ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld.2014.1121.274.tlr

TLR1 and *PRKAA1* Gene Polymorphisms in the Development of Atrophic Gastritis and Gastric Cancer

Gintare Dargiene[1], Greta Streleckiene[2], Jurgita Skieceviciene[1], Marcis Leja[3], Alexander Link[4], Thomas Wex[5], Limas Kupcinskas[1], Peter Malfertheiner[4], Juozas Kupcinskas[1]

1) Department of Gastroenterology and Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania;
2) Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania;
3) Institute for Clinical and Preventive Medicine, University of Latvia, Riga, Latvia;
4) Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany;
5) Medical Laboratory for Clinical Chemistry, Microbiology and Infectious Diseases, Department of Molecular Genetics, Otto-von-Guericke University, Magdeburg, Germany

Address for correspondence:
Juozas Kupcinskas, MD, PhD
Dept. Gastroenterology and Institute for Digestive Research
Lithuanian University of Health Sciences
Eiveniu str. 2
Kaunas, LT-50009
Lithuania
juozas.kupcinskas@lsmuni.lt

Received: 21.08.2018
Accepted: 08.11.2018

ABSTRACT

Background & Aims: Previous genome-wide association studies showed that genetic polymorphisms in toll-like receptor 1 (*TLR1*) and protein kinase AMP-activated alpha 1 catalytic subunit (*PRKAA1*) genes were associated with gastric cancer (GC) or increased *Helicobacter pylori* (*H. pylori*) infection susceptibility. The aim of this study was to evaluate the association between *TLR1* and *PRKAA1* genes polymorphisms and *H. pylori* infection, atrophic gastritis (AG) or GC in the European population.
Methods: Single-nucleotide polymorphisms (SNPs) were analysed in 511 controls, 340 AG patients and 327 GC patients. *TLR1* C>T (rs4833095) and *PRKAA1* C>T (rs13361707) were genotyped by the real-time polymerase chain reaction. *H. pylori* status was determined by testing for anti-*H. pylori* IgG antibodies in the serum.
Results: The study included 697 (59.2%) *H. pylori* positive and 481 (40.8%) *H. pylori* negative cases. We observed similar distribution of *TLR1* and *PRKAA1* alleles and genotypes in *H. pylori* positive and negative cases. *TLR1* and *PRKAA1* SNPs were not linked with the risk of AG. TC genotype of *TLR1* gene was more prevalent in GC patients compared to the control group (29.7% and 22.3% respectively, p=0.002). Carriers of TC genotype had a higher risk of GC (aOR=1.89, 95% CI: 1.26–2.83, p=0.002). A similar association was observed in a dominant inheritance model for *TLR1* gene SNP, where comparison of CC+TC vs. TT genotypes showed an increased risk of GC (aOR=1.86, 95% CI: 1.26–2.75, p=0.002). No association between genetic polymorphism in *PRKAA1* gene and GC was observed.
Conclusions: *TLR1* rs4833095 SNP was associated with an increased risk of GC in a European population, while *PRKAA1* rs13361707 genetic variant was not linked with GC. Both genetic polymorphisms were not associated with *H. pylori* infection susceptibility or the risk of AG.

Key words: *Helicobacter pylori* – *TRL1* – *PRKAA1* – gastric cancer – atrophic gastritis.

Abbreviations: AG: atrophic gastritis; AMPK: adenosine monophosphate-activated protein kinase; GC: gastric cancer; *H. pylori*: Helicobacter pylori; *PRKAA1*: protein kinase AMP-activated alpha 1 catalytic subunit; SNPs: single-nucleotide polymorphisms; *TRL1*: toll-like receptor 1.

INTRODUCTION

The global burden of gastric cancer (GC) remains exceptionally high, still ranking as a second leading cause of cancer related death worldwide [1]. Gastric cancer trends are dominated by the declining occurrence of non-cardia gastric cancer; however, rates of cardia cancer are increasing in Western countries [2, 3]. *H. pylori* induced chronic atrophic gastritis (AG) is the most consistent risk factor for development of GC [4]. Widely available data show that *H. pylori* gastritis is an infectious disease and leads to chronic active gastritis of varying severity in virtually all infected subjects [5]. The severity of *H. pylori* related diseases varies greatly among infected individuals, with the outcome governed by interactions between host genetic [6], epigenetic [7–10], apoptotic pathways [11] or environmental factors [12]. To date, molecular factors that may determine malignant transformation severity in gastric cancerogenesis are not completely understood [13–15]. It has been observed that environmental factors combining with low-penetrance susceptibility genes might be very important for cancer development [16, 17].

Epithelial cells of gastric mucosa are among the first cellular barriers for *H. pylori* in the gastrointestinal tract as

they recognize *H. pylori*-derived microbe-associated molecular patterns (MAMPs) through ligation of pattern recognition receptors (PRRs), especially the toll-like receptors (*TLR*s). The PRRs recognize bacterial lipopolysaccharides (LPS) and induce the secretion of pro-inflammatory molecules [18]. Polymorphisms of *TLR* genes can confer host susceptibility to *H. pylori* infection as well as to *H. pylori* related diseases by modulating the release of pro-inflammatory cytokines, which underline gastric inflammation and the immune response to *H. pylori* [19]. A recent genome-wide association study (GWAS) revealed significant association of single-nucleotide polymorphisms (SNPs) in the *TRL1* gene with susceptibility of *H. pylori* in an European descent population [20].

PRKAA1 is a gene that encodes adenosine monophosphate-activated protein kinase (AMPK). Adenosine monophosphate-activated protein kinase is the central metabolic switch found in all eukaryotes that govern glucose and lipid metabolism in response to nutrient and intracellular energy levels alterations. It has been implicated in a number of diseases related to energy metabolism, including cancer [16]. Therefore, genetic polymorphisms in *PRKAA1* might contribute to GC development by affecting the regulation of energy metabolism [21]. A Chinese GWAS identified that a SNP in *PRKAA1* (rs13361707) gene was associated with non-cardia GC in Chinese patients [22]. Further studies also showed significant gene-based association between *PRKAA1* and GC [16, 21, 23–25]. Moreover, one study found that *PRKAA1* polymorphisms may have an influence to *H. pylori* infection development and a synergetic effect on the risk of development of GC [26]. However, the direction of this association remains ambiguous and the polymorphism may even be inversely related to GC risk [27].

In this study we aimed to evaluate the association between *TLR1* C>T (rs4833095) and *PRKAA1* C>T (rs13361707) gene polymorphisms and the presence of *H. pylori* infection, AG or GC in European population.

METHODS

Study population

Patients and controls were recruited during 2005-2017 at three gastroenterology centers in Lithuania (Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas), Latvia (Riga East University Hospital) and Germany (Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg). Most of the patients came from our previous studies on genetic predisposition of cancer risk [28–31]. All patients in the control and AG groups underwent upper endoscopy with biopsies due to dyspeptic symptoms, but had no history of malignancy. Patients without atrophy or intestinal metaplasia on gastric biopsies based on the Sydney classification were included in the control group, while patients with premalignant conditions constituted the AG group. This consisted of patients with high-risk AG, defined as pan-gastritis (similar inflammatory scores in antrum and corpus), corpus-predominant gastritis with or without the presence of gastric atrophy, and intestinal metaplasia either in the antrum or corpus of the stomach [32]. All GC patients had histopathological documentation of gastric adenocarcinoma. The inclusion criterion for the study population was no previous history of *H. pylori* eradication. *H. pylori* status was determined by testing for anti-*H. pylori* IgG antibodies in serum using the ELISA method. The results were interpreted according to the locally established titers.

In total 1178 individuals were included in the study (511 controls, 340 AG patients and 327 GC patients): 634 subjects were from Latvia (383 controls, 112 AG, 139 GC), 302 from Germany (62 controls, 138 AG, 102 GC) and 242 from Lithuania (66 controls, 90 AG, 86 GC). All patients included in the study were of European descent. The study was approved by the Ethics Committee of the Lithuanian University of Health Sciences (Protocol no. BE-2-10), Central Medical Ethics Committee of Latvia (Protocol no. 01-29.1) and Ethics Committee of the Otto-von-Guericke University Magdeburg (Protocols No. 63/08 and 34/08). The research was carried out in accordance with the Helsinki Declaration. Informed consent was obtained from all the persons participating in the study.

DNA extraction and genotyping

Genomic DNA from samples was extracted from peripheral blood mononuclear cells using the salting-out method and stored at -20°C until analysis as described previously [33]. *TLR1* C>T rs4833095 and *PRKAA1* C>T rs13361707 SNPs were genotyped by the real-time polymerase chain reaction (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.

Statistical analysis

Statistical analysis was performed using the PLINK software (version 1.07). Distribution of observed and expected genotypes was examined for consistency with Hardy-Weinberg equilibrium. For comparisons of allele frequencies between controls and cases Pearson's goodness-of-fit test χ^2 was used. Logistic regression analysis with adjustment for age, sex and *H. pylori* infection presence was performed to estimate the association between SNP alleles and genotypes with development of AG, GC and *H. pylori* infection. Analysis was performed estimating allelic, genotypic, recessive and dominant models. Odds ratios and 95% confidence intervals are presented. Categorical data are expressed as absolute numbers with percentages. Age is shown as a mean with standard deviation. Differences were considered statistically significant when $p<0.05$.

RESULTS

Study group

Characteristics of the study groups are presented in Table I. Males were dominant in the GC group (65.4 %), while females were more prevalent in control (70.8 %) and AG (62.9 %) groups ($p<0.001$). Average age of controls was 47.16 years, in AG group 60.74 years and in GC group 65.49 years ($p<0.001$). Males were more prevalent in *H. pylori* positive (64.9%) than in *H. pylori* negative group (53.9%) (Table I). Country of origin was distributed equally between *H. pylori* positive and negative cases. *H. pylori* distribution (*H. pylori*

Table I. Characteristics of study groups

	Control group (n=511)	Atrophic gastritis group (n=340)	Gastric cancer group (n=327)	P value[1,2]	*H. pylori* negative cases (n=481)	*H. pylori* positive cases (n=697)	P value[1,2]
Gender (n, %)							
Males	149 (29.2)	127 (37.4)	214 (65.4)	< 0.001	169 (35.1)	321 (46.1)	< 0.001
Females	362 (70.8)	213 (62.6)	113 (34.6)		312 (64.9)	376 (53.9)	
Age (years)							
Mean ± SD	47.16±17.3	60.74±13.4	65.49±13.0	< 0.001	55.41±17.9	56.70±16.6	0.205
Country (n, %)							
Latvia	383 (75.0)	112 (32.9)	139 (42.5)	< 0.001	251 (52.2)	383 (55.0)	0.417
Germany	62 (12.1)	138 (40.6)	102 (31.2)		133 (27.7)	169 (24.2)	
Lithuania	66 (12.9)	90 (26.5)	86 (26.3)		97 (20.2)	145 (20.8)	

[1] Statistical analysis of age distribution performed using ANOVA; [2] Statistical analysis of gender and country distribution performed using the Chi-Square test.

positive and negative cases) in the control group was 52.3 % and 47.7 %, in AG group 59.4 % and 40.3%, and in GC group 69.7 % and 30.3 %,, respectively. In order to prevent potential confounding effects of gender, age and *H. pylori* infection presence, these factors were included as covariates in logistic regression analysis.

Hardy-Weinberg equilibrium

The studied SNPs were tested for Hardy-Weinberg equilibrium. Results are presented in Table II. Determined and expected frequency of two SNPs distribution of genotypes did not differ and were in agreement with HWE expectation rs4833095, p=1; rs13361707, p=0.653 (Table II).

Association of *TLR1* and *PRKAA1* SNPs with atrophic gastritis and gastric cancer

Table III summarizes frequencies of both SNPs alleles and genotypes in controls, AG and GC patients. *TLR1* rs4833095 allele C frequency in GC group was 17.0 %, while in the control group was 13.3 %. This increase was statistically significant and revealed an increased risk for GC (aOR =1.64, 95 % CI: 1.17-2.29, p=0.004, Table III). Furthermore, *TLR1* rs4833095 genotype TC was found in 29.7 % of GC patients compared to 22.3 % of controls (p=0.002). TC genotype and dominant inheritance models showed association with GC with aOD = 1.89, 95 % CI: 1.26-2.83, p = 0.002, and aOD=1.86, 95 % CI 1.26-2.75, p=0.002. Genotypes and alleles of *PRKAA1* rs13361707 were distributed similarly between controls, AG and GC patients. No significant associations were found (Table III).

Association of *TLR1* and *PRKAA1* SNPs with the presence of *H. Pylori* infection

Table IV summarizes frequencies of both SNPs (*TLR1* rs4833095 and *PRKAA1* rs13361707) alleles and genotypes in *H. pylori* negative and positive cases. Overall, genotypes and alleles frequencies of both polymorphisms did not differ between *H. pylori* positive and negative cases and no significant associations were determined (Table IV).

DISCUSSION

Genetic predisposition appears to be crucial for the development of different gastrointestinal cancers with the new risk loci being identified every year [6, 34]. We hypothesized that *TLR1* rs4833095 identified in a recent *H. pylori* susceptibility GWAS and *PRKAA1* rs13361707 identified in a GC GWAS may contribute to the progression of AG and GC in a population of European descent. We genotyped these two polymorphisms in 327 GC patients, 340 AG cases and 511 controls. We found that *TLR1* SNP rs4833095 was significantly associated with GC in a European descent population; however, this SNP was not linked with the susceptibility to *H. pylori* infection and was not associated with the risk of AG. Meanwhile, we found no associations between polymorphism in *PRKAA1* rs13361707 and *H. pylori* infection, neither in AG nor GC groups. To our best knowledge, this is the first replication study in European populations of the genetic variant *TLR1* rs4833095 identified in *H. pylori* susceptibility GWAS. It must be pointed out that these SNPs also have not been investigated previously in patients with *H. pylori* induced gastritis.

Table II. Analysis of Hardy-Weinberg equilibrium

Single nucleotide polymorphism (SNP)	Allele frequencies		Genotype distribution	Determined frequency of heterozygous allele	Expected frequency of heterozygous allele	p value
rs4833095	C (0.146)	T (0.854)	25/295/858 (CC/CT/TT)	0.250	0.250	1
rs13361707	T (0.264)	C (0.736)	79/465/634 (TT/TC/CC)	0.395	0.389	0.653

Table III. Genotype and allele frequencies of *TLR1* C>T (rs4833095), *PRKAA1* C>T (rs13361707) and association with atrophic gastritis and gastric cancer.

Alleles/ genotypes	Controls (n=511)	Atrophic gastritis group (n=340)			Gastric cancer group (n=327)		
	n (%)	n (%)	aOR (95% CI)	p	n (%)	aOR (95% CI)	p
rs4833095 *TLR1* C>T							
T	886 (86.7)	582 (85.6)			543 (83.0)		
C	136 (13.3)	98 (14.4)	1.19 (0.88-1.61)	0.254	111 (17.0)	1.64 (1.17-2.29)	**0.004**
TT	386 (75.5)	249 (73.2)	1 (Reference)		223 (68.2)	1 (Reference)	
TC	114 (22.3)	84 (24.7)	1.27 (0.89-1.82)	0.186	97 (29.7)	1.89 (1.26-2.83)	**0.002**
CC	11 (2.2)	7 (2.1)	1.08 (0.39-2.98)	0.882	7 (2.1)	1.62 (0.56-4.69)	0.371
CC+TC / TT			1.26 (0.89-1.77)	0.199		1.86 (1.26-2.75)	**0.002**
CC / TC+TT			1.02 (0.37-2.80)	0.969		1.36 (0.48-3.91)	0.564
rs13361707 *PRKAA1* C>T							
T	263 (25.7)	205 (30.1)	1.23 (0.97-1.57)	0.091	155 (23.7)	0.87 (0.65-1.16)	0.349
C	759 (74.3)	475 (69.9)			499 (76.3)		
TT	31 (6.1)	29 (8.5)	1.68 (0.92-3.07)	0.094	19 (5.8)	0.87 (0.40-1.86)	0.717
TC	201 (39.3)	147 (43.2)	1.17 (0.85-1.60)	0.336	117 (35.8)	0.82 (0.57-1.18)	0.288
CC	279 (54.6)	164 (48.3)	1 (Reference)		191 (58.4)	1 (Reference)	
TT+TC/ CC			1.23 (0.91-1.66)	0.181		0.83 (0.58-1.17)	0.287
TT / TC+CC			1.56 (0.87-2.82)	0.136		0.94 (0.45-1.99)	0.874

aOR, adjusted odds ratio; CI, confidence interval; significant p values are marked in bold.

Innate immune responses mediated by TLRs induce early inflammatory responses to pathogen and damage-associated molecular patterns. Genetic variation in TLRs has been associated with susceptibility and outcomes in a number of infectious and noninfectious disease states [35]. Association between TRL1 rs4833095 and tuberculosis susceptibility [36,

Table IV. Genotype and allele frequencies of *TLR1* C>T (rs4833095), *PRKAA1* C>T (rs13361707) and association with *H. Pylori* infection presence.

Alleles/genotypes	*H. pylori* negative cases (n=481)	*H. pylori* positive cases (n=697)			
	n (%)	n (%)	aOR	95% CI	p value
rs4833095 *TLR1* C>T					
T	819 (85.1)	1192 (85.5)			
C	143 (14.9)	202 (14.5)	0.966	(0.764-1.220)	0.770
TT	349 (72.6)	509 (73.0)	1 (Reference)		
TC	121 (25.2)	174 (25.0)	0.980	(0.747-1.284)	0.881
CC	11 (2.2)	14 (2.0)	0.871	(0.389-1.951)	0.737
CC + TC / TT			0.971	(0.746-1.262)	0.823
CC / TC + TT			0.876	(0.392-1.955)	0.746
rs13361707 *PRKAA1* C>T					
T	251 (26.1)	372 (26.7)	1.051	(0.870-1.271)	0.605
C	711 (73.9)	1022 (73.3)			
TT	29 (6.1)	50 (7.2)	1.230	(0.756-2.003)	0.405
TC	193 (40.1)	272 (39.0)	0.995	(0.778-1.271)	0.965
CC	259 (53.8)	375 (53.8)	1 (Reference)		
TT+TC / CC			1.025	(0.810-1.297)	0.836
TT / TC + CC			1.233	(0.766-1.985)	0.388

aOR, adjusted odds ratio; CI, confidence interval.

37], increased risk of IgA nephropathy [38], Crohn's disease and ulcerative colitis [39], susceptibility for alopecia areata [40], increased mortality in patients with sepsis following traumatic injury [35] have been found in various studies. The discovery of association between *TLR1* SNP rs4833095 and *H. pylori* seroprevalence in a GWAS in European descent population [20] was replicated in the Thai population: the *TLR1* rs4833095, C allele was associated with a significantly increased risk for *H. pylori* infection [19]. Moreover, the above mentioned SNP along with *TLR10* rs10004195 was associated with GC in *H. pylori* infected subjects in a Malaysian population [18]. Interestingly, in a Chinese population with a high risk of GC, TRL1 rs4833095 and *TLR10* rs10004195 were associated with a decreased risk of *H. pylori* infection and precancerous gastric lesions [41]. Our study also revealed an association between *TLR1* rs4833095 and GC, but we did not find the association of this SNP with a susceptibility to *H. pylori* infection or presence of AG.

PRKAA1 rs13361707 has been evaluated in several GC genetic association studies [16, 21, 24–27, 42–47], following identification of this SNP as a risk factor for non-cardia GC in GWAS in 2011 [22]. The systematic review and meta-analysis by Mocellin et al. (2015) supported the link between *PRKAA1* rs13361707 and an increased risk of non-cardia subtype GC [46]. Additionally, some studies have also reported associations between *PRKAA1* and GC [16, 21, 23, 24, 26]. Recently, *PRKAA1* SNP was identified in a GWAS in Iceland as a risk factor for GC [25]. In contrast, the results of a recent meta-analysis (2017) showed that *PRKAA1* rs13361707 was not significantly associated with a GC risk in an Asian population [27]. These findings support our study results since we did not find the *PRKAA1* gene association with the development of GC. Conflicting data of published studies might appear due to various causes. First of all, different ethnic backgrounds may impact the risk of different diseases [42, 48]. Secondly, GC risk is influenced both by environmental and genetic factors [25]. The study by Eom et al. [43] showed that *H. pylori* infection, CagA status, and *PRKAA1* polymorphisms were risk factors for GC in Koreans, and that the combination of two of these factors rather than their independent effects synergistically increased the risk. Finally, combined polymorphisms, instead of single low-penetrance variations in susceptibility, may lead to high-risk classification for specific populations [42].

This study has some limitations that need to be discussed. First of all, the small sample size and small subgroups of diffuse and intestinal cancer might be too small to carry out sub-group analyses. Due to the same reason, we could not perform sub-analyses between cancers located at different anatomical sites (e.g. cardia cancer versus distal GC). Furthermore, within the genetic association analysis of *H. pylori* IgG seropositivity, the presence of GC or AG might have a certain bias to the results and requires evaluation in larger independent cohorts. We also did not have complete data on other risk factors such as smoking and drinking status and we could not include them in the multifactorial analyses. Since published data on the two SNPs are partly contradictory, larger studies with subgroup analyses would be desirable.

CONCLUSIONS

TLR1 rs4833095 SNP is associated with an increased risk of GC in a population of European descent, while polymorphism in *PRKAA1* gene is not linked with the presence of GC. Both genetic polymorphisms have no association with *H. pylori* infection susceptibility and the risk of AG.

Conflicts of interest: No conflict of interest.

Authors' contributions: J.K., M.L., A.L., T.W., L.K. and P.M. conceived and designed the study; G.S and G.D. performed the DNA extraction and genotyping in the laboratory; G.S. and J.S analyzed the data; G.D., J.K. and G.S. wrote the paper.

Acknowledgments: Jurgita Skieceviciene, Greta Streleckiene were funded by Lithuanian research Council Grant No.: APP-2/2016. The authors also acknowledge Justina Arstikyte for her assistance with genotyping experiments in the lab.

REFERENCES

1. Colquhoun A, Arnold M, Ferlay J, Goodman KJ, Forman D, Soerjomataram I. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut 2015;64:1881–1888. doi:10.1136/gutjnl-2014-308915
2. Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends — An Update. Cancer Epidemiol Biomarkers Prev 2016;25:16–28. doi:10.1158/1055-9965.EPI-15-0578
3. Anderson WF, Rabkin CS, Turner N, Fraumeni JF, Rosenberg PS, Camargo MC. The Changing Face of Noncardia Gastric Cancer Incidence Among US Non-Hispanic Whites. J Natl Cancer Inst 2018;110:608-615. doi:10.1093/jnci/djx262
4. Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut 2015;64:1353-1367. doi:10.1136/gutjnl-2015-309252
5. Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis 2012;13:2–9. doi:10.1111/j.1751-2980.2011.00550.x
6. Kupcinskas J, Wex T, Link A, et al. PSCA and MUC1 gene polymorphisms are linked with gastric cancer and pre-malignant gastric conditions. Anticancer Res 2014;34:7167–7175.
7. Kupcinskaite-Noreikiene R, Skieceviciene J, Jonaitis L, et al. CpG island methylation of the MLH1, MGMT, DAPK, and CASP8 genes in cancerous and adjacent noncancerous stomach tissues. Medicina (Kaunas) 2013;49:361–366.
8. Steponaitiene R, Kupcinskas J, Langner C, et al. Epigenetic silencing of miR-137 is a frequent event in gastric carcinogenesis. Mol Carcinog 2016;55:376–386. doi:10.1002/mc.22287
9. Kupcinskas J, Steponaitiene R, Langner C, et al. LINE-1 hypomethylation is not a common event in preneoplastic stages of gastric carcinogenesis. Sci Rep 2017;7:4828. doi:10.1038/s41598-017-05143-0
10. Yang XX, He XQ, Li FX, Wu YS, Gao Y, Li M. Risk-association of DNA methyltransferases polymorphisms with gastric cancer in the Southern Chinese population. Int J Mol Sci 2012;13:8364–8378. doi:10.3390/ijms13078364
11. Zhang JY, Lin MT, Zhou MJ, et al. Combinational treatment of curcumin and quercetin against gastric cancer MGC-803 cells in vitro. Molecules 2015;20:11524–11534. doi:10.3390/molecules200611524

12. Nagashima H, Iwatani S, Cruz M, et al. Toll-like Receptor 10 in Helicobacter pylori Infection. J Infect Dis 2015;212:1666–1676. doi:10.1093/infdis/jiv270
13. Bornschein J, Leja M, Kupcinskas J, et al. Molecular diagnostics in gastric cancer. Front Biosci (Landmark Ed) 2014;19:312–338. doi:10.2741/4210
14. Ge Y, Liu H, Qiu X, et al. Genetic variants in PI3K / Akt / mTOR pathway genes contribute to gastric cancer risk. Gene 2018;670:130–135. doi:10.1016/j.gene.2018.05.093
15. Huang L, Yuan K, Liu J, et al. Polymorphisms of the TLR4 gene and risk of gastric cancer. Gene 2014;537:46–50. doi:10.1016/j.gene.2013.12.030
16. Qiu LX, He J, Cheng L, et al. Genetic variant of PRKAA1 and gastric cancer risk in an Eastern Chinese population. Oncotarget 2015;6:42661-42666. doi:10.18632/oncotarget.6124
17. Zhang W, Liang P, Wang W, et al. The influence of PSCA gene variation on its expression and gastric adenocarcinoma susceptibility in the northwest chinese population. Int J Mol Sci 2015;16:11648–11658. doi:10.3390/ijms160511648
18. Ravishankar Ram M, Goh KL, Leow AH, et al. Polymorphisms at locus 4p14 of Toll-like receptors TLR-1 and TLR-10 confer susceptibility to gastric carcinoma in Helicobacter pylori infection. PLoS One 2015;10:e0141865. doi:10.1371/journal.pone.0141865
19. Tongtawee T, Bartpho T, Kaewpitoon S, et al. Genetic polymorphisms in TLR1, TLR2, TLR4, and TLR10 of Helicobacter pylori-associated gastritis. Eur J Cancer Prev 2018;27:118-123. doi:10.1097/CEJ.0000000000000347
20. Mayerle J, den Hoed CM, Schurmann C, et al. Identification of genetic loci associated with helicobacter pylori serologic status. JAMA 2013;309:1912-1920. doi:10.1001/jama.2013.4350
21. Kim YD, Yim DH, Eom SY, et al. Risk of gastric cancer is associated with PRKAA1 gene polymorphisms in Koreans. World J Gastroenterol 2014;20:8592–8598. doi:10.3748/wjg.v20.i26.8592
22. Shi Y, Hu Z, Wu C, et al. A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. Nat Genet 2011;43:1215–1258. doi:10.1038/ng.978
23. Hu N, Wang Z, Song X, et al. Genome-wide association study of gastric adenocarcinoma in Asia: a comparison of associations between cardia and non-cardia tumours. Gut 2016;65:1611–1618. doi:10.1136/gutjnl-2015-309340
24. Hwang JY, Kim DH, Ji YI, et al. Recapitulation of previous genome-wide association studies with two distinct pathophysiological entities of gastric cancer in the Korean population. J Hum Genet 2013;58:233–235. doi:10.1038/jhg.2012.158
25. Helgason H, Rafnar T, Olafsdottir HS, et al. Loss-of-function variants in ATM confer risk of gastric cancer. Nat Genet 2015;47:906–910. doi:10.1038/ng.3342
26. Cai M, Dai S, Chen W, et al. Environmental factors, seven GWAS-identified susceptibility loci, and risk of gastric cancer and its precursors in a Chinese population. Cancer Med 2017;6:708–720. doi:10.1002/cam4.1038
27. Ni J, Shen N, Tang J, Ren K. Correlation between protein kinase catalytic subunit alpha-1 gene rs13361707 polymorphism and gastric cancer susceptibility in asian populations. Oncotarget 2017;8:68354–68364. doi:10.18632/oncotarget.19355
28. Kupcinskas J, Wex T, Link A, et al. Gene polymorphisms of micrornas in helicobacter pylori-induced high risk atrophic gastritis and gastric cancer. PLoS One 2014;9:e87467. doi:10.1371/journal.pone.0087467
29. Petkevicius V, Salteniene V, Juzenas S, et al. Polymorphisms of microRNA target genes IL12B, INSR, CCND1 and IL10 in gastric cancer. World J Gastroenterol 2017;23:3480–3487. doi:10.3748/wjg.v23.i19.3480
30. Kupcinskas L, Wex T, Kupcinskas J, et al. Interleukin-1B and interleukin-1 receptor antagonist gene polymorphisms are not associated with premalignant gastric conditions: A combined haplotype analysis. Eur J Gastroenterol Hepatol 2010;22:1189–1195. doi:10.1097/MEG.0b013e32833cf3d5
31. Kupcinskas J, Bruzaite I, Juzenas S, et al. Lack of association between miR-27a, miR-146a, miR-196a-2, miR-492 and miR-608 gene polymorphisms and colorectal cancer. Sci Rep 2014;4:5993. doi:10.1038/srep05993
32. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the developement of gastric cancer. N Engl J Med 2001;345:784–789. doi:10.1056/NEJMoa001999
33. Kupcinskas J, Gyvyte U, Bruzaite I, et al. Common Genetic Variants of PSCA, MUC1 and PLCE1 Genes are not Associated with Colorectal Cancer. Asian Pac J Cancer Prev 2015;16:6027–6032.
34. Campa D, Pastore M, Gentiluomo M, et al. Functional single nucleotide polymorphisms within the cyclin- dependent kinase inhibitor 2A/2B region affect pancreatic cancer risk. Oncotarget 2016;7:57011-57020. doi:10.18632/oncotarget.10935
35. Thompson CM, Holden TD, Rona G, et al. Toll-like receptor 1 polymorphisms and associated outcomes in sepsis after traumatic injury: A candidate gene association study. Ann Surg 2014;259:179–185. doi:10.1097/SLA.0b013e31828538e8
36. Schurz H, Daya M, Möller M, Hoal EG, Salie M. TLR1, 2, 4, 6 and 9 variants associated with tuberculosis susceptibility: A systematic review and meta-analysis. PLoS One 2015;10:e0139711. doi:10.1371/journal.pone.0139711
37. Zhang M, Tang M, Wang Y, et al. Variants of TLR1 associated with tuberculosis susceptibility in the Chinese Tibetan population but not in Han Chinese. Infect Genet Evol 2018;61:53-59. doi:10.1016/j.meegid.2018.02.021
38. Gao J, Wei L, Wei J, et al. TLR1 polymorphism rs4833095 as a risk factor for IgA nephropathy in a Chinese Han population: A case-control study. Oncotarget 2016;7:83031– 83039. doi:10.18632/oncotarget.12965
39. Bank S, Andersen PS, Burisch J, et al. Polymorphisms in the toll-Like receptor and the IL-23/IL-17 pathways were associated with susceptibility to inflammatory bowel disease in a danish cohort. PLoS One 2015;10:e0145302. doi:10.1371/journal.pone.0145302
40. Seok H, Suh DW, Jo B, et al. Association between TLR1 polymorphisms and alopecia areata. Autoimmunity 2014;47:372–377. doi:10.3109/08916934.2014.910769
41. Tang FB, Li ZX, Wang YM, et al. Toll-like receptor 1 and 10 polymorphisms, Helicobacter pylori susceptibility and risk of gastric lesions in a high-risk Chinese population. Infect Genet Evol 2015;31:263–269. doi:10.1016/j.meegid.2015.02.005
42. Yuan J, Li Y, Tian T, et al. Risk prediction for early-onset gastric carcinoma : a case-control study of polygenic gastric cancer in Han Chinese with hereditary background. Oncotarget 2016;7:33608-33615. doi:10.18632/oncotarget.9025
43. Eom SY, Hong SM, Yim DH, et al. Additive interactions between PRKAA1 polymorphisms and Helicobacter pylori CagA infection associated with gastric cancer risk in Koreans. Cancer Med 2016;5:3236–3335. doi:10.1002/cam4.926
44. Song HR, Kim HN, Kweon SS, et al. Genetic variations in the PRKAA1 and ZBTB20 genes and gastric cancer susceptibility in a korean population. Mol Carcinog 2013;52 Suppl 1:E155–E160. doi:10.1002/mc.22063

45. Li M, Huang L, Qiu H, et al. 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 2013;8:e74976. doi:10.1371/journal.pone.0074976
46. Mocellin S, Verdi D, Pooley KA, Nitti D. Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. Gut 2015;64:1209-1219. doi:10.1136/gutjnl-2015-309168
47. Dong Y, Chen J, Chen Z, et al. Evaluating the association of eight polymorphisms with cancer susceptibility in a han Chinese population. PLoS One 2015;10:e0132797. doi:10.1371/journal.pone.0132797
48. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature 2015;526:68–74. doi:10.1038/nature15393