RESEARCH COMMUNICATION

# Association of Genetic Polymorphisms at 1q22 but not 10q23 with Gastric Cancer in a Southern Chinese Population

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## Abstract

**Objective: Data from a recent genome-wide association studiesy of gastric cancer (GC) and oesophageal squamous cell carcinoma in Chinese living in the Taihang Mountains of north-central China suggest that 1q22 and 10q23 are susceptibility-associated regions for GC. However, this has not been confirmed in southern Chinese populations. The aim of this study was to investigate whether these polymorphisms at 1q22 and 10q23 are associated with the risk of GC in a southern Chinese population. Methods: We selected seven top significant associated single nucleotide polymorphisms (SNPs) at 1q22 and 10q23 and conducted a population-based case-control study in a southern Chinese population. Genotypes were determined using MassARRAYTM system (Sequenome, San Diego, CA). Results: Two SNPs at 1q22, rs4072037 and rs4460629, were significantly associated with a reduced risk of GC, best fitting the dominant genetic model. Logistic regression models adjusted for age and sex showed that rs4072037 AG and GG (OR=0.64, *P*=0.017, compared with AA) and rs4460629 CT and TT (OR=0.54, *P*=0.0016, compared with TT) significantly reduced the risk of GC. However, no significant results for the five SNPs at 10q23 were obtained in this study. Conclusion: These outcomes indicate that 1q22 is associated with GC susceptibility in this southern Chinese population, while an association for the locus at 10q23 was not confirmed.**

**Keywords:** 1q22 - 10q23 - single nucleotide polymorphism - gastric cancer

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## Introduction

Gastric cancer (GC) is the fourth most common cancer and second leading cause of cancer-related death worldwide (Crew and Neugut, 2006). Although the incidence and mortality of GC have decreased over the past few decades, the incidence and mortality rates are still high in East Asia, Eastern Europe, and South America (Jemal et al., 2011). The aetiology of GC remains unclear, although multiple factors are thought to play a role in gastric carcinogenesis, including diet, tobacco smoking, alcohol consumption, infection with Helicobacter pylori and other agents, and the accumulation of specific genetic alteration, including polymorphisms (Blaser, 2000; González et al., 2002; Peek and Blaser, 2002).

Genome-wide association studies, a powerful new approach to identifying susceptibility loci, utilise genotyping platforms that can type hundreds of thousands of single nucleotide polymorphisms (SNPs) simultaneously (Burton et al., 2007). GWAS currently has become a major strategy for identifying genetic susceptibility factors for polygenic diseases including cancers. To examine GC, a genome-wide association study (GWAS) in north-central China identified seven SNPs at 1q22 and 10q23 as being significantly associated with GC susceptibility in the initial scanning phase, though none of the two regions remain significant in the second phase (Abnet et al., 2010). After combined the two phases, a genome-wide association was observed at 10q23 tagged by a nonsynonymous SNP of rs2274223, but genetic variants at 1q22 defined by synonymous SNP of rs4072037 was failed to reach genome-wide significance with P-value of $4.22\times10^{-7}$.

Though the GC GWAS conducted by Abnet et al has been replicated in an eastern Chinese population (Zhang et al., 2011), and the significant associations with risk of GC were found for rs4072037 at 1q22 and rs2274223 at 10q23. As far as we know, these findings have not been confirmed in other independent studies. Here, we performed a case-control study in a southern Chinese population by genotyping seven top significant SNPs in GWAS (Abnet et al., 2010), including two SNPs (rs4072037 and rs4460629) at 1q22 and five SNPs located at 10q23 (rs753724, rs2274223, rs3765524, rs3781264, and rs11187842). Therefore, in this study, we first analyzed the prevalence of these SNPs at 1q22 and 10q23 in southern Chinese population, then to assess whether they may play a key role in GC or may be a suitable marker of GC.

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## Materials and Methods

*Subjects*

Patients and control subjects were obtained from individuals attending the outpatient and inpatient clinics of the First Affiliated Hospital of Nanchang University and the First Affiliated Hospital of Guangdong Medical College, southern of China. All patients were histologically confirmed to have GC. Controls were cancer-free individuals randomly selected from the hospital's outpatients. All patients and control subjects were recruited between 2007 and 2010. The study protocol was approved by the Clinical Research Ethics Committee of Southern Medical University. Informed consent was obtained from all participants.

*Genotyping*

Genomic DNA was extracted from the peripheral blood samples of the participants using a genomic DNA kit (Tiangen Biotech, Beijing, China). The primer sets, which included a pair of amplification primers and an extension primer for each SNP, were designed using Assay Design 3.1. Subsequently, the SNPs rs753724, rs2274223, rs3765524, rs3781264, rs4072037, rs4460629, and rs11187842 were analysed using the MassARRAY matrix-assisted laser desorption ionisation-time of flight mass spectrometry platform (Sequenom), following the manufacturer's instructions (www.sequenom.com).

*Statistical analysis*

Deviations from Hardy-Weinberg equilibrium were assessed using the web-based tool SNPStats (http://bioinfo.iconcologia.net/SNPStats). Codominant, dominant, recessive, and overdominant genetic models of inheritance were chosen to evaluate the associations between each SNP and GC. The association analysis based on unconditional logistic regression was carried out by calculating the odds ratio (OR) and 95% confidence interval (95% CI) after adjusting for age and sex for each SNP in every chosen genetic model; the significance level was set at 0.05. These tests were also implemented with SNPStats. The results from this calculator were consistent with those using SPSS 13.0 and have been summarised in another publication (Li et al., 2011). The linkage disequilibrium (LD) and pairwise LD coefficient were also implemented with SNPStats.

**Table 1. Characteristics of Study Subjects**

| Variable | Gastric cancer Cases, n (%) | Controls, n (%) |
|---|---|---|
| Study subjects | 249 (100) | 292 (100) |
| Sex | | |
| Male | 178 (71.5) | 175 (59.9) |
| Female | 71 (28.5) | 117 (40.1) |
| Age | | |
| Mean ± SD (years) | 54.82±12.47 | 58.65±16.14 |
| Median (years) | 56 | 61 |
| Tumor type | | |
| Adenocarcinoma | 166 (66.7) | |
| Myxoma | 10 (4.0) | |
| Unclassified | 73 (29.3) | |

## Results

*Subject characteristics*

A total of 249 GC patients and 292 healthy individuals were enrolled in this study. The GC patient including 233 cases diagnosed at the First Affiliated Hospital of Nanchang University and 16 cases diagnosed at the First Affiliated Hospital of Guangdong Medical College. The control series included 292 healthy subjects from the general population of Nanchang, Jiangxi province, southern China. The mean age of the GC patients was 54.82±12.47 years (mean±SD), while that of the normal control group was 58.65±16.14 years. Patient age was defined as the age at diagnosis, and control subject age was defined as the age at the time of recruitment. No significant difference were found between patients and controls in the distribution of age. Of the patients, 166 are adenocarcinoma and 10 are myxoma, whereas 73 patients had missing data. The details of the subject characteristics are listed in Table 1.

*GC and polymorphisms at 1q22 and 10q23*

All SNPs conformed to Hardy-Weinberg proportions in the controls. From Table 2, we see that the minor allele frequencies for the SNPs at 10q23 and 1q22 varied in the population. Differences in genotype distribution between the cases and controls were also observed for the seven selected SNPs. As shown in Table 3, logistic regression analysis after adjustment revealed that the rs4072037 G homozygote and heterozygote reduced the GC risk (AG: OR=0.64, *P*=0.058; AG and GG: OR=0.64, *P*=0.017) compared with the A homozygote (AA). Similarly, the rs4460629 T homozygote and T heterozygote had a significantly reduced GC risk (CT: OR=0.54, *P*=0.007; CT and TT: OR=0.54, *P*=0.002), compared with the C homozygote (CC). No significant association with susceptibility was observed for the SNPs on 10q23 (Table

**Table 2. Interethnic Comparisons of Minor Allele Frequencies (MAF) for SNPs at 10q23 and 1q22 in Our Subjects with HapMap and Published Data**

| Locus | MAF: our study control, case | MAF: Shanxi control, case | MAF: HapMap data Beijing | Japanese | European | African |
|---|---|---|---|---|---|---|
| rs4072037 | 0.207, 0.157 | 0.159, 0.125 | 0.167 | 0.174 | 0.517 | 0.213 |
| rs4460629 | 0.200, 0.135 | 0.142, 0.112 | 0.143 | 0.196 | 0.583 | 0.232 |
| rs753724 | 0.172, 0.159 | 0.147, 0.190 | 0.157 | 0.186 | 0.132 | 0.039 |
| rs11187842 | 0.173, 0.159 | 0.147, 0.190 | 0.157 | 0.183 | 0.132 | 0.035 |
| rs3765524 | 0.228, 0.229 | 0.207, 0.259 | 0.223 | 0.208 | 0.363 | 0.59 |
| rs2274223 | 0.228, 0.231 | 0.209, 0.259 | 0.219 | 0.203 | 0.385 | 0.554 |
| rs3781264 | 0.175, 0.165 | 0.152, 0.199 | 0.161 | 0.186 | 0.363 | 0.131 |

**Table 3. Association Between Gastric Cancer And SNP Genotypes in our Subjects**

| Locus | Geno-type | Case | Co-dominant Adjusted OR (95% CI) | Dominant Adjusted OR (95% CI) | *P* |
|---|---|---|---|---|---|
| 1q22: rs4072037 | AA | 181(73) | 1 | 1 | |
| | AG | 58(23) | 0.64 (0.43-0.94) | 0.64(0.44-0.93) | 0.017* |
| | GG | 10(4) | 0.64 (0.27-1.50) | | |
| rs4460629 | CC | 190(77) | 1 | 1 | |
| | CT | 49(20) | 0.54 (0.36-0.81) | 0.54 (0.37-0.79) | 0.002* |
| | TT | 9(4) | 0.55 (0.23-1.32) | | |
| 10q23: rs753724 | GG | 178(71) | 1 | 1 | |
| | GT | 63(25) | 1.04 (0.69-1.54) | 1.00 (0.68-1.46) | 0.99 |
| | TT | 8(3) | 0.77 (0.31-1.93) | | |
| rs2274223 | AA | 149(60) | 1 | 1 | |
| | AG | 85(34) | 1.12 (0.77-1.63) | 1.11 (0.78-1.58) | 0.56 |
| | GG | 15(6) | 1.05 (0.51-2.16) | | |
| rs3765524 | CC | 150(60) | 1 | 1 | |
| | CT | 84(34) | 1.09 (0.75-1.59) | 1.09 (0.76-1.54) | 0.65 |
| | TT | 15(6) | 1.04 (0.51-2.14) | | |
| rs3781264 | TT | 175(70) | 1 | 1 | |
| | TC | 66(27) | 1.07 (0.72-1.59) | 1.03 (0.71-1.50) | 0.88 |
| | CC | 8(3) | 0.78 (0.31-1.96) | | |
| rs11187842 | CC | 178(71) | 1 | 1 | |
| | CT | 63(25) | 1.03 (0.69-1.53) | 0.99 (0.68-1.44) | 0.95 |
| | TT | 8(3) | 0.77 (0.31-1.93) | | |

*P value <0.05

3).

## Discussion

This study evaluated the associations of genetic polymorphisms at 1q22 and 10q23 with GC susceptibility in a case-control analysis of 249 GC cases and 292 controls in a Chinese population. SNPs rs4072037 and rs4460629 at locus 1q22 were significantly associated with an altered risk of GC, while no significant result for the SNPs at 10q23 was found, unlike previous reports (Abnet et al., 2010; Zhang et al., 2011).

Regarding 1q22, Abnet et al observed a near-significant association between the rs4072037 G allele and GC risk (OR=0.71, $P=1.10\times10^{-6}$) in the first phase of their GWAS; no significant result was obtained in the second phase (OR=0.84, *P*=0.083) (Abnet et al., 2010). Zhang et al. (2011) reported a significantly reduced risk (OR=0.72, $P=2.98\times10^{-7}$) of GC for the G allele of rs4072037 in Mucin 1 (MUC1), and we found a similar result (reduced OR=0.64, *P*=0.017, on comparing AG+GG with AA). MUC1 is a highly polymorphic membrane-associated mucin that is often expressed aberrantly in cancer (Taylor-Papadimitriou et al., 2002). In addition, MUC1 was reported to bind to H. pylori (Lindén et al., 2004; McGuckin et al., 2007); MUC1 knockout mice are susceptible to H. pylori gastritis (McGuckin et al., 2007), and there is an altered pattern of expression of MUC1 in H. pylori gastritis (Vinall et al., 2002). Two previous studies also found genetic variations in MUC1 that were associated with GC susceptibility (Jia et al., 2010; Xu et al., 2009). Regarding rs4072037, the MUC1 A/G polymorphism in exon 2 was reported to control alternative splicing of the 5'-exon 2 region in both full-length transcripts and those lacking the polymorphic tandem repeat domain (Ng et al., 2008). Subsequently, the polymorphism was observed to disrupt the physiological functions of MUC1, which is important for protection of the gastric mucosa. This implies that SNP rs4072037 is a potential genetic factor leading to increased susceptibility to GC by altering MUC1 and its expression in populations carrying the A allele (Xu et al., 2009).

We also found that SNP rs4460629 at 1q22 was significantly associated with the risk of GC (OR=0.593, *P*=0.002), consistent with previous data (Abnet et al., 2010). The genes nearest this SNP are keratinocyte associated protein 2 (KRTCAP2) and tripartite motif containing 46 (TRIM46). Tripartite motif-containing (TRIM) family proteins have many biological activities, including cell differentiation, apoptosis, transcriptional regulation, and signalling pathways. Many reports suggest that TRIMs play a crucial role in immunity by regulating signalling pathways, including the RIG-I pathway (McNab et al., 2011). Of course, an interaction between MUC1 and TRIM46 may affect susceptibility to GC. In a GWAS of a Japanese population (Sakamoto et al., 2008), the SNP rs2070873 in TRIM46 was also identified as being associated with GC. In addition, a study of a Chinese population reported that the SNPs rs4072037 in MUC1 and rs2075570 in TRIM46 were both located in an extended LD block involving a region about 724 kb in length (Chr1: 153372506…154096135) (Zhang et al., 2011). We also found that the SNPs rs4072037 and rs4460629 were linked (D=0.1316, D´=0.9469, r=0.9039).

Regarding 10q23, Zhang et al confirmed that 10q23, tagged by rs2274223, increased susceptibility to GC in an eastern Chinses population, Nanjing (Zhang et al., 2011). But there no significant result for the SNPs at 10q23 was found in our study. SNP rs2274223 is localised to exon 26 of PLCE1, a member of the phospholipase C protein family that catalyses the hydrolysis of phosphatidylinositol-4, 5-bisphosphate to generate two intracellular signalling molecules: inositol 1,4,5-triphosphate (IP3) and

diacylglycerol (DAG). This enzyme interacts with the proto-oncogene ras (Bunney et al., 2009); participates in the regulation of cell growth, differentiation, apoptosis, and angiogenesis in the skin (Bai et al., 2004); and is involved in intestinal carcinogenesis (Bourguignon et al., 2006). We found no significant association with the SNPs at 10q23, which suggests a genetic difference between this Chinese population and others. Our data also suggest that the mutations on 10q23 perhaps have a smaller role in the genetic susceptibility to GC compared to 1q22.

In summary, we have provided an independent replication of the findings from the genome-wide association study in GC and ESCC. Our data show that 1q22 was a susceptibility-associated region for GC in the southern Chinese population. However, the association of 10q23 with GC was not confirmed. Considering the diverse SNP distributions identified in different populations, additional studies of causal variants of 1q22 and 10q23 and GC susceptibility as well as mechanistic studies are warranted.

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