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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-

Table 1 – Description of mucin single nucleotide polymorphism (SNP) and criteria for selection.

Gene	Position	[a]SNP ID	[b]MAF	[c]Validation	[d]FastSNP[34]	PolyPhen[35]	SIFT[36]	[e]SNPs3D[37,38] SVM Score	Previous findings Ref.
MUC1	T31T	rs4072037	0.42	No	n/a	n/a	n/a	n/a	22–25
MUC2	L58P	rs2856111	0.11	Yes	**Medium-high (3–4)**	**Possibly damaging**	Tolerated	**−0.90**	
MUC2	V116 M	rs11825977	0.21	Yes	**Medium-high (3–4)**	Benign	Tolerated	1.34	26
MUC5B	E34G	rs2672785	0.24	Yes	Low-medium (2–3)	Benign	Tolerated	n/a	22
MUC5B	R51 W	rs2075853	0.08	**Yes**	Low-medium (2–3)	Benign	Tolerated	n/a	
MUC5B	Intron 25/tagging	rs2014486	0.48	No	n/a	n/a	n/a	n/a	22
MUC6	V619 M	rs7481521	0.41	Yes	Low-medium (2–3)	Benign	Tolerated	1.68	22 LD with rs112841322

Abbreviation: LD, linkage disequilibrium; n/a, not applicable; SVM, support vector machine MAF, Minor allele frequency.
Parameters contributing to SNP selection are given in **bold**; main emphasis was placed on previous findings on gastric cancer risk concerning gene expression profiles and risk modulation and on prediction programs.[34–38]

[a] SNP ID according to NCBI dbSNP.
[b] MAFs are based on HapMap-CEU populations (Utah residents with northern and western European ancestries from the Centre d'Etude du Polymorphisme Humain (CEPH) collection).
[c] Validated as a non-synonymous coding SNP with MAF > 0.05 in HapMap-CEU populations.
[d] Risk ranking varies from 0 (no effect) to 5 (very strong effect).
[e] Negative SVM scores indicate SNPs that are classified as deleterious by the sequence profile method.[37,38].

Table 2 – Characteristics of study participants.

	Cases [a]N (%)	Controls [a]N (%)
Total	533 (100)	1054 (100)
Sex		
Male	223 (41.8)	440 (41.7)
Female	310 (58.2)	614 (58.3)
Age (years)		
50–54	44 (8.3)	87 (8.3)
55–59	59 (11.1)	117 (11.1)
60–64	132 (24.8)	265 (25.1)
65–69	164 (30.8)	325 (30.8)
70–74	134 (25.1)	260 (24.7)
Smoking		
Never	292 (58.1)	571 (57.1)
Former	158 (31.4)	308 (30.8)
Current	53 (10.5)	121 (12.1)
Alcohol (g/week)		
0	199 (41.4)	318 (33.1)
0 < and < 60	116 (24.1)	286 (29.8)
60–140	105 (21.8)	239 (24.9)
>140	61 (12.7)	117 (12.2)
Family history of gastric cancer		
Yes	46 (8.6)	63 (5.8)
Helicobacter pylori infection		
Positive	401 (80.8)	555 (57.2)

Chronic atrophic gastritis was defined as pepsinogen (PG) I < 70 ng/ml and PG I/PG II < 3.
[a] For smoking, alcohol and *Helicobacter pylori* infection, the sum does not add up to total due to missing values.

tional logistic regression was applied to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) adjusted for matching factors age and sex, using dominant and co-dominant genotype models. Tests for linear trend (additive genotype models) were also employed. All tests were two-sided and considered statistically significant with $P < 0.05$. As CAG strongly increases with age,[5] subgroup analyses included stratifications according to ages <65 and ⩾65 years. All analyses were carried out using Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc., Cary, USA).

Haploview was used to examine measures of LD (D′ and *r*-squared) between adjacent SNPs.[39] Power calculations were employed with the power and sample size software PS version 3.0.7.[41]

3. Results

At baseline, 9953 ESTHER participants were recruited to identify new ways of prevention and early detection of chronic diseases in the elderly. Because of a positive history of GC, seven individuals were excluded from the present analyses. Of the remaining 9444 participants with available PG concentrations (94.9%), 533 met the serological definition of CAG[5,30,31] and were selected for this study along with 1054 age- and sex-matched controls. Major characteristics of study participants are shown in Table 2. Among the analysed

Table 3 – Associations of *MUC1*, *MUC2*, *MUC5B* and *MUC6* single nucleotide polymorphisms (SNPs) and risk of chronic atrophic gastritis (CAG).

Gene name	SNP ID	SNP/Location	Genotype	CAG Cases N (%)	Controls N (%)	[a]OR (95% CI)	P
MUC1	rs4072037	T31T	AA	144 (28.2)	289 (29.6)	1	
			AG	237 (46.4)	464 (47.4)	1.03 (0.80, 1.32)	0.85
			GG	130 (25.4)	225 (23.0)	1.16 (0.86, 1.55)	0.34
			GG + AG	367 (71.8)	689 (70.4)	1.07 (0.84, 1.35)	0.59
							P_{trend} = 0.35
MUC2	rs2856111	L58P	TT	396 (75.1)	809 (77.9)	1	
			TC	124 (23.5)	214 (20.6)	1.18 (0.92, 1.52)	0.19
			CC	7 (1.3)	16 (1.5)	0.89 (0.37, 2.19)	0.81
			CC + TC	131 (24.9)	230 (22.1)	1.16 (0.91, 1.49)	0.23
							P_{trend} = 0.31
MUC2	rs11825977	V116M	GG	315 (60.6)	656 (63.1)	1	
			GA	181 (34.8)	341 (32.8)	1.11 (0.88, 1.39)	0.38
			AA	24 (4.6)	42 (4.0)	1.20 (0.71, 2.02)	0.49
			AA + GA	205 (39.4)	383 (36.9)	1.12 (0.90, 1.39)	0.32
							P_{trend} = 0.30
MUC5B	rs2672785	E34G	AA	324 (62.8)	653 (63.1)	1	
			AG	178 (34.5)	330 (31.9)	1.08 (0.86, 1.36)	0.49
			GG	14 (2.7)	52 (5.0)	0.54 (0.30, 0.99)	0.05
			GG + AG	192 (37.2)	382 (36.9)	1.01 (0.81, 1.26)	0.93
							P_{trend} = 0.49
MUC5B	rs2075853	R51W	CC	456 (88.0)	884 (85.6)	1	
			CT	59 (11.4)	144 (13.9)	0.79 (0.57, 1.09)	0.15
			TT	3 (0.6)	5 (0.5)	1.15 (0.27, 4.84)	0.85
			TT + CT	62 (12.0)	149 (14.4)	0.80 (0.58, 1.10)	0.17
							P_{trend} = 0.21
MUC5B	rs2014486	Tag/Intron25					
			GG	124 (24.0)	286 (27.7)	1	
			GA	279 (54.1)	523 (50.7)	1.24 (0.96, 1.60)	0.11
			AA	113 (21.9)	222 (21.5)	1.18 (0.87, 1.61)	0.29
			AA + GA	392 (76.0)	745 (72.3)	1.22 (0.96, 1.56)	0.11
							P_{trend} = 0.26
MUC6	rs7481521	V619M					
			AA	160 (30.1)	320 (30.7)	1	
			AG	265 (49.9)	490 (46.9)	1.08 (0.85, 1.37)	0.54
			GG	106 (20.0)	234 (22.4)	(0.67, 1.22)	0.50
			GG + AG	371 (69.9)	724 (69.3)	1.02 (0.81, 1.28)	0.85
							P_{trend} = 0.59

Abbreviation: CI, confidence interval; ID, identification; OR, odds ratio; SNP, single nucleotide polymorphism.
[a] ORs were adjusted for age and sex.

individuals, females were more common (58.2%), and median ages were 65 and 66 years for women and men, respectively. In contrast to an evenly distributed smoking status among cases and controls, alcohol consumption was more prevalent among controls (66.9% vs. 58.6% drinkers). Contrariwise, the proportion of individuals with a GC family history and an infection with *H. pylori* was more common among cases (Table 2).

The average call rate for the SNPs analysed was 97.6% (range: 93.9% to 99.3%), and it did not differ between cases and controls for any individual assay. Applying the most frequently used serological definition of CAG with PG I <70 ng/ml and PG I/PG II <3, allele frequencies among controls were consistent with HWE for all SNPs.

Table 3 summarises the results for the seven analysed SNPs. There was no evidence of a significant association with CAG risk for any of the investigated polymorphisms (Table 3). For each SNP, very similar ORs were obtained with modified serological definitions in sensitivity analyses (data not shown).

Considering associations among subgroups, i.e. CAG among subjects < and ⩾65 years of age, we found a dose-dependent, marginal association of MUC2 V116 M with an increased risk of CAG among individuals ⩾65 years of age (OR = 1.30, 95% CI = 0.97, 1.73; P = 0.08, Table 4). In addition, heterozygosity for the minor allele of MUC5B E34G showed a trend for association with an increased CAG risk among subjects ⩾65 years of age (OR = 1.32, 95% CI = 0.98, 1.77; P = 0.07, Table 4). By contrast, a dose-dependent, marginal association of this variant with a decreased risk of CAG was seen among subjects <65 years of age (OR = 0.78, 95% CI = 0.56, 1.10; P = 0.16, Table 4). Additional analyses comparing younger

Table 4 – Associations of *MUC1, MUC2, MUC5B* and *MUC6* SNPs among individuals < and > 65 years of age and risk of chronic atrophic gastritis (CAG).

Gene Name	SNP ID	SNP/Location	Genotype	Cases < 65 years N (%)	Controls < 65 years N (%)	[a]OR (95% CI)	P	Cases ⩾ 65 years N (%)	Controls ⩾ 65 years N (%)	[a]OR (95% CI)	P
MUC1	rs4072037	T31T	AA	64 (28.8)	134 (30.9)	1		80 (27.7)	155 (28.5)	1	
			AG	103 (46.4)	203 (46.8)	1.06 (0.73, 1.56)	0.75	134 (46.4)	261 (48.0)	0.99 (0.71, 1.40)	0.97
			GG	55 (24.8)	97 (22.4)	1.18 (0.75, 1.84)	0.48	75 (26.0)	128 (23.5)	1.13 (0.77, 1.68)	0.53
			GG + AG	158 (71.2)	300 (69.1)	1.10 (0.77, 1.57)	0.60	209 (72.3)	389 (71.5)	1.04 (0.76, 1.43)	0.81
							P_{trend} = 0.48				P_{trend} = 0.54
MUC2	rs2856111	L58P	TT	173 (74.6)	362 (78.2)	1		223 (75.6)	447 (77.6)	1	
			TC	56 (24.1)	96 (20.7)	1.22 (0.84, 1.78)	0.30	68 (23.1)	118 (20.5)	1.16 (0.82, 1.62)	0.40
			CC	3 (1.3)	5 (1.1)	1.27 (0.30, 5.39)	0.75	4 (1.4)	11 (1.9)	0.74 (0.23, 2.34)	0.60
			CC + TC	59 (25.4)	101 (21.8)	1.22 (0.85, 1.77)	0.28	72 (24.4)	129 (22.4)	1.12 (0.81, 1.56)	0.50
							P_{trend} = 0.29				P_{trend} = 0.66
MUC2	rs11825977	V116M	GG	139 (61.5)	275 (59.7)	1		176 (59.9)	381 (65.9)	1	
			GA	76 (33.6)	165 (35.8)	0.91 (0.65, 1.28)	0.58	105 (35.7)	176 (30.4)	1.29 (0.96, 1.74)	0.10
			AA	11 (4.9)	21 (4.6)	1.05 (0.49, 2.25)	0.90	13 (4.4)	21 (3.6)	1.35 (0.66, 2.75)	0.42
			AA + GA	87 (38.5)	186 (40.3)	0.92 (0.67, 1.28)	0.63	118 (40.1)	197 (34.1)	1.30 (0.97, 1.73)	0.08
							P_{trend} = 0.75				P_{trend} = 0.09
MUC5B	rs2672785	E34G	AA	155 (68.9)	293 (63.6)	1		169 (58.1)	360 (62.7)	1	
			AG	64 (28.4)	146 (31.7)	0.83 (0.58, 1.18)	0.29	114 (39.2)	184 (32.1)	1.32 (0.98, 1.77)	0.07
			GG	6 (2.7)	22 (4.8)	0.50 (0.20, 1.27)	0.15	8 (2.7)	30 (5.2)	0.57 (0.26, 1.27)	0.17
			GG + AG	70 (31.1)	168 (36.4)	0.78 (0.56, 1.10)	0.16	122 (41.9)	214 (37.3)	1.21 (0.91, 1.62)	0.19
							P_{trend} = 0.10				P_{trend} = 0.60
MUC5B	rs2075853	R51W	CC	202 (89.8)	398 (86.5)	1		254 (86.7)	486 (84.8)	1	
			CT	22 (9.8)	60 (13.0)	0.71 (0.42, 1.20)	0.20	37 (12.6)	84 (14.7)	0.84 (0.56, 1.28)	0.42
			TT	1 (0.4)	2 (0.4)	0.97 (0.09, 10.72)	0.98	2 (0.7)	3 (0.5)	1.28 (0.21, 7.77)	0.79
			TT + CT	23 (10.2)	62 (13.5)	0.72 (0.43, 1.20)	0.21	39 (13.3)	87 (15.2)	0.86 (0.57, 1.29)	0.46
							P_{trend} = 0.23				P_{trend} = 0.52
MUC5B	rs2014486	Tag/Intron25	GG	53 (23.6)	122 (26.6)	1		71 (24.4)	164 (28.6)	1	
			GA	113 (50.2)	239 (52.2)	1.10 (0.74, 1.63)	0.64	166 (57.0)	284 (49.6)	1.35 (0.96, 1.90)	0.08
			AA	59 (26.2)	97 (21.2)	1.42 (0.90, 2.25)	0.14	54 (18.6)	125 (21.8)	1.00 (0.65, 1.53)	1.00
			AA + GA	172 (76.4)	336 (73.4)	1.19 (0.82, 1.73)	0.36	220 (75.6)	409 (71.4)	1.24 (0.90, 1.72)	0.19
							P_{trend} = 0.14				P_{trend} = 0.84
MUC6	rs7481521	V619M	AA	80 (34.2)	152 (32.7)	1		80 (26.9)	168 (29.0)	1	
			AG	115 (49.2)	211 (45.4)	1.03 (0.72, 1.47)	0.87	150 (50.5)	279 (48.2)	1.13 (0.81, 1.58)	0.47
			GG	39 (16.7)	102 (21.9)	0.72 (0.46, 1.15)	0.17	67 (22.6)	132 (22.8)	1.07 (0.72, 1.59)	0.75
			GG + AG	154 (65.8)	313 (67.3)	0.93 (0.67, 1.30)	0.67	217 (73.1)	411 (71.0)	1.11 (0.81, 1.52)	0.52
							P_{trend} = 0.23				P_{trend} = 0.72

Abbreviation: CI, confidence interval; ID, identification; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] ORs were adjusted for age and sex.

cases (<65 years of age, N = 235) to the older controls (⩾65 years of age, N = 585) did not disclose any significant association for MUC1, MUC2 and MUC5B SNPs. Yet, we revealed a significant association of MUC6 V619 M with a decreased risk for homozygous carriers (OR = 0.62, 95% CI = 0.40, 0.97; P = 0.04) and a significant dose–response relation with the number of alleles (P_{trend} = 0.04).

4. Discussion

To our knowledge, this is the first population-based epidemiological candidate SNP study, addressing the association between mucin gene variants and the susceptibility to CAG, the well-established precursor of intestinal GC.[1–3] None of the seven investigated SNPs showed a statistically significant association with CAG.

The most widely used approach to identify low-risk susceptibility alleles in cancer has been to study candidate genes in biologically relevant pathways. Mucins, high molecular weight glycoproteins, are major components of the mucous viscous gel, covering and protecting surface epithelial tissues.[18] Aberrant expression of mucins is a common feature in inflammatory diseases and several types of cancers, such as GC.[11,18,22] While there are various methods to assess the effects of SNPs on diseases, including tagging (tag)SNP approaches or genome-wide association studies (GWAS), a candidate SNP approach was preferred,[42] focusing on seven most promising candidate SNPs for analysis on CAG risk. Of note, the prioritised SNPs had the highest likelihood of being functionally relevant, considering former associations with GC risk as well as employing diverse *in silico* methods (FastSNP, PolyPhen, SIFT and SNPs3D) (Table 1). Indeed, one of the SNPs (rs2014486) was non-coding, yet previously found to be associated with an increased GC risk.[22]

We, however, found no significant association between the analysed candidate SNPs and risk of CAG. The lack of significance may be either attributable to statistical power limitations, but it may also be a matter of null associations. In what way this finding is related to the recent shift towards gastric cardia tumor location remains elusive.[6]

Yet, one may not rule out that other (tag)SNPs within neighbouring sections of the given mucin genes are associated with CAG. In fact, MUC2 V116 M was marginally associated with an increased risk of CAG among individual's ⩾65 years of age (Table 4), but this finding appears to contradict the observations by Moehle et al. who showed a significant downregulation of *MUC2* in the colon in CD as a result of reduced mRNA expression by reason of V116 M.[26] Heterozygosity for the minor allele of MUC5B E34G which showed a trend for association with an increased CAG risk among subjects ⩾65 years of age (Table 4) supported a previously observed risk increase of this variant with GC,[22] but this association had been weak and not statistically significant. Besides, no stratification according to age had been performed in the study.[22]

The present study has both strengths and limitations. Strengths include the well-defined, homogeneous study population and the fairly large sample size. In addition, we included a number of SNPs for which an association with CAG is biologically plausible and/or has been previously reported and which may be functionally relevant. Despite its size, power was insufficient for detecting potential weak associations even without correction for multiple testing.[43] We had a power of 80% at a significance level of 0.05 to detect ORs ⩾1.39 (rs4072037), ⩾1.49 (rs2856111), ⩾1.38 (rs11825977), ⩾1.38 (rs2672785), ⩾1.61 (rs2075853), ⩾1.39 (rs2014486) and ⩾1.37 (rs7481521).[41]

Moreover, the present data need to be interpreted cautiously as the best serological definition of CAG is difficult to accomplish. Thus, Miki et al. suggested to use the PG I/PG II ratio for the definition of CAG and reported PG I alone to be specific, yet insufficiently sensitive,[44] and a series of studies agreed to the necessity to include the PG I/PG II ratio in the definition.[30,45]

In conclusion, this is the first comprehensive analysis of mucin gene variants and risk of CAG. We found no association between *MUC1*, *MUC2*, *MUC5B* and *MUC6* variants and CAG. Larger and statistically better powered studies that also allow for stratification by important covariates, such as age, are needed to disclose or exclude potential weak associations.

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Conflict of interest statement

None declared.

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