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Genetic variants at 1q22 and 10q23 reproducibly associated with gastric cancer susceptibility in a Chinese population

Hanze Zhang[1,2,†], Guangfu Jin[1,2,†], Huizhang Li[1], Chuanli Ren[3], Yanbing Ding[4], Qin Zhang[1], Bin Deng[4], Jianming Wang[1], Zhibin Hu[1,2], Yaochu Xu[1] and Hongbing Shen[1,2,*]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, China, [2]Section of Clinical Epidemiology, Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Cancer Center, Nanjing Medical University, Nanjing 210029, China, [3]Medical Lab, Northern Jiangsu People's Hospital, Yangzhou 225001, China and [4]Department of Gastroenterology, Yangzhou First People's Hospital, Yangzhou 225009, China

*To whom correspondence should be addressed. Tel: +86 25 868 62756; Fax: +86 25 865 27613;
Email: hbshen@njmu.edu.cn

Two recent genome-wide association studies reported significant associations of genetic variants at 1q22, 10q23 and 20p13 with gastric cancer (GC) risk in Chinese populations. However, these findings have not been confirmed in other independent studies. Here, we performed an independent case–control study in a Chinese population by genotyping three loci (rs4072037A>G at 1q22, rs2274223A>G at 10q23 and rs13042395C>T at 20p13) in 1681 GC cases and 1858 controls. We found that rs4072037 at 1q22 and rs2274223 at 10q23 were significantly associated with risk of GC with per allele odds ratio (OR) of 0.72 [95% confidence interval (CI): 0.63–0.81; $P = 2.98 \times 10^{-7}$] and 1.42 (95% CI: 1.27–1.58; $P = 9.68 \times 10^{-10}$), respectively. The association was more prominent for rs2274223 in female (OR = 1.86, 95% CI: 1.49–2.32) and gastric cardia adenocarcinoma (GCA) (OR = 1.71, 95% CI: 1.49–1.95). Furthermore, we combined the two single-nucleotide polymorphisms to evaluate the joint effect and found that the GC risk significantly increased with the number of risk allele increasing with a trend P value of 6.66×10^{-16}, and individuals with four risk alleles had a 3.28-fold (95% CI: 1.75–6.13) risk of GC compared with those having no risk alleles. However, no significant association was detected between rs13042395 at 20p13 and GC risk (OR = 1.04, 95% CI: 0.94–1.15; $P = 0.452$). In conclusion, our results indicate that genetic variants at 1q22 and 10q23 but not 20p13 may serve as candidate markers for GC susceptibility in the Chinese population.

Introduction

Gastric cancer (GC) is the second leading cause of cancer-related death around the world (1). *Helicobacter pylori* (*H.pylori*) infection is a well-established risk factor for GC and has been classified as a definite human carcinogen by International Agency of Research on Cancer (2). Besides, genetic factors substantially contribute to the gastric carcinogenesis (3), but the exact mechanism is still unclear. Over the last several years, as a powerful method to investigate the genetic determinants of complex diseases, genome-wide association studies (GWAS) have successfully identified hundreds of genetic markers that are related to the susceptibility of diseases (4). The GWAS of GC in Japanese population previously identified two single-nucleotide polymorphisms (SNPs) (rs2976392 and rs2294008 with high linkage disequilibrium) in prostate stem cell antigen (*PSCA*) gene at 8q24 that were significantly associated with GC risk (5), which was also well confirmed in Chinese populations (6,7).

Abbreviations: CI, confidence interval; GC, gastric cancer; ESCC, esophageal squamous cell carcinoma; GWAS, genome-wide association study; OR, odds ratio; confidence interval.

†These authors contributed equally to this work.

Recently, on the basis of Chinese population, Abnet *et al.* (8) performed a GWAS in 1625 GC cases, 1898 esophageal squamous cell carcinoma (ESCC) cases and 2100 controls and identified two clusters of SNPs at 1q22 and 10q23 that were significantly associated with risk of GC with P values of 2.33×10^{-9} and 1.10×10^{-6} in the initial scanning phase. However, none of these two regions remained significant ($P = 0.083$ and 0.120 for 1q22 rs4072037 and 10q23 rs2274223, respectively) in the second phase with a relatively small sample size (615 GC cases and 1202 controls). After combining the two phases, a genome-wide association was observed at 10q23 ($P = 8.40 \times 10^{-9}$), tagged by a non-synonymous SNP of rs2274223 with amino acid substitution of H1927R in phospholipase C epsilon 1 (*PLCE1*) gene, but genetic variants at 1q22, defined by synonymous SNP of rs4072037 in mucin 1 (*MUC1*) gene (T31T), was failed to reach genome-wide significance with P value of 4.22×10^{-7} (8). Interestingly, genetic variants at 10q23 were also significantly related to ESCC susceptibility (8). In the same period, Wang *et al.* (9) conducted a three-stage GWAS of ESCC in another Chinese population (GWAS: 1077 ESCC cases and 1733 controls; replication 1: 7673 cases and 11 013 controls; and replication 2: 303 cases and 537 controls) (9). They identified the same SNP of rs2274223 at 10q23 as well as a new SNP of rs13042395 at 20p13 for ESCC susceptibility, which was localized to 5′ flanking region of chromosome 20 open reading frame 54 (*C20orf54*). Both of these two SNPs were also associated with risk of gastric cardia adenocarcinoma (GCA) (10q23 rs2274223: $P = 1.74 \times 10^{-39}$; and 20p13 rs13042395: $P = 3.02 \times 10^{-3}$) when they used the same controls of ESCC replication 1 in GCA association study (2766 GCA cases and 11 013 controls) (9).

However, the GC GWAS conducted by Abnet *et al.* (8) was not well confirmed in their replication study partially due to the limited sample size (615 cases and 1202 controls), and the association results of GC risk observed by Wang *et al.* (9) are based on ESCC GWAS and the controls for GC were shared with ESCC association study, which might induce some potential bias. Therefore, it is helpful to confirm these findings in other independent studies with relatively large sample size. However, no replication studies have been reported to date. In this study, we conducted an independent case–control study with 1681 GC cases and 1858 controls in a Chinese population with high GC incidence in Jiangsu Province of eastern China to test the associations of rs2274223 (10q23), rs13042395 (20p13) and rs4072037 (1q22) with risk of GC.

Materials and methods

Subjects

This case–control study was approved by the institutional review board of Nanjing Medical University. As described previously (6), incident gastric cancer cases were recruited in the areas with relatively high incidence of GC, including cities of Yangzhong and Yixing from January 2004 to July 2005, and cities of Yangzhou and Nanjing from October 2006 to June 2010 in Jiangsu province, eastern China. The criteria for the recruitment of GC cases include: (i) self-reported Han Chinese; (ii) at least 5 years local residents; (iii) newly histopathologically diagnosed as primary gastric cancer; (iv) without a previous malignant tumor in any other organs and (v) without any antitumor therapy before recruitment, including chemotherapy, radiotherapy and so on. Any patients who were eligible to the above criteria and consented to participate in the study and to provide blood sample were included. As a result, a total of 1681 incident GC cases were included in this study with a response rate of 87.3%. Tumors located within 20 mm distal to the gastro-esophageal junction were defined as GCA (10). According to the criterion of Lauren classification, most GC cases were divided into two subgroups, intestinal type and diffuse type, and those with mixed type or difficult to determine were denoted with unclassified. On the basis of frequency matching for age (5 years interval), sex and residential area (city) to the cases, the controls were randomly selected from a pool of >30 000 cancer-free individuals who participated in the community-based screening program for non-infectious diseases conducted in

Jiangsu province, with an overall response rate of 83.8%. After signing an informed consent, each subject was interviewed face-to-face to obtain demographic data and information on related risk factors using a structure questionnaire, including tobacco smoking and alcohol consuming. Subsequently, an ~5 ml venous blood sample was collected from each subject. Finally, 1681 incident GC cases and 1858 frequency-matched controls were included in this study. Individuals who smoked one cigarette per day for over 1 year were considered as smokers, and those who had three or more alcohol drinks a week for over 6 months were defined as alcohol drinkers.

Genotyping

Genotyping was performed using the TaqMan allelic discrimination assay on the platform of 7900HT Real-time PCR System (Applied Biosystems, Foster City, CA). Two negative controls were included in each 384-well reaction plate and the genotyping results were determined by using SDS 2.3 Allelic Discrimination Software (Applied Biosystems). Moreover, to confirm genotyping results, 5% of samples (84 cases and 92 controls) were randomly selected to repeat and the accordance rate reached 100%.

Statistical analyses

Hardy–Weinberg equilibrium between SNPs was evaluated using the goodness-of-fit χ^2 test among the control subjects. Two-sided χ^2 tests were used to evaluate differences in the distributions of demographic characteristics, selected variables and genotypes between the cases and controls. Logistic regression analyses were employed to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between genetic variants and gastric cancer risk. The trend test was performed using logistic regression analysis. The heterogeneity of association between subgroups was assessed using the chi-square-based *Q*-test. All statistical analyses were performed with Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, NC).

Results

Characteristics of the 1681 GC cases and the 1858 controls are shown in Table I. No significant differences were detected on age, sex, smoking and drinking status between the cases and controls ($P = 0.884$, 0.245, 0.198 and 0.072, respectively). Of the 1681 GC cases, 1070 (63.6%) were classified as intestinal type, 312 (18.6) diffuse type and 299 (17.8%) unclassified type. The cases consisted of 822 (48.9%) cardia GC cases and 725 (43.1%) non-cardia GC cases, whereas 134 (8.0%) were unclassified.

Genotyping call rates were 98.6, 99.3 and 99.2% for rs4072037 (1q22), rs2274223 (10q23) and rs13042395 (20p13), respectively. The observed genotype frequencies for these variants were all consistent with Hardy–Weinberg equilibrium in controls ($P = 0.335$ for rs4072037A>G, $P = 0.451$ for rs2274223A>G and $P = 0.284$ for rs13042395C>T). Significant differences of genotype distributions between cases and controls were observed for rs4072037 and rs2274223 ($P = 2.49 \times 10^{-7}$ and1.88×10^{-8}, respectively) but not for rs13042395 ($P = 0.681$). As shown in Table II, logistic regression analysis revealed that individuals with variant alleles of rs4072037 and rs2274223 were significantly associated with altered risk of GC (adjusted per-allele OR = 0.72, 95% CI: 0.63–0.81 for rs4072037A>G; adjusted per-allele OR = 1.42, 95% CI: 1.27–1.58 for rs2274223A>G, respectively), with *P* values of 2.98×10^{-7} and 9.68×10^{-10}, respectively. However, no significant association was observed between rs13042395 and risk of GC (adjusted per-allele OR = 1.04, 95% CI: 0.94–1.15; $P = 0.452$).

Furthermore, stratification analyses were conducted to evaluate the potential association of genetic variants of these three SNPs with risk of subtype GC or that in subgroup populations. As shown in Figure 1, variant allele of rs4072037 (G) was consistently associated with a significantly decreased risk of gastric cancer among all strata (Figure 1A), whereas the rs2274223-G allele had stronger effect on female (OR = 1.86, 95% CI: 1.49–2.32) and GCA (OR = 1.71, 95% CI: 1.49–1.95) than male (OR = 1.30, 95% CI: 1.14–1.48) and non-cardia GC (OR = 1.15, 95% CI: 0.99–1.34), with *P* values of 0.006 and 0.0001 for heterogeneity test, respectively (Figure 1B). However, there were no obvious evidences of significant associations between rs13042395C>T and GC risk among all subgroups.

To demonstrate the independence of the associations of above two SNPs (rs2274223 and rs4072037) with GC risk, we conducted the logistic regression analysis by adjusting either of the two loci and found that the strength of association changed little for both loci (rs4072037: OR = 0.71, 95% CI: 0.63–0.81; rs2274223: OR = 1.44, 95% CI: 1.28–1.61), indicating that rs2274223 and rs4072037 were independently associated with GC susceptibility. Furthermore, as shown in Table III, we combined these two loci on an allele manner to detect the potential joint effects and found that the risk of GC significantly increased with the number of risk allele increasing (trend $P = 6.66 \times 10^{-16}$). Individuals with four risk alleles had a 3.28-fold (95% CI: 1.75–6.13) increased risk of GC compared with those having none of the risk alleles. Similar results were observed when we combined individuals carrying 0 or 1 risk alleles as the reference group (Table III).

Discussion

In this study, we evaluated the associations of genetic variants at 1q22 (rs4072037A>G), 10q23 (rs2274223A>G) and 20p13 (rs13042395C>T) with GC susceptibility in an independent case–control study with 1681 GC cases and 1858 controls in a high risk Chinese population. We found that rs4072037A>G and rs2274223A>G but not rs13042395C>T were significantly associated with altered risk of gastric cancer in our population. The effect appeared to be stronger in females and in GCA for rs2274223A>G. Furthermore, rs2274223 and rs4072037 could independently but cumulatively affect the risk of GC.

The GWAS of GC conducted by Abnet *et al.* (8) observed significant association between rs4072037-G allele and gastric cancer risk (OR = 0.71, 95% CI: 0.62–0.82) approaching the significant level of genome-wide association ($P = 1.10 \times 10^{-6}$) in their initial phase, but the association was not significant in the second phase (OR = 0.84, 95% CI: 0.69–1.02) with *P* value of 0.083 in 615 cases and 1202 controls, which might be due to the small sample size in part. The results of current study also revealed a reduced risk of GC for rs4072037-G (OR = 0.72, 95% CI: 0.63–0.81) with *P* value of 2.98×10^{-7}. Interestingly, the first GC GWAS performed in Japanese population also identified the same region (defined by rs2075570 and rs2070803) with the *P* values around 1×10^{-7}, which, however, was not investigated in depth (5). As shown in supplementary Figure 1 (available at *Carcinogenesis* Online), the above-mentioned SNPs (rs4072037, rs2070803 and rs2075570) locate in an extended LD block with the region size ~724 kb (Chr1:153372506–154096135), containing ~20 genes. Of these genes, the most notable gene *MUC1* is a membrane-associated mucin and expresses on the apical surface of glandular epithelium, playing critical functions in protection of epithelial surfaces by forming a protective mucous barrier with secreted mucins and signaling the presence of adverse conditions in the extracellular environment (11,12). Genetic variants in *MUC1* have been associated with the risk of gastric cancer in previous studies with a candidate gene approach (13,14). Jia *et al.* (13) found that six tag SNPs across the *MUC1* region were all significantly associated with risk of GC in Polish, and the rs4072037 AA genotype was associated with a 2.2-fold increased GC risk compared with GG genotype. Similar results were also reported in a Chinese study with 138 GC cases and 241 controls (14). More interestingly, rs4072037, a synonymous SNP in exon 2 of *MUC1*, was observed to disrupt the physiological functions of MUC1 (14), which might be due to alternative splicing of the 5′-region of exon 2 controlled by rs4072037 (15) and ultimately result in failure to the physiological protection of gastric mucosa. However, both fine-mapping studies in this region and further functional studies about rs4072037 are warranted to unravel the causal variants at 1q22 for gastric carcinogenesis.

Genetic variants on 10q23 region were identified as genetic markers for both GCA and ESCC susceptibility by two GWAS (8,9) simultaneously, which was also clearly confirmed in our independent

population. The SNP rs2274223 is localized to the 26th exon of *PLCE1* and results in a substitution of His to Arg at codon 1927 of *PLCE1*, a member of the phospholipase C protein family. PLCE1 interacts with the proto-oncogene *ras* and acts as an effector of guanosine triphosphatases (Ras, Rap1 and Rap2) (16) involving regulation of cell growth, differentiation, apoptosis and angiogenesis (17). A role of oncogene has been associated with *PLCE1* in skin (18) and intestinal (19) carcinogenesis through inflammation signaling pathways. Immunohistochemical analysis revealed a higher positive rate of PLCE1 in tumor tissues of GCA and ESCC than that in normal tissues (9). Besides, *NOC3L*, involving in the control of DNA replication during mitotic clonal expansion (20), is in the same linkage disequilibrium area as the susceptible loci at 10q23 (Chr10:95961456–96115639) with a region size∼154 kb (supplementary Figure 2 is available at *Carcinogenesis* Online). Even though, it is unclear which variant is the causal locus in such a big linkage disequilibrium region, and further studies are warranted to fine map this region based on resequencing data and to determine the causal loci.

The SNP rs13042395, localized to 20p13, was significantly associated with ESCC risk in a GWAS scan followed by two replication studies in Chinese populations, and it was also significantly associated with GCA risk when sharing the controls of ESCC cases (9). However, in this independent case–control study, we failed to observe a significant association of this SNP with risk of either overall GC or subgroup of GCA. The possibility may be that the association between 20p13 variants and GCA risk was very modest and our subgroup analysis did not have enough statistical power to detect it. Another explanation is that this association is not real in gastric cancer. Larger studies are warranted to clarify the associations of this 20p13 region with GC risk.

In addition, logistic regression analysis supported that rs2274223 and rs4072037 were independently contribute to GC susceptibility and a cumulative effect of rs2274223 and rs4072037 on the risk of GC was observed in our population. Both SNPs showed the potential to predict the risk of GC in future in combination with traditional risk factors of GC in Chinese population and they may be used to build the risk prediction model to serve as the genetic test for high risk population of GC.

One of the strengths in the current study is the large sample size (1681 cases and 1858 controls) from a relatively homogeneous population, which made us confident to validate the GWAS findings. For another, based on an independent Chinese study, we firstly confirmed the regions of 10q23 and 1q22 as susceptible regions for GC in Chinese population but suspended the candidate region of 20p13 for further evaluation in future studies. However, the limitations in this study need to be addressed. First of all, we recruited GC cases from hospitals and selected controls from communities, which might not well represent the whole population and might result in potential selection bias. Second, because the data of *H.pylori* infection was not available in this study, it is difficult for us to adjust the potential confounding bias from *H.pylori* infection as well as to evaluate the potential gene–environmental interaction.

In conclusion, this study evaluated the findings reported by recent GWAS in an independent population and provided strong evidence

Table I. Selected characteristics between gastric cancer cases and controls

Variable	Case (n = 1681) N (%)	Control (n = 1858) N (%)	P value[a]
Age (years)			0.884
<60	717 (42.6)	788 (42.4)	
≥60	964 (57.4)	1070 (57.6)	
Sex			0.245
Male	1238 (73.6)	1336 (71.9)	
Female	443 (26.4)	522 (28.1)	
Smoking status			0.198
Never	828 (52.8)	1022 (55.0)	
Ever	740 (47.2)	836 (45.0)	
Drinking status			0.072
Never	1132 (72.3)	1291 (69.5)	
Ever	434 (27.7)	567 (30.5)	
Histological types[b]			
Intestinal type	1070 (63.6)		
Diffuse type	312 (18.6)		
Unclassified	299 (17.8)		
Tumor sites[c]			
Cardia cancer	822 (48.9)		
Non-cardia cancer	725 (43.1)		
Unclassified	134 (8.0)		

[a]Two-sided χ^2 test.
[b]Including 89 mixed and 210 unknown.
[c]Including 22 mixed and 112 unknown.

Table II. Distribution of genotypes of SNPs at chromosomes 1q22, 20p13 and 10q23 and their associations with gastric cancer risk

Chr.	SNP	Controls	Cases			Cardia cancer			Non-cardia cancer		
		N (%)	N (%)	Adjusted OR (95% CI)[a]	P value[a]	N (%)	Adjusted OR (95% CI)[a]	P value[a]	N (%)	Adjusted OR (95% CI)[a]	P value[a]
1q22	rs4072037	n = 1833	n = 1658			n = 809			n = 717		
	AA	1203 (65.6)	1230 (74.2)	1.00		579 (71.6)	1.00		551 (76.9)	1.00	
	AG	556 (30.3)	382 (23.0)	0.67 (0.58–0.79)	6.0×10^{-7}	206 (25.4)	0.78 (0.64–0.94)	0.011	147 (20.5)	0.56 (0.45–0.70)	2.11×10^{-7}
	GG	74 (4.1)	46 (2.8)	0.63 (0.43–0.92)	0.016	24 (3.0)	0.67 (0.41–1.08)	0.098	19 (2.6)	0.61 (0.36–1.02)	0.061
	AG/GG	630 (34.4)	428 (25.8)	0.67 (0.57–0.77)	1.06×10^{-7}	230 (28.4)	0.77 (0.64–0.92)	0.004	166 (23.1)	0.57 (0.46–0.70)	8.15×10^{-8}
	Per allele[b]			0.72 (0.63–0.81)	2.98×10^{-7}		0.79 (0.68–0.93)	0.004		0.64 (0.53–0.76)	6.56×10^{-7}
10q23	rs2274223	n = 1848	n = 1665			n = 812			n = 720		
	AA	1122 (60.7)	867 (52.1)	1.00		373 (46.0)	1.00		421 (58.5)	1.00	
	AG	643 (34.8)	664 (39.9)	1.38 (1.19–1.59)	1.25×10^{-5}	355 (43.7)	1.64 (1.37–1.96)	5.53×10^{-8}	259 (36.0)	1.15 (0.95–1.38)	0.155
	GG	83 (4.5)	134 (8.0)	2.13 (1.59–2.85)	3.69×10^{-7}	84 (10.3)	3.09 (2.23–4.30)	1.85×10^{-11}	40 (5.5)	1.34 (0.89–2.02)	0.155
	AG/GG	726 (39.3)	798 (47.9)	1.46 (1.28–1.68)	5.17×10^{-8}	439 (54.0)	1.81 (1.53–2.15)	9.20×10^{-12}	299 (41.5)	1.17 (0.98–1.40)	0.091
	Per allele[b]			1.42 (1.27–1.58)	9.68×10^{-10}		1.71 (1.49–1.95)	9.77×10^{-15}		1.15 (0.99–1.34)	0.065
20p13	rs13042395	n=1841	n=1668			n= 814			n= 721		
	CC	741 (40.2)	654 (39.2)	1.00		310 (38.1)	1.00		290 (40.2)	1.00	
	CT	837 (45.5)	760 (45.6)	1.03 (0.89–1.19)	0.722	384 (47.2)	1.10 (0.91–1.32)	0.328	318 (44.1)	0.98 (0.81–1.19)	0.844
	TT	263 (14.3)	254 (15.2)	1.09 (0.88–1.33)	0.440	120 (14.7)	1.15 (0.88–1.48)	0.305	113 (15.7)	1.01 (0.77–1.33)	0.918
	CT/TT	1100 (59.8)	1014 (60.8)	1.04 (0.91–1.20)	0.571	504 (61.9)	1.11 (0.93–1.32)	0.248	431 (59.8)	0.99 (0.82–1.19)	0.904
	Per allele[b]			1.04 (0.94–1.15)	0.452		1.08 (0.95–1.22)	0.239		1.00 (0.88–1.14)	0.991

[a]Adjusted for age, sex, smoking and drinking status.
[b]Derived from trend test (d.f. = 1).

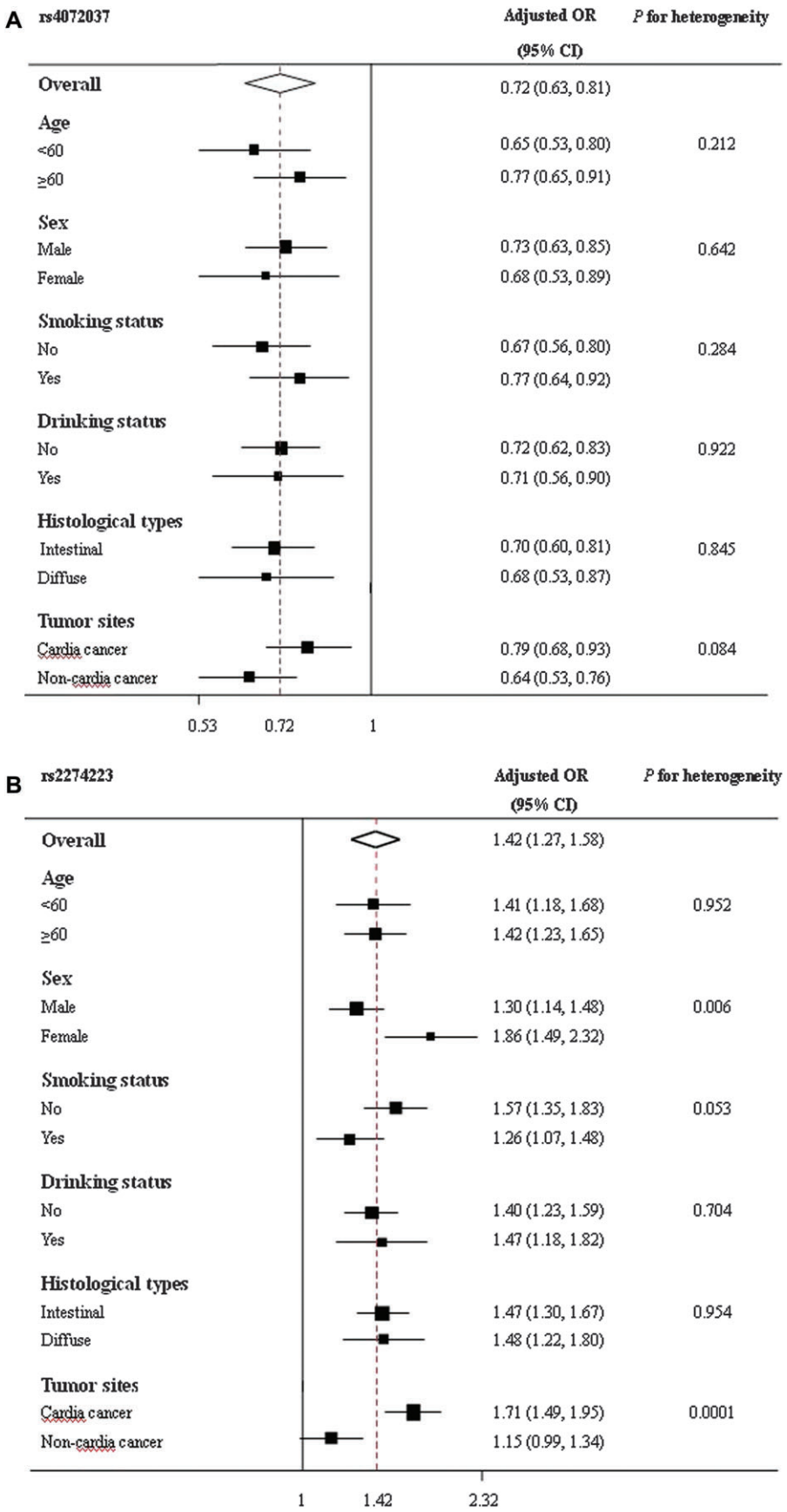

Fig. 1. The effects of SNPs rs4072037 at 1q22 (**A**) and rs2274223 at 10q23 (**B**) on gastric cancer risk among different subgroups. *P* values are from heterogeneity tests.

Table III. Joint effect of SNPs at 1q22 (rs4072037) and 10q23 (rs2274223) on gastric cancer risk

Risk allele number[a]	Case (N = 1651) N (%)	Control (N = 1828) N (%)	Adjusted OR (95% CI)[b]	P value[b]	Adjusted OR (95% CI)[c]	P value[c]
0	21 (1.3)	41 (2.2)	1.00		1.00	
1	217 (13.1)	376 (20.6)	1.06 (0.61–1.85)	0.829		
2	793 (48.0)	915 (50.1)	1.62 (0.95–2.76)	0.079	1.53 (1.26–1.85)	1.23×10^{-5}
3	526 (31.9)	442 (24.2)	2.25 (1.31–3.88)	3.3×10^{-3}	2.13 (1.73–2.63)	9.69×10^{-13}
4	94 (5.7)	54 (2.9)	3.28 (1.75–6.13)	2.0×10^{-4}	3.10 (2.13–4.52)	3.71×10^{-9}
Trend			1.42 (1.31–1.55)	6.66×10^{-16}	1.45 (1.33–1.58)	4.44×10^{-16}

[a]rs4072037-A and rs2274223-G alleles were assumed as risk alleles.
[b]The reference group was those with '0' risk allele.
[c]The reference group was those with 0 or '1' risk allele.

supporting that genetic variants at 10q23 and 1q22, defined by rs2274223 and rs4072037, respectively, were independent susceptible regions for GC in Chinese population. However, rs13042395 at 20p13 was not associated with the susceptibility of GC in our population. Further studies by fine-mapping the susceptible region and annotating the functional significance of the variants are needed to clarify the genetic mechanism of gastric carcinogenesis.

Supplementary material

Supplementary Figures 1 and 2 can be found at http://carcin.oxfordjournals.org/

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