* polymorphisms.[22–27]

To clarify the potential role of genetic variation in *MUC1*, *MUC2*, *MUC5B* and *MUC6* with respect to CAG risk, associations between putative functional SNPs in these genes were explored, using a large population-based study from Germany.

2. Material and methods

2.1. Study population

The present analyses are based on baseline data of ESTHER, a large population-based cohort study, initiated to investigate new avenues of prevention and early detection of chronic diseases in the elderly. Details of the study design have been described previously.[5,28] In brief, 9953 participants (age range: 50–74 years of age; mean: 62 years) were recruited between July 2000 and December 2002 by their general practitioners during a general health check-up in Saarland, a federal state in the south-west of Germany. The study was approved by the ethics committees of the medical faculty of the University of Heidelberg and the medical board of the state of Saarland. Written informed consent was obtained from each participant. The present analyses are restricted to 533 participants without gastric cancer who were serologically defined as CAG cases (age range: 50–74 years of age; mean: 64.7 years; serological definition see below) and a stratified random sample of 1054 controls (age range: 50–74 years of age; mean: 64.6 years).[28,29] Controls were frequency-matched to cases by 5-year age groups and sex.

2.2. Data collection

A standardised questionnaire was completed by every participant, providing information on socio-demographic characteristics, health status, family history and lifestyle factors. Serum samples were obtained from all participants and stored at −80 °C and, according to the study protocol and informed consent, blood samples were collected, mailed to the study centre and stored at −80 °C until analysis.

2.3. Serological examinations

Serum concentrations of pepsinogen (PG) I and II were measured by ELISA (Biohit, Helsinki, Finland). CAG was defined by applying the most frequently used serological definition, with PG I <70 ng/ml and PG I/PG II <3.[5,30,31] For sensitivity analyses, we used alternative cut-points to delineate CAG [(PG I <70 ng/ml and PG I/PG II <4.5) as well as (PG I <70 ng/ml and PG I/PG II <2)].[32]

2.4. Selection of single nucleotide polymorphisms (SNPs)

Candidate SNPs were selected by means of well-defined methods and criteria: Public literature resources and databases – NCBI PubMed and dbSNP – were searched for CAG- and GC-related candidate genes and for previous associations with gastrointestinal malignancies. In addition, SNPs were tested for evolutionary conservation among human, mouse and rat (WU-BLAST2),[33] and putative functional effects of the non-synonymous SNPs were predicted by FastSNP[34], PolyPhen[35], SIFT[36] and SNPs3D[37,38] as ancillary information and/or affirmation of selection (Table 1). SNPs with a minor allele frequency (MAF) $\geqslant 0.05$ in the HapMap CEU population (Utah residents with northern and western European ancestries from the Centre d'Etude du Polymorphisme Humain (CEPH) collection) were included in the study. As genotyping of the previously analysed rs1128413 on GC risk[22] was not feasible, we selected rs7481521, being adjacent to rs1128413. With $D' = 1.0$ and an r-squared value of 0.98, strong linkage disequilibrium (LD) between the variants was assured.[39] The final selection comprised MUC1 T31T (rs4072037), MUC2 L58P (rs2856111), MUC2 V116M (rs11825977), MUC5B E34G (rs2672785), MUC5B R51W (rs2075853), *MUC5B* rs2014486 (intron 25/tagging) and MUC6 V619M (rs7481521).

2.5. Genotyping

Sequenom's MassARRAY® system (Sequenom, San Diego, USA) was applied for genotyping, performing iPLEX® single base primer extension and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry as described elsewhere.[40] Genotyping calls were made in real time with the MassARRAY® RT software. A random selection of >5% of all samples was genotyped twice for quality control.

2.6. Statistics

Hardy–Weinberg equilibrium (HWE) in controls was tested by comparing observed and expected genotype frequencies, using Pearson's χ^2-tests with one degree of freedom. Uncondi-