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

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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>C at 1q22, rs2274223A>G at 1q22 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: 1.27–1.59; \( P = 9.68 \times 10^{-10} \)], respectively. The association was more prominent for rs2274223 in female (OR = 1.96, 95% CI: 1.80–2.14) and gastric cardia adenocarcinoma (GCA) (OR = 2.39, 95% CI: 2.09–2.75) than for gastric esophago-gastric junction (OC) (OR = 1.49, 95% CI: 1.34–1.65). 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 \( 2.33 \times 10^{-9} \) and \( 1.10 \times 10^{-6} \) 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 (\( P = 8.40 \times 10^{-6} \)), 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 \( 4.22 \times 10^{-7} \) (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: \( P = 1.74 \times 10^{-10} \); 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

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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 (\( P = 1.10 \times 10^{-4} \)) 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 \( \times 10^{-7} \). Interestingly, the first GC GWAS performed in Japanese population also identified the same region (defined by rs2070803 and rs2070809) with the \( P \) values of 0.004, 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 rs2070850) 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 extra-cellular 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 A/A genotype was associated with a 2.2-fold increased GC risk compared with GC 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 S'-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 \textit{PLCE1} and results in a substitution of His to Arg at codon 1927 of \textit{PLCE1}, a member of the phospholipase C protein family. \textit{PLCE1} interacts with the proto-oncogene \textit{ras} and acts as an effector of guanosine triphosphatases (\textit{Ras}, \textit{Rap1} and \textit{Rap2}) (16) involving regulation of cell growth, differentiation, apoptosis and angiogenesis (17). A role of oncogene has been associated with \textit{PLCE1} in skin (18) and intestinal (19) carcinogenesis through inflammation signaling pathways. Immunohistochemical analysis revealed a higher positive rate of \textit{PLCE1} in tumor tissues of GCA and ESCC than that in normal tissues (9). Besides, \textit{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.

\begin{table}[h]
\centering
\caption{Selected characteristics between gastric cancer cases and controls} 
\begin{tabular}{|l|c|c|c|}
\hline
Variable & Case & Control & \textit{P} value$^a$ \\
\hline
\textit{Age (years)} & & & 0.884 \\
<60 & 717 (42.6) & 788 (42.4) & \\
\geq 60 & 964 (57.4) & 1070 (57.6) & \\
\hline
\textit{Sex} & & & 0.245 \\
Male & 1238 (73.6) & 1336 (71.9) & \\
Female & 443 (26.4) & 222 (28.1) & \\
\hline
\textit{Smoking status} & & & 0.198 \\
Never & 828 (52.8) & 1022 (55.0) & \\
Ever & 740 (47.2) & 836 (45.0) & \\
\hline
\textit{Drinking status} & & & 0.072 \\
Never & 1132 (72.3) & 1291 (69.5) & \\
Ever & 434 (27.7) & 567 (30.5) & \\
\hline
\textit{Histological types}$^b$ & & & \\
Unclassified & 134 (8.0) & & \\
\textit{Tumor sites}$^c$ & & & \\
Intestinal type & 1070 (63.6) & & \\
Diffuse type & 312 (18.6) & & \\
Unclassified & 299 (17.8) & & \\
\hline
\end{tabular}
\end{table}

The $^b$including 89 mixed and 210 unknown.

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

\begin{table}[h]
\centering
\caption{Distribution of genotypes of SNPs at chromosomes 1q22, 20p13 and 10q23 and their associations with gastric cancer risk} 
\begin{tabular}{|l|c|c|c|c|c|c|c|c|}
\hline
\textit{Chr. SNP} & \textit{Controls} & \textit{Cases} & \textit{N} & \textit{N} & \textit{Adjusted OR (95\% CI)$^a$} & \textit{P value$^a$} & \textit{N} & \textit{N} & \textit{Adjusted OR (95\% CI)$^a$} \\
\hline
1q22 & rs4072037 & & & & & & & \\
AA & 1203 (65.6) & 1230 (74.2) & 1.00 & & & & & \\
AG & 556 (30.3) & 382 (23.0) & 0.67 (0.58-0.79) & 6.0 \times 10^{-7} & & & & \\
GG & 74 (4.1) & 46 (2.8) & 0.63 (0.43-0.92) & 0.016 & & & & \\
AG/\textit{GG} & 630 (34.4) & 428 (25.8) & 0.67 (0.57-0.77) & 1.06 \times 10^{-7} & 0.72 (0.63-0.81) & 2.98 \times 10^{-7} & & & \\
\textit{Per allele}$^b$ & & & & & & & & \\
\textit{Cardia cancer} & & & & & & & & \\
\hline
1q23 & rs7224223 & & & & & & & \\
AA & 1122 (60.7) & 876 (52.1) & 1.00 & & & & & \\
AG & 643 (34.8) & 664 (39.9) & 1.38 (1.19-1.59) & 1.25 \times 10^{-7} & 355 (43.7) & 1.64 (1.37-1.96) & 5.35 \times 10^{-6} & & \\
GG & 83 (4.5) & 134 (8.0) & 2.13 (1.59-2.85) & 3.69 \times 10^{-7} & 84 (10.3) & 3.09 (2.23-4.30) & 1.85 \times 10^{-6} & & \\
AG/\textit{GG} & 726 (39.3) & 798 (47.9) & 1.46 (1.28-1.68) & 5.17 \times 10^{-7} & 439 (54.0) & 1.81 (1.53-2.15) & 9.20 \times 10^{-12} & & \\
\textit{Per allele}$^b$ & & & & & & & & \\
\textit{Ever} & & & & & & & & \\
\hline
20p13 & rs13042395 & & & & & & & \\
CC & 741 (40.2) & 654 (39.2) & 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 & & \\
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 & & \\
\textit{Per allele}$^b$ & & & & & & & & \\
\hline
\end{tabular}
\end{table}

$^a$Adjusted for age, sex, smoking and drinking status.

$^b$Derived from trend test (d.f. = 1).
### A. rs4072037

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Adjusted OR (95% CI)</th>
<th>P for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.72 (0.63, 0.81)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>0.65 (0.53, 0.80)</td>
<td>0.212</td>
</tr>
<tr>
<td>≥60</td>
<td>0.77 (0.65, 0.91)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>0.73 (0.63, 0.83)</td>
<td>0.642</td>
</tr>
<tr>
<td>Female</td>
<td>0.68 (0.53, 0.89)</td>
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</tr>
<tr>
<td>Smoking status</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>0.67 (0.56, 0.80)</td>
<td>0.284</td>
</tr>
<tr>
<td>Yes</td>
<td>0.77 (0.64, 0.92)</td>
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</tr>
<tr>
<td>Drinking status</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>0.72 (0.62, 0.83)</td>
<td>0.922</td>
</tr>
<tr>
<td>Yes</td>
<td>0.71 (0.56, 0.90)</td>
<td></td>
</tr>
<tr>
<td>Histological types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>0.70 (0.60, 0.81)</td>
<td>0.845</td>
</tr>
<tr>
<td>Diffuse</td>
<td>0.68 (0.53, 0.87)</td>
<td></td>
</tr>
<tr>
<td>Tumor sites</td>
<td></td>
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<tr>
<td>Cardia cancer</td>
<td>0.79 (0.68, 0.93)</td>
<td>0.084</td>
</tr>
<tr>
<td>Non-cardia cancer</td>
<td>0.64 (0.53, 0.76)</td>
<td></td>
</tr>
</tbody>
</table>

### B. rs2274223

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Adjusted OR (95% CI)</th>
<th>P for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.42 (1.27, 1.58)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>1.41 (1.18, 1.68)</td>
<td>0.952</td>
</tr>
<tr>
<td>≥60</td>
<td>1.42 (1.23, 1.63)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.30 (1.14, 1.48)</td>
<td>0.006</td>
</tr>
<tr>
<td>Female</td>
<td>1.86 (1.49, 2.32)</td>
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</tr>
<tr>
<td>Smoking status</td>
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</tr>
<tr>
<td>No</td>
<td>1.57 (1.35, 1.83)</td>
<td>0.053</td>
</tr>
<tr>
<td>Yes</td>
<td>1.26 (1.07, 1.46)</td>
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</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.40 (1.23, 1.59)</td>
<td>0.704</td>
</tr>
<tr>
<td>Yes</td>
<td>1.47 (1.18, 1.82)</td>
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</tr>
<tr>
<td>Histological types</td>
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</tr>
<tr>
<td>Intestinal</td>
<td>1.47 (1.30, 1.67)</td>
<td>0.954</td>
</tr>
<tr>
<td>Diffuse</td>
<td>1.40 (1.22, 1.80)</td>
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<tr>
<td>Tumor sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia cancer</td>
<td>1.71 (1.49, 1.93)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Non-cardia cancer</td>
<td>1.15 (0.99, 1.34)</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Risk allele number</th>
<th>Case (N = 1651)</th>
<th>Control (N = 1828)</th>
<th>Adjusted OR (95% CI)</th>
<th>P valueb</th>
<th>Adjusted OR (95% CI)c</th>
<th>P valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21 (1.3)</td>
<td>41 (2.2)</td>
<td>1.00</td>
<td>0.829</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>217 (13.1)</td>
<td>376 (20.6)</td>
<td>1.06 (0.61–1.85)</td>
<td>0.079</td>
<td>1.53 (1.26–1.85)</td>
<td>1.23 × 10⁻⁵</td>
</tr>
<tr>
<td>2</td>
<td>795 (48.0)</td>
<td>915 (50.1)</td>
<td>1.62 (0.95–2.76)</td>
<td>3.3 × 10⁻³</td>
<td>2.13 (1.73–2.63)</td>
<td>9.69 × 10⁻¹³</td>
</tr>
<tr>
<td>3</td>
<td>526 (31.9)</td>
<td>442 (24.2)</td>
<td>2.25 (1.31–3.88)</td>
<td>2.0 × 10⁻⁴</td>
<td>3.10 (2.13–4.52)</td>
<td>3.71 × 10⁻⁹</td>
</tr>
<tr>
<td>4</td>
<td>94 (5.7)</td>
<td>54 (2.9)</td>
<td>3.28 (1.75–6.13)</td>
<td>6.66 × 10⁻¹⁶</td>
<td>1.45 (1.33–1.58)</td>
<td>4.44 × 10⁻¹⁶</td>
</tr>
<tr>
<td>Trend</td>
<td></td>
<td></td>
<td>1.42 (1.31–1.55)</td>
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</tr>
</tbody>
</table>

¹rs4072037-A and rs2274223-G alleles were assumed as risk alleles.

²The reference group was those with ‘0’ risk allele.

³The reference group was those with 0 or ‘1’ risk allele.

Supplementary material

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

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References


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