RESEARCH ARTICLE

Common Genetic Variants of PSCA, MUC1 and PLCE1 Genes are not Associated with Colorectal Cancer

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Abstract

Background: Polymorphisms of genes encoding PSCA, PLCE1 and MUC1 have been associated with the risk of different cancers in genome wide association studies (GWAS). Up to date there are limited data on the role of these genetic alterations in colorectal cancer (CRC) development. The aim of this study was to evaluate potential associations between single nucleotide polymorphisms (SNPs) of genes encoding PSCA, PLCE1 and MUC1 and the presence of CRC in European populations. Materials and Methods: Gene polymorphisms were analyzed in 574 European subjects (controls: n=382; CRC: n=192). PSCA C>T (rs2294008), PSCA G>A (rs2976392), MUC1 A>G (rs4072037) and PLCE1 A>G (rs2274223) SNPs were genotyped by RT-PCR. Results: The distribution of genotypes for all four SNPs was in line with the Hardy-Weinberg equilibrium (rs2294008, P=0.153; rs2976392, P=0.269; rs4072037, P=0.609; rs2274223, P=0.858). The distribution of genotypes and alleles of PSCA C>T, PSCA G>A, MUC1 A>G and PLCE1 A>G SNPs was similar among controls and CRC patient groups (P>0.05). GG genotype of MUC1 SNP was more frequent in CRC patients (24.0%) than in controls (20.2%); however, this association failed to reach significance (OR-1.45, P=0.15). Overall, in the present study SNPs of PSCA (rs2294008, rs2976392), MUC1 (rs4072037) and PLCE1 (rs2274223) genes were not associated with the presence of CRC. Conclusions: Gene polymorphisms of PSCA, PLCE1 and MUC1 genes are not associated with the presence of CRC in European subjects.

Keywords: Colorectal cancer - gene polymorphism - PSCA - PLCE1 - MUC1

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Introduction

Despite evident clinical advances during the last decades, colorectal cancer (CRC) remains the second most common malignancy and the second most common cause of cancer-related mortality, with 447,000 annual new cases diagnosed and 215,000 deaths only in Europe (Ferlay et al., 2013). Early diagnosis and CRC prevention still remain a major challenge in clinical practise and urge the search of novel disease markers. It is known that both environmental and genetic factors are involved in the development of CRC (Armaghany et al., 2012). Frequent genetic variations - single nucleotide polymorphisms (SNPs) - may play an important role in colorectal carcinogenesis. Detecting single nucleotide variations in genes associated with CRC and using them as disease markers may improve cancer diagnosis and treatment planning in the near future (Nassiri et al., 2013).

Alterations of prostate stem cell antigen (PSCA), mucin 1 (MUC1) and phospholipase C epsilon 1 (PLCE1) gene expression have been noted in gastrointestinal, bladder, prostate, breast cancers and are implicated in the processes of carcinogenesis (Zotter et al., 1988; Reiter et al., 1998; Sakamoto et al., 2008; Fu et al., 2012). PSCA gene was originally identified as a prostate cell surface specific marker and later was found to be overexpressed in several other human solid cancers (Reiter et al., 1998; Amara et al., 2001; Cao et al., 2005; Kawaguchi et al., 2010). It was also revealed that PSCA may be involved in cell proliferation, adhesion, migration and survival - the processes which are altered in carcinogenesis (Saffran et al., 2001; Eshel et al., 2002). MUC1 gene encoded protein is a transmembrane molecule, which is abundantly expressed in normal glandular epithelial cells (Zotter et al., 1988). MUC1 expression is significantly increased in malignant cells and overexpression, aberrant intracellular localization or changes in glycolisation of this protein were noted in colorectal, prostate, ovarian and breast carcinomas (Byrd and Bresalier, Wang et al., 2007; Creaney et al., 2008; Ghosh et al., 2013). PLCE1 gene encodes a phospholipase which is associated with intracellular signaling (Rhee, 2001). Subsequently,

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several studies demonstrated the importance of PLCE1 in carcinogensis of skin, bladder and colorectal carcinomas (Bai et al., 2004; Wang et al., 2008; Ou et al., 2010).

Potential carcinogenic role of the SNPs of the genes described above was identified in several genome wide association studies (GWAS). The most recent GWAS analysis performed by Tanikawa and colleagues in Japanese population revealed correlation between C allele of rs2294008 in PSCA gene with increased risk of duodenal and gastric ulcers (Tanikawa et al., 2012, 2013). Interestingly, CT and TT genotypes of the same SNP were associated with increased risk of gastric cancer in Japanese and Chinese populations, together with variants in other susceptibility loci including rs2976392 in PSCA gene and rs4072037 in MUC1 (Shi et al., 2011; Li et al., 2012a; Tanikawa et al., 2012). Other two GWAS in Chinese population identified rs2274223 variant of PLCE1 gene as the risk factor for esophageal squamous cell carcinoma and gastric adenocarcinoma (Abnet et al., 2010; Wang et al., 2010). Currently, there are very few studies analyzing the association between these SNPs and CRC risks. Rs2274223 of PLCE1 gene was found to have protective effect against CRC development in Chinese population (Li et al., 2012c; Wang et al., 2014), but no significant associations were reported for rs4072037 and rs2294008 variants (Li et al., 2012c; Smith et al., 2012). Recent meta-analysis have confirmed the role of these SNPs for the development of different cancers (Hao et al., 2013; Umar et al., 2013; Zheng et al., 2013), outlining the need of studies on CRC patients.

As discussed above, PSCA, MUC1 and PLCE1 genes have an established role in carcinogenesis. SNPs of these genes have been related to gastric and other gastrointestinal diseases in different GWAS studies and meta-analysis. It is well known that mechanisms of developing different cancers may share common triggers and pathways (Bunney and Katan, 2006). Therefore, we conducted a case-control genotyping study evaluating the association between rs2294008, rs2976392, rs4072037, rs2274223 and the risk of developing CRC. To our best knowledge this is the first report aimed at evaluating the role of rs4072037 and rs2274223 for CRC development in the European population. The four SNPs of PSCA, MUC1 and PLCE1 genes were analysed in 382 controls and 192 CRC patients of Caucasian ethnicity.

Materials and Methods

Study population

Patients and controls were recruited during the years 2006-2013 at two university hospitals in Lithuania (Department of Gastroenterology and Department of Surgery; Lithuanian University of Health Sciences, Kaunas) and Latvia (Riga East University Hospital and Digestive Diseases Centre GASTRO, Riga). All CRC cases and control subjects were genetically unrelated. Control subjects were healthy individuals included from the out-patient departments who were referred to gastroenterologists due to dyspeptic symptoms; the inclusion criteria for controls was no history of previous malignancy. Diagnosis and staging of CRC was confirmed by standard laboratory, endoscopic and radiological methods. All CRC patients had histological verification of colorectal adenocarcinoma and were recruited from the out-patient and stationary departments. In total 574 (382 controls, 192 CRC) individuals were included in the study. All patients were of Caucasian origin in a case-control study. There were 223 subjects in Latvian group (160 controls, 63 CRC) and 351 subjects in Lithuanian group (229 controls, 129 CRC). Detailed clinical characteristics of CRC and control study groups are provided in Table 1.

DNA extraction and genotyping

DNA extraction and genotyping methods are outlined in detail in our previous genotyping studies (Kupcinskas et al., 2011, 2014). In short, genomic DNA from samples was extracted using phenol-chloroform extraction method from peripheral blood mononuclear cells. DNA samples were stored at −20°C until analysis. PSCA C>T (rs2294008), PSCA G>A (rs2976392), MUC1 A>G (rs4072037) and PLCE1 A>G (rs2274223) SNPs were genotyped by using predesigned TaqMan® assays with a 7500TM real-time cycler, in accordance with the manufacturer's instructions (Life Technologies, CA, USA). Genotype assignments were manually confirmed by visual inspection with the SDS 2.0.5 software compatible with the TaqMan® system. After initial genotyping, 5% of all samples in each group were included in repetitive analysis, which showed 100% concordance rate. Dubious samples had triplet repetitive analysis. Samples that failed to genotype were recorded as undetermined.

Statistical analysis

All subjects were classified into two study groups:

Table 1. Clinical Characteristics of Colorectal Cancer Patients and Healthy Controls

		Controls (n=382)	CRC (n=192)	P value
Age	Mean ± SD	63.74 ± 10.13	67.20 ± 10.28	<0.001
Gender				
	Male, n (%)	102 (26.7%)	109 (56.8%)	<0.001
	Female, n (%)	280 (73.3%)	83 (43.2%)	
TNM staging				
	I, n (%)	-	9 (5%)	
	II, n (%)	-	86 (45%)	
	III, n (%)	-	61 (32%)	
	IV, n (%)	-	18 (9%)	
	Unknown, n (%)	-	18 (9%)	
T	1/2	-	27 (14%)	
	3	-	85 (44%)	
	4	-	61 (32%)	
	Unknown	-	19 (10%)	
N	0	-	96 (50%)	
	1	-	53 (28%)	
	2	-	23 (12%)	
	Unknown	-	20 (10%)	
M	0	-	153 (80%)	
	1	-	23 (12%)	
	Unknown	-	16 (8%)	

CRC - colorectal cancer

controls (n=382) and CRC (n=192). Age was compared using unpaired Student's t-test and is presented as mean and standard deviations. Quality and statistical analysis of the genotyping data were performed using PLINK software version 1.07 (Purcell et al., 2007). Hardy-Weinberg equilibrium (HWE) was assessed for each of the four SNPs. Differences in allele frequencies between cases and controls were calculated in the combined Lithuanian and Latvian study sample. Association between control and CRC groups with gene polymorphisms was calculated using logistic regression analysis with adjustment for age, gender, country of birth and presented as odds ratios (OR) with 95% confidence intervals (CI). The relative risks for SNPs were studied using allelic, genotypic, dominant and recessive models. Adjusted significance threshold α=0.0125 (0.05/4) was used to eliminate the potential influence of multiple comparisons.

Results

Characteristics of the subjects

In total 574 individuals were included in the study - 382 controls and 192 CRC patients. There were 223 subjects in Latvian group (160 controls, 63 CRC) and 351 subjects in Lithuanian group (229 controls, 129 CRC). There were no significant differences in terms of gender, age or genotype distribution between Lithuanian and Latvian groups (data not shown); therefore, respective CRC patients and controls were merged in common groups for further analyses. The percentage of males was 56.8% in CRC group, while in control group males accounted only for 26.7% (P<0.001). Control subjects were significantly younger (63.7 ± 10.1) than CRC patients (67.2 ± 10.3) by approximately 3.5 years (P<0.001). Since the distribution of subjects in control and CRC groups differed significantly according to the age and gender, these covariates covariates were included in all further calculations of statistical analysis to eliminate the potential bias.

Associations of PSCA, MUC1 and PLCE1 SNPs and the risk of CRC

The observed genotype frequencies for all 4 SNPs were concordant with Hardy-Weinberg equilibrium (rs2294008 P=0.153; rs2976392 P=0.269; rs4072037 P=0.609; rs2274223 P=0.858). The frequencies of genotypes and alleles among controls and CRC patients are presented in Table 2. Overall distribution of genotypes and alleles of the investigated SNPs was similar between the two groups. The distribution of genotypes of PSCA (CC, CT, TT) was 26.5%, 50.1%, 23.3% and 31.4%, 40.3%, 28.3% in control and CRC groups, respectively. PSCA G>A SNP genotype

Table 2. Distribution of PSCA, MUC1 and PLCE1 Polymorphisms and Association with CRC Incidence

SNP	Genotypes/Alleles	Controls (n=382)		CRC (n=192)				
		n	%	n	%	aOR	95% CI	P value
PSCA C>T a	CC	100	26.5	60	31.4	1		
rs2294008	CT	189	50.1	77	40.3	0.74	(0.49-1.18)	0.173
	TT	88	23.3	54	28.3	1.09	(0.68-1.75)	0.710
	CC vs CT + TT					0.85	(0.58-1.26)	0.441
	CC + CT vs TT					1.31	(0.87-1.95)	0.189
	Allele C	389	51.6	197	51.6			
	Allele T	365	48.4	185	48.4	1.00	(0.78-1.28)	0.995
PSCA G>A b	GG	99	27.2	56	29.3	1		
rs2976392	GT	180	49.5	84	44.0	0.91	(0.59-1.39)	0.666
	TT	85	23.4	51	26.7	1.13	(0.69-1.84)	0.610
	GG vs GT + TT					0.98	(0.66-1.46)	0.936
	GG + GT vs TT					1.20	(0.80-1.81)	0.375
	Allele G	378	51.9	196	51.3			
	Allele T	350	48.1	186	48.7	1.02	(0.80-1.33)	0.845
MUC1 A>G c	AA	105	29.0	47	24.5	1		
rs4072037	AG	184	50.8	99	51.6	1.22	(0.79-1.87)	0.360
	GG	73	20.2	46	24.0	1.45	(0.87-2.43)	0.152
	AA vs AG + GG					1.29	(0.86-1.93)	0.223
	AA + AG vs GG					1.28	(0.83-1.95)	0.264
	Allele A	394	54.4	193	50.3			
	Allele G	330	45.6	191	49.7	1.18	(0.92-1.51)	0.187
PLCE1 A>G d	AA	147	39.1	77	40.1	1		
rs2274223	AG	173	46.0	91	47.4	0.98	(0.67-1.44)	0.930
	GG	56	14.9	24	12.5	0.86	(0.49-1.51)	0.601
	AA vs AG + GG					0.95	(0.66-1.37)	0.799
	AA + AG vs GG					0.87	(0.52-1.46)	0.597
	Allele A	467	62.1	245	63.8			
	Allele G	285	37.9	139	36.2	0.93	(0.72-1.20)	0.575

CRC, colorectal cancer; aOR, adjusted OR (age, sex, country); CI, confidence interval; n, sample size; a 6 individuals failed to be genotyped for rs2294008; b 19 individuals failed to be genotyped for rs2976392; c 20 individuals failed to be genotyped for rs4072037; d 6 individuals failed to be genotyped for rs2274223

distribution (GG, GA, AA) was 27.2%, 49.5%, 23.4% in control group and 29.3%, 44.0%, 26.7% in CRC group, respectively. MUC1 A>G SNP had similar distribution of AA, AG and GG genotypes between controls (29.0%, 50.8%, 20.2%, respectively) and CRC patients (24.5%, 51.6%, 24.0%). PLCE1 A>G SNP genotypes (AA, AG, GG) had similar distribution between control (29.1%, 46.0%, 14.9%, respectively) and CRC groups (40.1%, 47.4%, 12.5%, respectively).

Logistic regression analysis using dominant, recessive and genotypic models did not reveal significant associations between rs2294008, rs2976392, rs4072037 or rs2274223 and CRC development (Table 2). GG genotype of MUC1 SNP was more frequent in CRC patients (24.0%) than in controls (20.2); however, this association failed to reach required significance level (OR-1.45, P=0.15). No association between alleles and the risk of CRC was observed for all four PSCA, MUC1 and PLCE1 polymorphisms as well (Table 2).

Discussion

Our present study did not show any potential relation between SNPs of PSCA, MUC1, PLCE1 genes and CRC risk in European population. This case-control study included 382 controls and 192 CRC patients; however, the distribution of rs2294008, rs2976392, rs4072037 and rs2274223 alleles and genotypes among the groups did not reveal significant differences. The polymorphisms selected for our present study have been associated with various cancers in recent GWAS studies. Since different cancers may share common pathogenetic pathways (Bunney and Katan, 2006), we hypothesized that SNPs that have been implicated in other gastrointestinal carcinomas may also play a role in CRC development. To date, there are very few papers which have assessed this relationship in CRC patients (Li et al., 2012b; Smith et al., 2012; Wang et al., 2014). Furthermore, to our best knowledge this is the first report aimed at evaluating the role of rs4072037 and rs2274223 for CRC development in European population.

PSCA gene polymorphisms rs2294008 and rs2976392 are the most studied of all four investigated polymorphisms in cancer related case-control studies. It is well known that these polymorphisms are in linkage disequilibrium (LD) within PSCA gene (Sakamoto et al., 2008). These two SNPs were identified to be remarkably associated with diffuse-type gastric cancer in GWAS (Sakamoto et al., 2008) and positive repetitive results were published in later studies (Lu et al., 2010; Lochhead et al., 2011; Shi et al., 2011; Rizzato et al., 2013). Rs2294008 was also associated with the risk of bladder cancer susceptibility in Asian individuals (Fu et al., 2012). In vitro experiments revealed the importance of rs2294008 on transcriptional activity, which may even have clinical implications in the future (Kohaar et al., 2013). To date, there was only one attempt to associate rs2294008 polymorphisms of PSCA gene with CRC in European subjects. Smith and colleagues performed a study in 388 CRC patients and 496 controls and found no associations of this polymorphism and the risk of CRC (Smith et al., 2012). Our data support the findings of the later study, as rs2294008 and rs2976392 were not related with CRC development. Based on the currently available data, these SNPs are unlikely to have a role in a CRC development in Caucasians, but larger studies including patients from different ethnical backgrounds are desirable.

MUC1 A>G SNP (rs4072037) was associated with gastric cancer development in GWAS analysis. MUC1 gene polymorphism increased diffuse-type gastric cancer risk with an OR of 1.66 and genotypes of this SNP affected promoter activity (Saeki et al., 2011). A recent large meta-analysis study of 6,580 gastric cancer cases and 10,324 controls including both Asian and European populations showed that G allele of rs4072037 polymorphism was associated with decreased risk of gastric cancer (OR = 0.72) (Zheng et al., 2013). Recent publications showed that genetic variation of MUC1 may be associated with ovarian cancer (Williams et al., 2014) and esophageal cancer (Palmer et al., 2012). In the only case-control study including CRC patients (Li et al., 2012c) rs4072037 showed a marginal association in Chinese population (OR 0.69, P=0.06). The findings of our work show that MUC1 SNP is not related to CRC development in European population; however, currently no comparison of our results to the other Caucasian populations can be made and further studies are warranted.

The studies conducted in Chinese population found that rs2274223 of PLCE1 gene was associated with CRC development (Li et al., 2012b; Wang et al., 2014). A case-control research including 417 CRC patients and 416 controls reported that carriers of AG and GG genotypes had an increased risk for CRC. This study also showed that lower levels of PLCE1 mRNA were observed both in carriers of AG and GG genotypes (Wang et al., 2014). Similar results were obtained in a study by Li and colleagues which reported a sigificant association between rs2274223 and CRC (Li et al., 2012b). The results of our study do not support these findings as the distribution differences of genotypes and alleles between CRC and control groups were not present. Inconsistent findings between previous studies and our results might be related to ethnical differences as this is a common phenomenon in genetic associations studies (Dura et al., 2013). Unfortunately, due to the lack of studies, no comparison for our results can be made in terms of other Caucasian populations. GWAS conducted on gastric and esophageal cancer patients have shown an evident role of rs2274223 gene polymorphism (Abnet et al., 2010; Wang et al., 2010); these findings were confirmed in subsequent replication studies (Palmer et al., 2012) and recent meta-analysis (Hao et al., 2013; Umar et al., 2013). Nevertheless, the ultimate role of this SNP in CRC development needs further investigation in large scale studies.

The results of this study suggest that none of the four investigated polymorphisms in PSCA, MUC1 and PLCE1 genes are associated with the risk of CRC. Rs2294008, rs2976392, rs4072037 and rs2274223 variants do not appear as potential diagnostic markers for CRC. Our results are discordant with the results presented by Li and colleagues which associated rs2274223 with CRC risks, but the different outcomes of the studies could be related

to different ethnical backgrounds (Li et al., 2012b).

We admit that our case-control genotyping study has some limitations. First of all, the size of the population is not large and may be underpowered to detect subtle associations. Although there were gender and age differences between CRC and control groups, these factors were included as covariates in the statistical logistic regression analysis, thus minimizing the potential bias. Due to a relatively small number of individuals we were not able to carry out a subanalysis stratifying CRC patients according to TNM stage, tumor localization in the colon, differentiation grade etc. Survival data were available only for a small part of subjects; therefore, we did not perform SNP-survival association analysis. Our study was not designed to evaluate the potential gene expression changes in relation to the SNPs and this might be aimed in further research projects. The results of our study did not reveal significant associations; nevertheless, this data may contribute to the future meta-analysis of case-control studies in the field.

In conclusion, gene polymorphisms of PSCA, PLCE1 and MUC1 genes are not associated with the presence of CRC in European subjects..

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References

Abnet CC, Freedman ND, Hu N, et al (2010). A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet*, **42**, 764-7.

Amara N, Palapattu GS, Schrage M, et al (2001). Prostate stem cell antigen is overexpressed in human transitional cell carcinoma. *Cancer Res*, **61**, 4660-5.

Armaghany T, Wilson JD, Chu Q, Mills G (2012). Genetic alterations in colorectal cancer. *Gastrointest Cancer Res*, **5**, 19-27.

Bai Y, Edamatsu H, Maeda S, et al (2004). Crucial role of phospholipase Cepsilon in chemical carcinogen-induced skin tumor development. *Cancer Res*, **64**, 8808-10.

Byrd JC, Bresalier RS (2004). Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev*, **23**, 77-99.

Bunney TD, Katan M (2006). Phospholipase C epsilon: linking second messengers and small GTPases, *Trends Cell Biol*, **16**, 640-8.

Cao D, Ji H, Ronnett BM (2005). Expression of mesothelin, fascin, and prostate stem cell antigen in primary ovarian mucinous tumors and their utility in differentiating primary ovarian mucinous tumors from metastatic pancreatic mucinous carcinomas in the ovary. *Int J Gynecol Pathol*, **24**, 67-72.

Creaney J, Segal A, Sterrett G, et al (2008). Overexpression and altered glycosylation of MUC1 in malignant mesothelioma. *Br J Cancer*, **98**, 1562-9.

Dura P, Bregitha CVV, te Morsche RHM, et al (2013). 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**, 417-9.

Eshel R, Zanin A, Kapon D, et al (2002). Human Ly-6 antigen E48 (Ly-6D) regulates important interaction parameters between endothelial cells and head-and-neck squamous carcinoma cells. *Int J Cancer*, **98**, 803-10.

Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al (2013). Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *Eur J Cancer*, **49**, 1374-403.

Fu Y-P, Kohaar I, Rothman N, et al (2012). Common genetic variants in the PSCA gene influence gene expression and bladder cancer risk. *Proc Natl Acad Sci*, **109**, 4974-9.

Ghosh SK, Pantazopoulos P, Medarova Z, Moore A. (2013). Expression of underglycosylated MUC1 antigen in cancerous and adjacent normal breast tissues. *Clin Breast Cancer*, **13**, 109-18.

Hao N-B, He Y-F, Zhang D, et al (2013). PLCE1 polymorphism and upper gastrointestinal cancer risk: a meta-analysis. *PLoS One*, **8**, 67229.

Kawaguchi T, Sho M, Tojo T, et al (2010). Clinical significance of prostate stem cell antigen expression in non-small cell lung cancer. *Jpn J Clin Oncol*, **40**, 319-26.

Kohaar I, Porter-Gill P, Lenz P, et al (2013). Genetic variant as a selection marker for anti-prostate stem cell antigen immunotherapy of bladder cancer. *J Natl Cancer Inst*, **105**, 69-73.

Kupcinskas J, Wex T, Bornschein J, et al (2011). Lack of association between gene polymorphisms of Angiotensin converting enzyme, Nod-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of Helicobacter pylori-induced premalignant gastric lesions and gastric cancer in Caucasians. *BMC Med Genet*, **12**, 112.

Kupcinskas J, Wex T, Link A, et al (2014). Gene polymorphisms of micrornas in helicobacter pylori-induced high risk atrophic gastritis and gastric cancer. *PLoS One*, **9**, 87467.

Li F, Zhong M-Z, Li J-H, et al (2012a). Case-control study of single nucleotide polymorphisms of PSCA and MUC1 genes with gastric cancer in a Chinese. *Asian Pac J Cancer Prev*, **13**, 2593-6.

Li FX, Yang XX, He XQ, et al (2012b). Association of 10q23 with colorectal cancer in a Chinese population. *Mol Biol Rep*, **39**, 9557-62.

Li F-X, Yang X-X, He X-Q, et al (2012c). Association of 10q23 with colorectal cancer in a Chinese population. *Mol Biol Rep*, **39**, 9557-62.

Lochhead P, Frank B, Hold GL, et al (2011). Genetic variation in the prostate stem cell antigen gene and upper gastrointestinal cancer in white individuals. *Gastroenterology*, **140**, 435-41.

Lu Y, Chen J, Ding Y, et al (2010). 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**, 2183-9.

Nassiri M, Kooshyar MM, Roudbar Z, et al (2013). MINI-REVIEW genes and SNPs associated with non-hereditary and hereditary colorectal cancer. *Asian Pac J Cancer Prev*, **14**, 5609-14.

Ou L, Guo Y, Luo C, et al (2010). RNA interference suppressing PLCE1 gene expression decreases invasive power of human bladder cancer T24 cell line. *Cancer Genet Cytogenet*, **200**, 110-9.

Palmer AJ, Lochhead P, Hold GL, et al (2012). Genetic variation in C20orf54, PLCE1 and MUC1 and the risk of upper gastrointestinal cancers in Caucasian populations. *Eur J Cancer Prev*, **21**, 541-4.

Purcell S, Neale B, Todd-Brown K, et al (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*, **81**, 559-75.

Reiter RE, Gu Z, Watabe T, et al (1998). Prostate stem cell antigen: a cell surface marker overexpressed in prostate

cancer. *Proc Natl Acad Sci U S A*, **95**, 1735-40.
Rhee SG (2001). Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem*, **70**, 281-312.
Rizzato C, Kato I, Plummer M, et al (2013). Genetic variation in PSCA and risk of gastric advanced preneoplastic lesions and cancer in relation to Helicobacter pylori infection. *PLoS One*, **8**, 73100.
Saeki N, Saito A, Choi IJ, et al (2011). A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. *Gastroenterology*, **140**, 892-902.
Saffran DC, Raitano AB, Hubert RS, et al (2001). Anti-PSCA mAbs inhibit tumor growth and metastasis formation and prolong the survival of mice bearing human prostate cancer xenografts. *Proc Natl Acad Sci U S A*, **98**, 2658-63.
Sakamoto H, Yoshimura K, Saeki N, et al (2008). Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet*, **40**, 730-40.
Shi Y, Hu Z, Wu C, et al (2011). A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. *Nat Genet*, **43**, 1215-8.
Smith C, Lochhead P, Basavaraju U, et al (2012). Lack of association between the rs2294008 polymorphism in the prostate stem cell antigen gene and colorectal neoplasia: a case-control and immunohistochemical study. *BMC Res Notes*, **5**, 371.
Tanikawa C, Matsuo K, Kubo M, et al (2013). Impact of PSCA variation on gastric ulcer susceptibility. *PLoS One*, **8**, 63698.
Tanikawa C, Urabe Y, Matsuo K, et al (2012). A genome-wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population. *Nat Genet*, **44**, 430-4.
Umar M, Upadhyay R, Mittal B (2013). PLCE1 rs2274223 A>G polymorphism and cancer risk: a meta-analysis. *Tumour Biol*, **34**, 3537-44.
Wang L, Ma J, Liu F, et al (2007). Expression of MUC1 in primary and metastatic human epithelial ovarian cancer and its therapeutic significance. *Gynecol Oncol*, **105**, 695-702.
Wang L-D, Zhou F-Y, Li X-M, et al (2010). Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. *Nat Genet*, **42**, 759-63.
Wang Q, Chen P, Chen D, et al. (2014). Association between phospholipase C epsilon gene (PLCE1) polymorphism and colorectal cancer risk in a Chinese population. *J Int Med Res*, **42**, 70-81.
Wang X, Zbou C, Qiu G, et al (2008). Screening of new tumor suppressor genes in sporadic colorectal cancer patients. *Hepatogastroenterology*, **55**, 2039-44.
Williams K a, Terry KL, Tworoger SS, et al (2014). Polymorphisms of MUC16 (CA125) and MUC1 (CA15.3) in relation to ovarian cancer risk and survival. *PLoS One*, **9**, 88334.
Zheng L, Zhu C, Gu J, et al (2013). 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**, 5791-6.
Zotter S, Hageman P, Lossnitzer A, et al (1988). Tissue and tumour distribution of human polymorphic epithelial mucin. *Cancer Rev*, **11**, 55-101.