PSCA and *MUC1* Gene Polymorphisms Are Linked with Gastric Cancer and Pre-malignant Gastric Conditions

JUOZAS KUPCINSKAS[1,2], THOMAS WEX[3,4], ALEXANDER LINK[3], RUTA BARTUSEVICIUTE[2], MILDA DEDELAITE[2], GINTARE KEVALAITE[2], MARCIS LEJA[5,6,7], JURGITA SKIECEVICIENE[1], GEDIMINAS KIUDELIS[1,2], LAIMAS JONAITIS[1,2], LIMAS KUPCINSKAS[1,2] and PETER MALFERTHEINER[3]

[1]*Institute for Digestive Research, and* [2]*Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas, Lithuania;*
[3]*Department of Gastroenterology, Hepatology and Infectious Diseases, and* [4]*Medical Laboratory for Clinical Chemistry, Microbiology and Infectious Diseases, Department of Molecular Genetics, Otto von Guericke University, Magdeburg, Germany;*
[5]*Faculty of Medicine, University of Latvia, Riga, Latvia;*
[6]*Digestive Diseases Center GASTRO, Riga, Latvia;*
[7]*Riga East University Hospital, Riga, Latvia*

Abstract. *Background/Aim: Genome-wide association studies revealed a link between gastric cancer (GC) and single nucleotide polymorphisms (SNPs) of prostate stem cell antigen (PSCA), phospholipase C epsilon-1 (PLCE1) and mucin-1 (MUC1) genes. Herein, we aimed to evaluate associations between PSCA (C>T, rs2294008; G>A, rs2976392), MUC1 (C>T, rs4072037) and PLCE1 (A>G, rs2274223) SNPs and GC or high-risk gastritis (HRAG). Materials and Methods: Using TaqMan system, SNPs were genotyped in 252 patients with GC, 136 patients with HRAG and 246 controls. Results: PSCA rs2294008 allele T was linked with risk of GC (odds ratio (OR)=1.88, p<0.001) and HRAG (OR=1.49, p=0.009). Allele A of PSCA rs2976392 was associated with development of GC (OR=1.88, p<0.001) and HRAG (OR=1.56, p<0.01). MUC1 rs4072037 allele G was protective against development of GC (OR=0.64, p=0.0005), while no differences were found for PLCE1 rs2274223. Conclusion: Polymorphisms of PSCA (rs2976392, rs2294008) and MUC1 (rs4072037) genes are linked with GC and HRAG.*

Gastric cancer (GC) is one of the most common causes of cancer-related death worldwide (1). Most recent reports on cancer reveal a declining incidence of GC in Western countries; nevertheless, the mortality associated with GC remains very high (1). The pathways of GC pathogenesis have merged into a complex picture, which involves *Helicobacter pylori* infection as well as genetic, epigenetic and other co-founding factors (2). A genome-wide association study (GWAS) approach has been applied in various diseases including cancer. The first GWAS on GC was published in 2008 and identified an association with a single nucleotide polymorphism (SNP) of prostate stem cell antigen (*PSCA*) gene (3). A few GWAS studies confirmed this association and revealed new susceptibility loci at mucin-1 (*MUC1*) and phospholipase C epsilon-1 (*PLCE1*) genes (3, 4).

Gene SNPs detected in the aforementioned GWAS studies have been implicated in various cancer-related molecular pathways. *PSCA* gene encodes a cell membrane glycoprotein responsible for cellular activation (3). *PSCA* gene is expressed in the epithelium of the stomach and it is particularly down-regulated in gastric tissue with intestinal metaplasia (3). *MUC1* gene encodes a cell membrane protein that plays a role in forming protective mucous barriers on stomach epithelial surfaces and is essential in intracellular signaling (5). Different studies have shown that *MUC1* is involved in the regulation of *H. pylori*-induced chronic gastritis (5). *PLCE1* gene encodes an enzyme that catalyzes hydrolysis which generates second messengers affecting the cell cycle (6). The *PLCE1*-related signaling network has an impact on several critical carcinogenetic processes, including proliferation, cell survival, metabolism, and tumor growth (6). The changes of encoding sequences of these genes have functional roles and may influence the process of carcinogenesis.

Correspondence to: Professor Limas Kupcinskas, Head of Department of Gastroenterology, Lithuanian University of Health Sciences, Eiveniu str. 2, Kaunas, LT-50009, Lithuania. Tel/Fax: +370 37326508, e-mail: l.kupcinskas@gmail.com

Key Words: PSCA, PLCE1, MUC1, SNP, gene polymorphism, gastric cancer, gastritis.

Due to the large number of traits analyzed, GWAS results need replication in independent cohorts of patients in different populations for validation of these findings. Most of the replication studies on GWAS results in patients with GC have been carried-out on Asian populations, while representative data on Europeans are lacking.

In the study, we analyzed four SNPs reported in GC GWAS studies: *PSCA* G>A (rs2976392; NM_005672.4: c.133+80G>A), *PSCA* C>T (rs2294008; NM_005672.4:c.-26C>T), *MUC1* A>G (rs4072037, NM_001204295.1, c.93G>A, pThr31=) and *PLCE1* A>G (rs2274223, NM_016341.3:c.5780A>G, p.His1925Arg). The study population consisted of 252 patients with GC, 136 patients with high-risk gastritis (HRAG) and 246 controls from Lithuania and Latvia, countries which are still distinguished by a high prevalence of *H. pylori* infection and GC (7, 8). Only two studies have previously analyzed the role of *PSCA* SNPs in patients with GC precursor state, *i.e.* atrophic gastritis (9, 10). Furthermore, *MUC1* A>G (rs4072037) and *PLCE1* A>G (rs2274223) gene polymorphisms have not previously been studied in premalignant gastric conditions.

Materials and Methods

Study population. Patients and controls were recruited during 2006-2013 at two gastroenterology Centers in Lithuania (Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas) and Latvia (Riga East University Hospital). HRAG patients and controls were included from the out-patient departments of these Institutes. The inclusion criterion for controls was no history of previous malignancy. Patients with HRAG were defined as in our previous genotyping articles (11, 12) based on criteria suggested by Uemura *et al*. (13) and Meining *et al*. (14). Patients with HRAG had undergone upper endoscopy with thorough histological analysis of stomach mucosa according to Sydney classification (15). All patients with GC had histological verification of gastric adenocarcinoma and were recruited from out-patient and in-patient departments. *H. pylori* status was determined with anti-*H. pylori* IgG antibodies in serum.

In total, 634 individuals were included in the study (246 controls, 136 HRAG and 252 GC); 285 came from the Latvian group (100 controls, 127 GC and 58 HRAG) and 349 from the Lithuanian group (146 controls, 125 GC and 78 HRAG). All patients in the present study were of European descent. The study was approved by the Ethics Committees of the Lithuanian University of Health Sciences (Protocol Nr. BE-2-10) and Central Medical Ethics Committee of Latvia (Protocol Nr. 01-29.1). All patients and controls gave their informed consent to take part in the study.

DNA extraction and genotyping. Genomic DNA from samples was extracted from peripheral blood mononuclear cells using a salting-out method and stored at −20˚C until analysis as described in our previous studies (12, 14). *PSCA* G>A (rs2976392), *PSCA* A>G (rs2294008), *MUC1* A>G (rs4072037) and *PLCE1* A>G (rs2274223) SNPs were genotyped by real-time PCR (RT-PCR), using TaqMan® assays with a 7500™ real-time cycler, in accordance with the manufacturer's instructions (Life Technologies, Carlsbad, California, USA). Dubious samples underwent repetitive genotyping analysis.

Table I. *Characteristics of the participant groups within the study.*

	Controls (n=246)	GC (n=252)	HRAG (n=136)	ANOVA (age)* Chi-squared test *p*-value
Age (years)				
Mean±SD	63.2±9.5	65±11.5	64.6±10.4	NS
Gender				
Male	59 (23.8%)	152 (61.7%)	42 (30.9%)	<0.001
Female	187 (76.2%)	96 (38.2%)	94 (69.1%)	
H. pylori				
Positive	192 (78.1%)	103 (40.8%)	106 (77.9%)	<0.001
Negative	52 (21.1%)	29 (11.5%)	30 (22.1%)	
Unknown	2 (0.8%)	120 (47.6%)		
GC Lauren type				
Intestinal		82 (32.5%)		
Diffuse		74 (29.3%)		
Mixed		29 (11.5%)		
Data unavailable		96 (38.1%)		

GC: Gastric cancer; HRAG: high-risk gastritis; *H. pylori*: *Helicobacter pylori*. *Statistical analysis was performed for all three groups.

Statistical analysis. Age is shown as means with standard deviations, and was compared using ANOVA and unpaired Student's *t*-test. Statistical analysis of the genotyping data was performed using PLINK software version 1.07 (16). All SNPs underwent assessment for Hardy–Weinberg equilibrium (HWE). Association of HRAG and GC with gene polymorphisms was calculated using logistic regression analysis with adjustment for age, gender, and country of birth, with 95% confidence intervals (CI). Disease risk related to SNPs was assessed using genotypic, allelic, recessive and dominant models. *p*-Values were adjusted for multiple testing (significance threshold α=0.0125; 0.05/4).

Results

Characteristics of participants. All participants were classified into three study groups: controls (n=246), HRAG (n=136) and GC (n=252). The characteristics of control, GC and HRAG groups are presented in Table I. The participants differed significantly regarding age and gender distribution between the groups. Males were predominant in GC and accounted for 61.7% in the GC group, while in the control and HRAG groups they constituted 23.8% and 30.9%, respectively. Controls were of similar age to HRAG and GC groups (Table I). To eliminate the potential bias of differences in age and gender distribution among the groups, these parameters were included as co-variates in further logistic regression analysis. A total of 40.9% of patients were positive for *H. pylori* in the GC group; however, *H. pylori* IgG status was not available in around 47.6% of individuals in the GC group. Over 78.1% of those in the control group

Table II. *Genotypic and allelic frequencies of prostate stem cell antigen (PSCA), mucin-1 (MUC1) and phospholipase C epsilon-1 (PLCE1) single nucleotide polymorphisms (SNPs) in controls, and patients with gastric cancer (GC) and high-risk gastritis (HRAG).*

	Controls (n=246*)		GC (n=252*)					HRAG (n=136*)				
SNP	n	%	n	%	aOR	95% CI	*p*-Value	n	%	aOR	95% CI	*p*-Value
PSCA C>T rs2294008												
CC	64	26.3	33	13.2	1			23	17.2	1		
CT	123	50.6	116	46.2	0.54	0.35-0.82	0.004	66	49.3	1.42	0.86-2.33	0.170
TT	56	23.1	102	40.6	3.70	2.15-6.35	2.2e-006	45	33.5	2.21	1.19-4.11	0.012
CC *vs*. CT+TT					2.22	1.51-3.34	6.3e-005			1.62	1.01-2.59	0.044
CC+CT *vs*. TT					2.56	1.59-4.16	0.0001			1.76	1.03-3.02	0.037
Allele C	251	51.6	182	36.3				112	41.8			
Allele T	235	48.4	320	63.7	1.88	2.42-7.70	1.1e-006	156	58.2	1.49	1.01-2.01	0.009
PSCA G>A rs2976392												
GG	62	26.7	34	13.7	1			22	16.7	1		
GA	116	50.0	113	45.4	0.54	0.35-0.83	0.005	64	48.5	0.68	0.41-1.13	0.140
AA	54	23.3	102	40.9	3.57	2.08-6.25	4.2e-006	46	34.8	2.43	1.28-4.54	0.006
GG *vs*. GA+AA					2.22	1.49-3.34	8.8e-005			1.72	1.07-2.78	0.027
GG+GA *vs*. AA					2.43	1.53-4.11	0.0002			1.92	1.09-3.34	0.022
Allele G	240	51.7	181	36.4				108	40.9			
Allele A	224	48.3	317	63.6	1.88	1.47-2.43	1.6e-006	156	59.1	1.56	1.14-2.13	0.004
MUC1 A>G rs4072037												
AA	37	15.9	81	32.5	1			35	26.9	1		
AG	121	52.2	103	41.4	0.96	0.63-1.49	0.864	64	49.3	0.77	0.46-1.29	0.312
GG	74	31.9	65	26.1	0.39	0.23-0.65	0.0004	31	23.8	0.42	0.22-0.79	0.007
AA *vs*. AG+GG					0.71	0.48-1.06	0.096			0.65	0.39-1.06	0.083
AA+AG *vs*. GG					0.39	0.25-0.62	4.8e-005			0.50	0.29-0.85	0.010
Allele A	269	53.2	265	58.0				134	51.6			
Allele G	195	46.8	233	42.0	0.64	0.49-0.81	0.0005	126	48.4	0.68	0.51-0.93	0.013
PLCE1 A>G rs2274223												
AA	91	37.8	94	37.6	1			56	41.5	1		
AG	116	48.1	126	50.4	1.05	0.71-1.55	0.803	57	42.2	0.80	0.51-1.28	0.354
GG	34	14.1	30	12.0	0.88	0.49-1.56	0.652	22	16.3	1.11	2.11-0.34	0.729
AA *vs*. AG+GG					1.01	0.69-1.46	0.951			0.87	0.56-1.34	0.537
AA+AG *vs*. GG					0.85	0.49-1.45	0.5558			1.25	0.69-2.26	0.446
Allele A	298	61.8	314	62.8				169	62.6			
Allele G	184	38.2	186	37.2	0.96	0.74-1.24	0.7528	101	37.4	0.96	0.71-1.32	0.835

*Fnal numbers for each genotype differ due to missing analysis in 8-23 cases (for details see results). aOR: Adjusted odds ratio; CI: confidence interval.

and 77.9% in the HRAG group were *H. pylori*-positive. Histological classification for diffuse and intestinal GC types was available for 73.4% of patients with GC (Table I).

Hardy-Weinberg equilibrium. Overall, samples from six individuals failed genotyping analyses for *PSCA* rs2294008, 21 for *PSCA* rs2976392, 23 for *MUC1* rs4072037 and eight for *PLCE1* rs2274223 repeatedly and were excluded from the study. All SNPs within the study were in HWE: *PSCA* rs2294008, p=0.807; *PSCA* rs2976392, p=0.511; *MUC1* rs4072037, p=0.168; *PLCE1* rs2274223, p=0.733.

PSCA, MUC1 and PLCE1 gene polymorphisms in GC and HRAG. The frequencies of alleles and genotypes of *PSCA* rs2294008 and rs2976392, *MUC1* rs4072037, *PLCE1* rs2274223 in control, GC and HRAG groups is presented in Table II. Allele T of *PSCA* rs2294008 was less frequent in controls (48.4%) than in patients with GC (63.7%, odds ratio

(OR)=1.88, $p=1.1\times10^{6}$) and with HRAG (58.2%, OR=1.49, $p<0.01$). Similarly, TT genotype of *PSCA* rs2294008 was more frequent among patients with GC (40.6%, OR=3.70, $p<0.001$) and HRAG (33.5%, OR=2.21, $p=0.012$) than in controls (23.1%). *PSCA* rs2976392 was noted for a higher frequency of allele A in GC (63.6%, $p<0.001$) and HRAG groups (59.1%, $p=0.004$) when compared to controls (48.3%). Genotype AA of *PSCA* rs2976392 was associated with higher risk of GC both in dominant ($p=0.0002$) and recessive models ($p<0.001$) (Table II). *MUC1* rs4072037 allele G was associated with decreased risk of GC (OR=0.64, $p=00005$). Genotype GG of *MUC1* rs4072037 was also linked with lower risk of GC in AA *vs*. GG (OR= 0.39, $p=0.004$), as well as in the dominant model (OR=0.39, $p<0.001$). A similar link of *MUC1* rs4072037 was also detected in patients with HRAG, where GG genotype (23.8%) was less frequent than in controls (OR=0.42%, $p=0.007$). *PLCE1* rs2274223 alleles and genotypes were distributed equally among control, GC and HRAG groups (Table II).

Association analysis of PSCA, MUC1 and PLCE1 SNPs with diffuse and intestinal-type GC. Allelic and genotypic distribution for the four investigated SNPs among controls and different histological subtypes of GC (intestinal and diffuse) are presented in Table III. This sub-analysis was limited only to those patients for whom histological classification of GC subtype was available. Allele T of *PSCA* rs2294008 was more frequent in diffuse-type GC (59.6%, $p=0.017$) than in controls (23.1%), but this tendency was much more profound for intestinal-type GC (66.5%, OR=2.13, $p<0.001$). A similar observation was made for the TT genotype, which was associated with higher risk of intestinal-type GC both in dominant (OR=2.22, $p=0.0049$) and recessive (OR=4.17, $p=0.001$) comparisons. Allele A of *PSCA* G>A SNP was more common in diffuse-type GC (58.8%) *vs*. controls (51.7%, $p=0.026$). However, comparison of allele A frequencies revealed greater differences between controls (51.7%) and intestinal-type GC (66.7%, OR=2.17, $p=0.001$). Intestinal-type GC was also denoted by higher frequency of AA genotype (42.0%) than controls (26.7%, $p=0.0009$). *MUC1* A>G allele G was associated with reduced risk of diffuse-type GC (OR=0.52, $p=0.0006$), but not of intestinal-type GC (OR=1.31, $p=0.136$). Association analysis also showed that GG *MUC1* genotype was associated with reduced risk of diffuse-type GC in a dominant model (OR=0.34, $p=0.0003$). *PLCE1* A>G SNP was not associated with the presence of intestinal- or diffuse-type GC in all statistical models that were applied, with similar distributions of alleles and genotypes among cases and controls (Table III).

Association analysis between H. pylori and PSCA, MUC1 and PLCE1 SNPs. The analysis of genotypic and allelic distribution among *H. pylori*-positive and -negative individuals for four SNPs of *PSCA, MUC1* and *PLCE1* genes is presented in Table IV. Overall, alleles and genotypes in this sub-analysis were distributed equally among *H. pylori*-positive and -negative individuals for all polymorphisms that have been investigated in this study. There was a tendency for higher distribution of GG genotype (20.4%) and G allele (46.2%) in *H. pylori*-positive individuals when compared to controls (14.5% and 42.7%, respectively); however, the significance remained below the required threshold (Table IV).

Discussion

Multiple SNPs have been linked with gastrointestinal malignancies in recent GWAS studies (17), but the validation data in different populations are still scarce. In the current study, we evaluated *PSCA* C>T rs2294008, *PSCA* G>A rs2294008, *MUC1* A>G rs4072037 and *PLCE1* A>G (rs2274223) SNPs in an Eastern European cohort with GC and premalignant gastric conditions. The major finding of the current study is the significant association of PSCA and *MUC1* SNPs not only with GC, but also with its precursor states, elucidating potential genetic predisposition that might be involved in early stages of gastric cancer development. To the best of our knowledge, there are only two studies that have assessed the role of these *PSCA* SNPs in patients with GC precursor state, *i.e*. atrophic gastritis (9, 10). Furthermore, this is the first article to analyze *MUC1* A>G (rs4072037) and *PLCE1* A>G (rs2274223) SNPs in pre-malignant gastric conditions.

From all SNPs investigated in our study, *PSCA* gene polymorphisms rs2294008 and rs2976392 have probably been extensively studied most. It is well-known that these polymorphisms are in linkage disequilibrium within the *PSCA* gene (3). These two SNPs were linked with diffuse-type GC in GWAS (3). Later studies confirmed this association in other populations (4, 9, 10), and suggested that these genetic alterations are also linked with intestinal-type GC (18). rs2294008 has been linked with the risk of different types of cancer, including bladder cancer (19) and esophageal cancer (20). A recent meta-analysis by Gu *et al*. including 18,820 GC cases and 35,766 controls from 16 studies confirmed the association for rs2294008 and rs2976392 of *PSCA* gene (21). Furthermore, a study by Tanikawa *et al*. showed a link between these PSCA SNPs with the presence of duodenal ulcer (22). Functional studies have shown that rs2294008 influences transcriptional activity and may even have a clinical implication in the treatment of bladder cancer (23). The potential implications of *PSCA* SNPs in the clinical setting are of interest for further research in GC. Based on the currently available data, these *PSCA* SNPs are clearly linked with development of GC and the results of our present study support these findings. Our data suggest that TT genotype for rs2294008 and AA genotype

Table III. *Genotypic and allelic frequencies of prostate stem cell antigen (PSCA), mucin-1 (MUC1) and phospholipase C epsilon-1 (PLCE1) single nucleotide polymorphisms (SNPs) in controls, and patients with diffuse-type and intestinal-type gastric cancer (GC).*

	Controls (n=246)		Diffuse-type GC (n=74)					Intestinal-type GC (n=82)				
SNP	n	%	n	%	aOR	95% CI	*p*-Value	n	%	aOR	95% CI	*p*-Value
PSCA C>T rs2294008												
CC	64	26.3	10	13.7	1			7	8.5	1		
CT	123	50.6	39	53.4	1.35	0.74-2.44	0.334	41	50.0	1.64	0.93-2.94	0.0917
TT	56	23.1	24	32.9	2.78	1.21-6.26	0.015	34	41.5	5.56	2.27-14.3	0.0002
CC *vs*. CT+TT					1.64	0.72-2.86	0.095			4.17	1.79-9.98	0.0010
CC+CT *vs*. TT					2.27	1.10-4.76	0.027			2.22	1.27-3.85	0.0049
Allele C	251	51.6	59	40.4				55	33.5			
Allele T	235	48.4	87	59.6	1.59	1.09-2.33	0.017	109	66.5	2.13	1.47-3.13	5.9e-005
PSCA G>A rs2976392												
GG	54	23.3	11	14.9	1			7	8.6	1		
GA	116	50.0	39	52.7	1.33	0.72-2.44	0.361	40	49.4	1.61	0.89-2.86	0.1135
AA	62	26.7	24	32.4	2.56	1.12-5.56	0.024	34	42.0	5.57	2.19-14.5	0.0002
GG *vs*. GA+AA					1.59	083-2.86	0.116			4.17	1.79-9.96	0.0009
GG+GA *vs*. AA					2.13	1.04-4.35	0.039			2.19	1.24-3.86	0.0059
Allele G	224	48.3	61	41.2				54	33.3			
Allele A	240	51.7	87	58.8	1.52	1.05-2.22	0.026	108	66.7	2.17	1.49-3.13	5.4e-005
MUC1 A>G rs4072037												
AA	37	15.9	27	36.5	1			22	27.2	1		
AG	121	52.2	32	43.2	0.79	0.41-1.59	0.514	35	43.2	0.95	0.51-1.76	0.8682
GG	74	31.9	15	20.3	0.29	0.14-0.61	0.0012	24	29.6	0.45	0.21-0.93	0.0133
AA *vs*. AG+GG					0.56	0.30-1.06	0.0783			0.46	0.25-0.87	0.0167
AA+AG *vs*. GG					0.34	0.19-0.61	0.0003			0.76	0.42-1.35	0.345
Allele A	269	53.2	86	58.1				79	48.8			
Allele G	195	46.8	62	41.9	0.52	0.36-0.76	0.0006	83	51.2	1.31	0.91-1.88	0.136
PLCE1 A>G rs2274223												
AA	91	37.8	33	44.6	1			30	37.5	1		
AG	116	48.1	33	44.6	0.77	0.44-1.45	0.369	40	50.0	0.98	0.43-2.29	0.978
GG	34	14.1	8	10.8	0.65	0.28-1.58	0.349	10	12.5	0.99	0.42-2.29	0.978
AA *vs*. AG+GG					0.74	0.44-1.27	0.285			0.95	0.44-2.06	0.892
AA+AG *vs*. GG					0.75	0.33-1.72	0.503			1.06	0.62-1.82	0.838
Allele A	298	61.8	99	66.9				100	62.5			
Allele G	184	38.2	49	33.1	0.80	0.54-1.18	0.264	60	37.5	0.97	0.67-1.41	0.879

aOR: Adjusted odds ratio; CI: confidence interval.

for rs2976392 are linked with different histological subtypes of GC; however, the strength of association was higher for intestinal-type GC. It is worth mentioning that our results are in line with previously published studies suggesting that these SNPs are important in the early stages of GC development (9, 10).

MUC1 A>G SNP (rs4072037) has been linked with increased risk of diffuse-type gastric cancer, with an OR of 1.66 in GWAS (24). Several publications have linked this genetic variation of *MUC1* with other cancer types, including ovarian (25) and esophageal (26) cancer. A recent large meta-analysis study of almost 6,580 GC cases and 10,324 controls has confirmed this association, both in Asian and European populations (27). Our results clearly confirm those findings. Subgroup analysis of samples according to subtype of GC showed that G allele and GG genotype have been associated with reduced risk of diffuse-type GC, while association with intestinal type-GC was marginal. The most important finding from our analyses is the significant association of *MUC1* rs4072037 with advanced precancerous

Table IV. *Genotypic and allelic frequencies of prostate stem cell antigen (PSCA), mucin-1 (MUC1) and phospholipase C epsilon-1 (PLCE1) single nucleotide polymorphisms (SNPs) among Helicobacter pylori-positive and -negative individuals.*

	H. pylori-negative, n=111		*H. pylori*-positive, n=397				
Genotype	n	(%)	n	(%)	OR	95% CI	*p*-Value
PSCA C>T							
rs2294008							
CC	32	29.1	122	30.7	1		
CT	55	50.0	193	48.6	0.67	0.39-1.17	0.165
TT	23	20.9	82	20.7	0.76	0.38-1.47	0.413
CC vs. CT+TT					0.95	0.53-1.70	0.875
CC+CT vs. TT					0.69	0.41-1.18	0.178
Allele C	119	54.1	437	55.0			
Allele T	101	45.9	357	45.0	0.96	0.71-1.29	0.803
PSCA G>A							
rs2976392							
GG	32	30.2	121	31.4	1		
GA	54	50.9	182	47.3	0.65	0.37-1.45	0.138
AA	20	18.9	82	21.3	0.87	0.44-1.73	0.691
GG vs. GA+AA					1.11	0.61-2.03	0.733
GG+GA vs. AA					0.72	0.42-1.21	0.211
Allele G	118	55.7	424	55.1			
Allele A	94	44.3	346	44.9	1.02	0.75-1.39	0.877
MUC1 A>G							
rs4072037							
AA	32	29.1	107	28.0	1		
AG	62	56.4	197	51.6	0.77	0.46-1.42	0.207
GG	16	14.5	78	20.4	1.46	0.88-2.98	0.086
AA vs. AG+GG					1.55	0.97-3.09	0.061
AA+AG vs. GG					0.98	0.59-1.65	0.949
Allele A	126	57.3	411	53.8			
Allele G	94	42.7	353	46.2	1.28	0.95-1.73	0.111
PLCE1 A>G							
rs2274223							
AA	42	37.8	153	38.9	1		
AG	55	49.5	184	46.8	1.04	0.62-1.72	0.883
GG	14	12.6	56	14.2	1.01	0.45-2.15	0.992
AA vs. AG+GG					0.98	0.48-1.99	0.961
AA+AG vs. GG					1.03	0.63-1.67	0.899
Allele A	139	62.6	490	62.3			
Allele G	83	37.4	296	37.7	1.01	0.74-1.38	0.941

aOR: Adjusted odds ratio; CI: confidence interval.

gastric mucosal alterations, HRAG, which has not been assessed previously.

The studies conducted mostly in Asian populations have linked rs2274223 of *PLCE1* gene with GC and esophageal cancer (28, 29); these findings were confirmed in subsequent replication studies (26) and recent meta-analyses (30, 31), as well as being linked with survival in pw GC (32). We did not observe any significant association between *PLCE1* rs2274223 and GC or its precursors. The results of our study show a similar distribution of rs2274223 genotypes and alleles between GC, HRAG and control groups. Differences between our findings and those of previous studies might be related, at least partially, to ethnical differences as this is a common phenomenon in genetic association studies, while further highlighting the need for validation analyses in different population cohorts (33).

In a certain genetic background, *H. pylori* infection may trigger GC development probably at different levels, and

following this hypothesis, only a small proportion of individuals infected with *H. pylori* infection actually do develop GC (2). Since significant associations between PSCA and MUC1 SNPs with risk of premalignant gastric conditions have been observed, we further evaluated whether these genetic alterations are linked to *H. pylori* infection. One study has shown that *MUC1* rs4072037 and *PLCE1* rs2274223 SNPs may increase GC risk in combination with *H. pylori* seropositivity (34). The analysis of genotypic and allelic distribution among *H. pylori*-positive and *H. pylori*-negative individuals did not reveal any significant differences between these two groups. A recent GWAS has revealed certain genetic predisposition for *H. pylori* seropositivity (35). Based on our results, shared association of GC and HRAG with *PSCA* and *MUC1* gene SNPs is not *H. pylori*-dependent but might be related to other factors or related carcinogenic pathways.

It is worth pointing-out that the frequencies of genotypes observed in our study for all four SNPs are in line with those of previous studies and PubMed SNP database for European populations (9, 26, 33). Our present study carries certain limitations. The number of individuals is not large in our case–control study; therefore, certain tendencies that have been observed in this study could gain significance in a larger group of patients. There were differences in age and gender distribution between GC, HRAG and control groups, but these factors were included as covariates in the statistical logistic regression analysis in order to eliminate potential influence. Because of the low number of participants, we were not able to stratify risk with respect to subgroups of GC in terms of survival, TNM stage, tumor localization and differentiation grade. In addition, here we provided only an insight into the association between SNPs and clinicopathological characteristics, while further studies are needed to evaluate the interplay with gene expression in the background of those genotypes and alleles. Indeed, our results will provide additional data for further analysis on the topic, especially for *PLCE1* rs2274223 which has been reported to have more inconsistent results.

Conclusion

SNPs of *PSCA* (rs2976392, rs2294008) and *MUC1* (rs4072037) genes are linked with susceptibility of GC and HRAG, while no significant association was determined for *PLCE1* rs2274223.

Acknowledgements

The Authors would like to thank Raminta Grigaliunaite for help with genotyping in the laboratory. This project was supported by a grant from the Research Council of Lithuania No. MIP-14418 and in part by a grant from the BMBF-0315905D in the frame of ERA-Net PathoGenoMics.

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90.

2 Bornschein J and Malfertheiner P: *Helicobacter pylori* and Gastric Cancer. Dig Dis *32*: 249-264, 2014.

3 Sakamoto H, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, Saito D, Sugimura H, Tanioka F, Kato S, Matsukura N, Matsuda N, Nakamura T, Hyodo I, Nishina T, Yasui W, Hirose H, Hayashi M, Toshiro E, Ohnami S, Sekine A, Sato Y, Totsuka H, Ando M, Takemura R, Takahashi Y, Ohdaira M, Aoki K, Honmyo I, Chiku S, Aoyagi K, Sasaki H, Ohnami S, Yanagihara K, Yoon K-A, Kook M-C, Lee Y-S, Park SR, Kim CG, Choi IJ, Yoshida T, Nakamura Y and Hirohashi S: Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. Nat Genet *40*: 730-740, 2008.

4 Shi Y, Hu Z, Wu C, Dai J, Li H, Dong J, Wang M, Miao X, Zhou Y, Lu F, Zhang H, Hu L, Jiang Y, Li Z, Chu M, Ma H, Chen J, Jin G, Tan W, Wu T, Zhang Z, Lin D and Shen H: A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. Nat Genet *43*: 1215-1218, 2011.

5 Lillehoj EP, Guang W, Ding H, Czinn SJ and Blanchard TG: *Helicobacter pylori* and gastric inflammation: Role of MUC1 mucin. J Pediatr Biochem *2*: 125-132, 2012.

6 Wadhwa R, Song S, Lee J-S, Yao Y, Wei Q and Ajani JA: Gastric cancer-molecular and clinical dimensions. Nat Rev Clin Oncol *10*: 643-655, 2013.

7 Kupcinskas L, Rasmussen L, Jonaitis L, Kiudelis G, Jørgensen M, Urbonaviciene N, Tamosiunas V, Kupcinskas J, Miciuleviciene J, Kadusevicius E, Berg D and Andersen LP: Evolution of *Helicobacter pylori* susceptibility to antibiotics during a 10-year period in Lithuania. APMIS *121*: 431-436, 2013.

8 Kupcinskas J and Leja M: Management of *Helicobacter pylori*-related diseases in Baltic States. Dig Did 32: 295-301, 2014.

9 Rizzato C, Kato I, Plummer M, Muñoz N and Canzian F: Genetic variation in PSCA and risk of gastric advanced preneoplastic lesions and cancer in relation to *Helicobacter pylori* infection. PLoS One *8*: e73100, 2013.

10 Lochhead P, Frank B, Hold GL, Rabkin CS, Ng MTH, Vaughan TL, Risch HA, Gammon MD, Lissowska J, Weck MN, Raum E, Müller H, Illig T, Klopp N, Dawson A, McColl KE, Brenner H, Chow W-H and El-Omar EM: Genetic variation in the prostate stem cell antigen gene and upper gastrointestinal cancer in white individuals. Gastroenterology *140*: 435-441, 2011.

11 Kupcinskas J, Wex T, Link A, Leja M, Bruzaite I, Steponaitiene R, Juzenas S, Gyvyte U, Ivanauskas A, Ancans G, Petrenkiene V, Skieceviciene J, Kupcinskas L and Malfertheiner P: Gene polymorphisms of micrornas in *Helicobacter pylori*-induced high-risk atrophic gastritis and gastric cancer. PLoS One *9*: e87467, 2014.

12 Kupcinskas L, Wex T, Kupcinskas J, Leja M, Ivanauskas A, Jonaitis LV, Janciauskas D, Kiudelis G, Funka K, Sudraba A, Chiu H-M, Lin J-T and Malfertheiner P: Interleukin-1B and interleukin-1 receptor antagonist gene polymorphisms are not associated with premalignant gastric conditions: a combined haplotype analysis. Eur J Gastroenterol Hepatol *22*: 1189-1195, 2010.

13 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N and Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med *345*: 784-789, 2001.

14 Meining A, Bayerdörffer E, Müller P, Miehlke S, Lehn N, Hölzel D, Hatz R and Stolte M: Gastric carcinoma risk index in patients infected with *Helicobacter pylori*. Virchows Arch *432*: 311-314, 1998.

15 Dixon MF, Genta RM, Yardley JH and Correa P: Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol *20*: 1161-1181, 1996.

16 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ and Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet *81*: 559-575, 2007.

17 Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L and Parkinson H: The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res *42*: D1001-1006, 2014.

18 Lu Y, Chen J, Ding Y, Jin G, Wu J, Huang H, Deng B, Hua Z, Zhou Y, Shu Y, Liu P, Hu Z, Shen J, Xu Y and Shen H: Genetic variation of PSCA gene is associated with the risk of both diffuse- and intestinal-type gastric cancer in a Chinese population. Int J Cancer *127*: 2183-2189, 2010.

19 Fu Y-P, Kohaar I, Rothman N, Earl J, Figueroa JD, Ye Y, Malats N, Tang W, Liu L, Garcia-Closas M, Muchmore B, Chatterjee N, Tarway M, Kogevinas M, Porter-Gill P, Baris D, Mumy A, Albanes D, Purdue MP, Hutchinson A, Carrato A, Tardón A, Serra C, García-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Diver WR, Gapstur SM, Thun MJ, Virtamo J, Chanock SJ, Fraumeni JF, Silverman DT, Wu X, Real FX and Prokunina-Olsson L: Common genetic variants in the *PSCA* gene influence gene expression and bladder cancer risk. Proc Natl Acad Sci USA *109*: 4974-4979, 2012.

20 Dai N, Zheng M, Wang C, Ji Y, Du J, Zhu C, He Y, Zhu M, Zhu X, Sun M, Dai J, Ma H, Chen J, Hu Z, Gu H, Jin G and Shen H: Genetic variants at 8q24 are associated with risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Sci *105*: 731-735, 2014.

21 Gu X, Zhang W, Xu L and Cai D: Quantitative assessment of the influence of prostate stem cell antigen polymorphisms on gastric cancer risk. Tumour Biol *35*: 2167-2174, 2014.

22 Tanikawa C, Urabe Y, Matsuo K, Kubo M, Takahashi A, Ito H, Tajima K, Kamatani N, Nakamura Y and Matsuda K: A genome-wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population. Nat Genet *44*: 430-434, S1-2, 2012.

23 Kohaar I, Porter-Gill P, Lenz P, Fu Y-P, Mumy A, Tang W, Apolo AB, Rothman N, Baris D, Schned AR, Ylaya K, Schwenn M, Johnson A, Jones M, Kida M, Silverman DT, Hewitt SM, Moore LE and Prokunina-Olsson L: Genetic variant as a selection marker for anti-prostate stem cell antigen immunotherapy of bladder cancer. J Natl Cancer Inst *105*: 69-73, 2013.

24 Saeki N, Saito A, Choi IJ, Matsuo K, Ohnami S, Totsuka H, Chiku S, Kuchiba A, Lee Y-S, Yoon K-A, Kook M-C, Park SR, Kim Y-W, Tanaka H, Tajima K, Hirose H, Tanioka F, Matsuno Y, Sugimura H, Kato S, Nakamura T, Nishina T, Yasui W, Aoyagi K, Sasaki H, Yanagihara K, Katai H, Shimoda T, Yoshida T, Nakamura Y, Hirohashi S and Sakamoto H: A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. Gastroenterology *140*: 892-902, 2011.

25 Williams KA, Terry KL, Tworoger SS, Vitonis AF, Titus LJ and Cramer DW: Polymorphisms of MUC16 (CA125) and MUC1 (CA15.3) in relation to ovarian cancer risk and survival. PLoS One *9*: e88334, 2014.

26 Palmer AJ, Lochhead P, Hold GL, Rabkin CS, Chow W-H, Lissowska J, Vaughan TL, Berry S, Gammon M, Risch H and El-Omar EM: Genetic variation in *C20orf54, PLCE1* and *MUC1* and the risk of upper gastrointestinal cancers in Caucasian populations. Eur J Cancer Prev *21*: 541-544, 2012.

27 Zheng L, Zhu C, Gu J, Xi P, Du J and Jin G: Functional polymorphism rs4072037 in MUC1 gene contributes to the susceptibility to gastric cancer: evidence from pooled 6,580 cases and 10,324 controls. Mol Biol Rep *40*: 5791-5796, 2013.

28 Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu X-O, Yuan J-M, Zheng W, Dawsey SM, Dong LM, Lee MP, Ding T, Qiao Y-L, Gao Y-T, Koh W-P, Xiang Y-B, Tang Z-Z, Fan J-H, Wang C, Wheeler W, Gail MH, Yeager M, Yuenger J, Hutchinson A, Jacobs KB, Giffen CA, Burdett L, Fraumeni JF, Tucker MA, Chow W-H, Goldstein AM, Chanock SJ and Taylor PR: A shared susceptibility locus in *PLCE1* at 10q23 for gastric adenocarcinoma and eso-phageal squamous cell carcinoma. Nat Genet *42*: 764-767, 2010.

29 Wang L-D, Zhou F-Y, Li X-M, Sun L-D, Song X, Jin Y, Li J-M, Kong G-Q, Qi H, Cui J, Zhang L-Q, Yang J-Z, Li J-L, Li X-C, Ren J-L, Liu Z-C, Gao W-J, Yuan L, Wei W, Zhang Y-R, Wang W-P, Sheyhidin I, Li F, Chen B-P, Ren S-W, Liu B, Li D, Ku J-W, Fan Z-M, Zhou S-L, Guo Z-G, Zhao X-K, Liu N, Ai Y-H, Shen F-F, Cui W-Y, Song S, Guo T, Huang J, Yuan C, Huang J, Wu Y, Yue W-B, Feng C-W, Li H-L, Wang Y, Tian J-Y, Lu Y, Yuan Y, Zhu W-L, Liu M, Fu W-J, Yang X, Wang H-J, Han S-L, Chen J, Han M, Wang H-Y, Zhang P, Li X-M, Dong J-C, Xing G-L, Wang R, Guo M, Chang Z-W, Liu H-L, Guo L, Yuan Z-Q, Liu H, Lu Q, Yang L-Q, Zhu F-G, Yang X-F, Feng X-S, Wang Z, Li Y, Gao S-G, Qige Q, Bai L-T, Yang W-J, Lei G-Y, Shen Z-Y, Chen L-Q, Li E-M, Xu L-Y, Wu Z-Y, Cao W-K, Wang J-P, Bao Z-Q, Chen J-L, Ding G-C, Zhuang X, Zhou Y-F, Zheng H-F, Zhang Z, Zuo X-B, Dong Z-M, Fan D-M, He X, Wang J, Zhou Q, Zhang Q-X, Jiao X-Y, Lian S-Y, Ji A-F, Lu X-M, Wang J-S, Chang F-B, Lu C-D, Chen Z-G, Miao J-J, Fan Z-L, Lin R-B, Liu T-J, Wei J-C, Kong Q-P, Lan Y, Fan Y-J, Gao F-S, Wang T-Y, Xie D, Chen S-Q, Yang W-C, Hong J-Y, Wang L, Qiu S-L, Cai Z-M and Zhang X-J: Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at *PLCE1* and *C20orf54*. Nat Genet *42*: 759-763, 2010.

30 Hao N-B, He Y-F, Zhang D, Luo G, Chen B-J, Zhang Y and Yang S-M: *PLCE1* polymorphism and upper gastrointestinal cancer risk: a meta-analysis. PLoS One *8*: e67229, 2013.

31 Umar M, Upadhyay R and Mittal B: *PLCE1* rs2274223 A>G polymorphism and cancer risk: a meta-analysis. Tumour Biol *34*: 3537-3544, 2013.

32 Luo D, Gao Y, Wang S, Wang M, Wu D, Wang W, Xu M, Zhou J, Gong W, Tan Y and Zhang Z: Genetic variation in *PLCE1* is associated with gastric cancer survival in a Chinese population. J Gastroenterol *46*: 1260-1266, 2011.

33 Dura P, Bregitha CV V, te Morsche RHM, Roelofs HMJ, Kristinsson JO, Wobbes T, Witteman BJM, Tan ACITL, Drenth JPH and Peters WHM: GWAS-uncovered SNPs in *PLCE1* and *RFT2* genes are not implicated in Dutch esophageal adenocarcinoma and squamous cell carcinoma etiology. Eur J Cancer Prev *22*: 417-419, 2013.

34 Li M, Huang L, Qiu H, Fu Q, Li W, Yu Q, Sun L, Zhang L, Hu G, Hu J and Yuan X: *Helicobacter pylori* infection synergizes with three inflammation-related genetic variants in the GWASs to increase risk of gastric cancer in a Chinese population. PLoS One *8*: e74976, 2013.

35 Mayerle J, den Hoed CM, Schurmann C, Stolk L, Homuth G, Peters MJ, Capelle LG, Zimmermann K, Rivadeneira F, Gruska S, Völzke H, de Vries AC, Völker U, Teumer A, van Meurs JBJ, Steinmetz I, Nauck M, Ernst F, Weiss F-U, Hofman A, Zenker M, Kroemer HK, Prokisch H, Uitterlinden AG, Lerch MM, Kuipers EJ and Kuipers E: Identification of genetic loci associated with *Helicobacter pylori* serologic status. JAMA *309*: 1912-1920, 2013.

Received July 27, 2014
Revised October 25, 2014
Accepted October 30, 2014