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Genetic variants in Fas signaling pathway genes and risk of gastric cancer

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Abstract

Populations in north central China are at high risk for gastric cancers (GC), and altered FAS-mediated cell signaling and/or apoptosis may contribute to this risk. We examined the association of 554 single nucleotide polymorphisms (SNPs) in 53 Fas signaling-related genes using a pathway-based approach in 1758 GC cases (1120 gastric cardia adenocarcinomas (GCA) and 632 gastric noncardia adenocarcinomas (GNCA)), and 2111 controls from a genome-wide association study (GWAS) of GC in ethnic Chinese. SNP associations with risk of overall GC, GCA and GNCA were evaluated using unconditional logistic regressions controlling for age, sex and study. Gene- and pathway-based associations were tested using the adaptive rank-truncated product (ARTP) method. Statistical significance was evaluated empirically by permutation. Significant pathway-based associations were observed for Fas signaling with risk of overall GC (P = 5.5E-04) and GCA (P = 6.3E-03), but not GNCA (P = 8.1E-02). Among examined genes in the Fas

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signaling pathway, MAP2K4, FAF1, MAPK8, CASP10, CASP8, CFLAR, MAP2K1, CAP8AP2, PAK2 and *IKBKB* were associated with risk of GC (nominal $P < 0.05$), and *FAF1* and *MAPK8* were significantly associated with risk of both GCA and GNCA (nominal $P < 0.05$). Our examination of genetic variation in the Fas signaling pathway is consistent with an association of altered Fas signaling and/or apoptosis with risk of GC. As one of the first attempts to investigate a pathway-level association, our results suggest that these genes and the Fas signaling pathway warrant further evaluation in relation to GC risk in other populations.

Keywords

Gastric cancer; gastric cardia; gastric noncardia; Fas signaling; genetic variants; GWAS; single nucleotide polymorphisms; pathway genes

INTRODUCTION

Gastric carcinoma (GC) is the fourth most common malignancy worldwide with an estimated incidence of 934,000 new cases per year. [1, 2] Furthermore, this incidence is geographically varied with more than 42% of GC patients occurring in China alone. [3] Globally, approximately 738,000 patients with GC die annually making GC the second most common cause of cancer-related deaths. [2, 4] This cancer also continues to have very poor survival, primarily because most patients present with advanced disease and treatment options are limited. [5, 6]

Populations from the Shanxi Province and Linxian in north central China are at very high risk for GC including gastric cardia adenocarcinoma (GCA) that arises in the top 3cm of the stomach, and gastric noncardia adenocarcinoma (GNCA), that arises more distally in the stomach. Previous studies have reported several risk factors associated with higher risk of GC in these populations including age, male gender, *Helicobacter pylori* (*H. pylori*) infection, [7] consumption of salted and nitrated foods, low levels of antioxidants, low consumption of fresh fruit, vegetables and eggs, [4, 8-10] tooth loss, [11] and thermal damage due to consumption of scalding hot foods. [4] In contrast, smoking and alcohol are not major risk factors. [4, 10]

In addition to environmental risk factors, data on family history of GC and genome-wide association studies [12-14] in these high risk populations suggest the importance of genetic susceptibility. To date, five susceptibility loci at 1q22, 3q13, 5p13, 19q23 and 20p12 have reached genome-wide significance in scans conducted in Han Chinese; specifically three loci have been associated with risk of GCA and two with GNCA. [12-15] Pathway-based analysis of genome-wide association study (GWAS) data is a complementary approach to identify pathways or groups of genes enriched with cancer associated SNPs whose individual effect sizes may be too small to be detected by standard methods.

The ability to avoid apoptosis and ensure continued proliferation and survival of premalignant and early tumor cells is likely to be an early and important event facilitating the development of cancer. Fas is a death domain-containing member of the TNFR (Tumor Necrosis Factor Receptor) superfamily and it has a central role in the physiological

regulation of apoptosis. Although activated Fas (FasL-Fas system) has been appreciated mainly with respect to its death-inducing function, which is mediated via proteolytic enzymes called 'caspases' (CASP).[16] Fas signaling may also transduce proliferative and activating signals, through nuclear factor-kappaB (NF-kB) activation and other mechanisms.[17] In mice, during early infection with *H.pylori*, Fas-mediated apoptosis depletes parietal and chief cell populations, leading to architectural distortion. Thus, the deregulation of FAS signaling may be an early and necessary trait for GC development and also important for *H.pylori* infection.[17, 18]

Genetic variation may alter the expression or activity of proteins in the FAS signaling pathway, potentially altering cell proliferation, apoptosis, and survival, and thus susceptibility to GC. Therefore, we evaluated 53 candidate genes associated with FAS signaling including genes downstream of Fas, initiator caspases and signal transduction effectors using ad hoc analysis of the first phase of a genome-wide association study (GWAS) of gastric cancer conducted in a high risk Chinese population. We present data here suggesting that overall Fas signaling and specific genes contained therein may be important for GC development and type of GC in high risk Chinese individuals.

METHODS & ANALYSES

Study Population

This study reports a further statistical analysis of the first phase of a genome-wide association study of GC conducted in ethnic Chinese, full details of which have been described elsewhere.[13] Briefly, participants for were drawn from two studies, the Shanxi Upper Gastrointestinal Cancer Genetics Project (Shanxi) and the Linxian Nutrition Intervention Trial (NIT), a prospective cohort. The Shanxi study controls were individually matched on age and sex for the case-control portion, whereas the NIT controls were selected as a case-cohort and frequency matched on age and sex. For the Shanxi and NIT studies, tumor anatomic location (cardia and noncardia) was known for all cases and >85% of cases had pathological confirmation. All GCAs were located in the proximal 5 cm of the stomach. Risk factor information for Shanxi and NIT were obtained by interview. The NCI Special Studies Institutional Review Board approved the overall GWAS.

Gene and SNP Selection for Fas Signaling Pathway

An inherent limitation of pre-processed pathway databases is the subjective interpretation of the curator. Therefore, to obtain as comprehensive a pathway as possible at the time of this study, genes associated with Fas signaling (Fas receptor and ligand, effector caspases, and downstream effectors, collectively referred to here as Fas signaling pathway genes) were identified *apriori* from the literature [16-28] and cross-referenced with the Biocarta fas signaling pathway (cd95) database (BioCarta_pid_faspathway and http://cgap.nci.nih.gov/Pathways/BioCarta/h_fasPathway) to confirm pathway information. Using this approach we identified 53 genes containing 668 unique SNPs from the GWAS. The 53 genes examined in this study are listed in Table 2.

Genotyping, Quality Control, and Exclusions

DNAs were genotyped as part of the GWAS at the Core Genotyping Facility of the National Cancer Institute's Division of Cancer Epidemiology and Genetics as previously described [13]. Data is available upon request from the NIH Data Access Committee (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000361.v1.p1). An overall subject completion rate of 85% was applied to cases and controls in the combined population for all assays analyzed. We excluded SNPs with <95% completion and <95% concordance, and a minor allele frequency (MAF) <1%. After exclusion criteria were applied, 550 unique SNPs in 53 FAS signaling pathway genes remained for analysis in GC (Supplementary Table 1); 548 SNPs for GCA, and 540 for GNCA (Supplementary Tables 3-4). Linkage disequilibrium (LD) in the combined data was further computed between any two SNPs in the same gene among the combined controls using Haploview (http://www.broad.mit.edu/mpg/haploview/).

Statistical Analyses

To investigate variation in Fas signaling pathway genes and risk of GC in the GWAS data, we carried out individual SNP-, gene- and pathway-based analyses for GCA and GNCA subtypes as well as GC overall. SNP-based analyses of each individual study as well as the combined population were tested under the additive model, and odds ratios and 95% confidence intervals were calculated using unconditional logistic regression with adjustment for age (10 year categories), sex and study in primary models. For some SNPs we used a dominant model because of the low frequency of the homozygous genotype in our population. In secondary models we also adjusted for alcohol, smoking, *H.pylori* and family history of UGI cancer

All *P*-values for SNPs are nominal except where otherwise specified. SNP-based analyses were performed using STATA version 9.0 and program language R (http://www.r-project.org/). After excluding SNPs with pairwise LD $r^2>0.80$ in controls, a Bonferroni-corrected threshold of $P < 1.44E-04$ was calculated using 345 independent SNP signals.

We conducted a gene-based analysis to evaluate the association between a candidate gene/region and cancer risk. The test statistic used was the minP statistic that was the minimum *P*-value among all *P*-values from the single SNP analysis conducted within the candidate gene. The *P*-value for the gene-based analysis (called gene *P* value) can be evaluated through a bootstrap procedure.[29] Lastly, we conducted pathway analysis to evaluate the association between the candidate genes included in the Fas signaling pathway and cancer risk. The pathway analysis was based on the ARTP method and was implemented in the R package ARTP (http://dceg.cancer.gov/bb/tools/artp). The ARTP method aims at maximizing the association signal by combining gene-level *P*-values from a set of selected genes within the pathway into the test statistic and uses a bootstrap procedure to estimate its *P*-value, and has been shown to account properly for the type I error.[29] The bootstrap procedure is used for the purpose of generating datasets under the null hypothesis while keeping the correlation among SNPs the same as that in the observed dataset. The *P*-value for both the gene-based and pathway analyses was estimated by 20,000 parametric bootstrap

steps. We also considered a more stringent Bonferroni-corrected significance threshold for gene-based analysis to account for testing 53 genes (P=9.43×10^{-4}, 0.05/53 genes).

RESULTS

Population Characteristics

In the present study we analyzed genotype data from 1,758 GC cases and 2,111 controls. Detailed characteristics and risk factors for GC in both NIT and Shanxi samples have been previously reported. [4, 11] A summary of demographic, risk factor, and anatomical site information for each individual study and the combined study population is shown in Table 1. In the combined population, cases were more likely to be male, drink alcohol, smoke, and have a family history of UGI cancer compared to controls. The mean age for cases of GCA, GNCA, and GC overall was higher in Shanxi compared to NIT, the proportion of male GC cases was also greater in Shanxi compared to NIT. A higher percentage of participants from the Shanxi study were ever drinkers and smokers, while participants from the NIT study had a stronger family history of UGI cancer.

Fas Signaling Pathway and GC Risk

Pathway-based analysis for all 53 genes involved in Fas signaling was significantly associated with risk of GC (P = 5.5E-04) (Table 2).

Gene-based analyses identified ten genes associated with overall risk of GC (ARTP P<0.05) (Table 2) including *MAP2K4* (P = 0.0038), FAF1 (P = 0.0033), MAPK8 (P = 0.0041), *CASP10* (P=0.011), *CASP8* (P= 0.012), *CFLAR* (P = 0.015), *MAP2K1* (P= 0.0185), *CASP8AP2* (P = 0.02), *PAK2* (P = 0.0476) and *FKBP* (P= 0.048). P values for the remaining 43 FAS signaling pathway genes and their most significant SNPs are shown in Table 2 and Supplementary Table 2. However, these genes did not remain significant after Bonferroni correction for multiple comparisons.

Seventy SNPs in introns and/or non-coding gene regions across 24 Fas signaling pathway genes (including: ARHGDIB, BID, CASP6, CASP7, CASP8, CASP8AP2, CASP10, CFLAR, CRADD, DFFB, FAF1, IKBKB, MAP2K1, MAP2K4, MAP3K5, MAPK8, NFKB2, PAK1, PAK2, PARP1, PRKDC, RAF1, RB1, and UBE2I) were significantly associated (P<0.05) with risk of GC in the combined population (Supplementary Table 1). The effect size and direction of SNPs were similar in both individual studies (Supplementary Table 1). After accounting for LD (r^2 ≥0.80), the 70 significant SNPs were shown to represent 34 independent or separate signals. We identified two SNPs in *MAP2K4* and four SNPs in *FAF1* that were significant at the P < 0.001 level. *MAP2K4* rs9788973 (T allele) (per allele OR: 1.18, 95% CI: 1.08-1.29, P=0.0003) was shown to be in strong LD (r^2≥ 0.95) with rs7216812 (C allele), which was also associated (P = 0.0005) with increased risk of GC cancer. *FAF1* rs1846522 (A allele), rs7543272 (C allele), rs12089041 (T allele), and rs3789587 (T allele) were significantly associated with reduced risk of GC (Supplementary Table 1). Strong LD r^2 =0.96 was observed between both *FAF1* rs1846522 and rs12089041, and rs1846522 and rs3789587, respectively. However, no individual SNP remained significant after Bonferroni correction for multiple comparisons.

Further adjustment for smoking, alcohol, and family history of UGI cancer did not alter these results (data not shown). *H.pylori* serology data were available only for NIT study participants, however, *H.pylori* seropositivity was essentially universal, which precluded a meaningful evaluation of the results.

Fas Signaling Pathway and Risk of GCA and GNCA

Genetic variation in the FAS signaling pathway was significantly associated with risk of GCA (P = 6.3E-03), but not GNCA (P = 8.0E-02) in our high risk population (Table 3). Gene-based analyses identified some shared susceptibility loci for both GCA and GNCA. *FAF1* and *MAPK8* were significantly associated with risk of both GCA (P = 0.0265 and 0.0412, respectively) and GNCA (P = 0.0436 and 0.0017, respectively) (Table 3). A number of potential cancer-specific loci were also identified between GCA and GNCA. *CASP8, CASP10, CFLAR, and MAP2K1* were significantly associated with risk of GCA only (P < 0.02), while *MAP2K4* and *IKBKB* were only significantly associated with GNCA (P < 0.05) (Table 3). However no SNP remained significant after correction for multiple comparisons. The most significant SNP in each of the 55 genes in the FAS signaling pathway for GCA and GNCA is shown in Supplementary Tables 3 and 4, respectively.

DISCUSSION

We evaluated the impact of genetic variation in the overall Fas signaling pathway with risk of GC using an ad hoc analysis of the first phase of a genome-wide association study (GWAS) of gastric cancer performed in a high risk Chinese population. The genes examined in this pathway encode proteins involved in FAS receptor-ligand binding, initiator and effector caspases, signaling and downstream regulatory and structural proteins.

When all 55 candidate Fas signaling genes were considered, we observed a significant pathway-based association with overall GC risk (P = 3.5E-04) and GCA risk (P = 6.3E-03), but not GNCA risk (P = 8.0E-02). Furthermore, we found evidence that genetic variation in ten individual genes significantly contributed to overall GC risk in this population. In particular, *FAF1* and *MAPK8* were significantly associated with both GCA and GNCA risk; *CASP10, CASP8, CFLAR and MAP2K1* were significantly associated with risk of GCA; and *MAP2K4* and *IKBKB* were significantly associated with GNCA. Polymorphisms in these genes have been previously examined for risk association in a number of cancers in both Chinese and Caucasian populations (summary presented in Supplementary Table 5). However, with the exception of *IKBKB* rs5029748,[30] which was associated with a reduced risk of GC (per allele OR: 0.90; 95%CI: 0.81-0.98) and GNCA (per allele OR: 0.86; 95%CI: 0.75-0.97) in our study; we failed to replicate any of these previously-reported observations.

The lack of a pathway-based association for the Fas signaling genes with GNCA may reflect the smaller number of GNCA cases (n = 632) genotyped in this study population. Alternatively, this result may reflect differences in Fas signaling (apoptosis *vs.* proliferation) in the development of the GC subtypes in our high-risk Chinese population. In support of this proposal, Boroumand-Noughabi and colleagues [31] found a significantly higher serum level of soluble FasL in Iranian patients with GNCA versus those with GCA (P = 0.005), suggesting difference in the efficacy of apoptosis in different gastric subtype tumors and/or

patient immune response to the subtypes. Also, other data suggests that GCA is distinguished from GNCA by differences in risk factors, [32] tumor characteristics, [33] patterns of mRNA profiling and protein expression [34, 35] and genetic alterations. [36] As well as being anatomically adjacent, GCA and esophageal squamous cell carcinoma(ESCC) occur at epidemic rates in this study population, share some etiological risk factors as well a GWAS risk variant in the *PLCE1* gene. [13] We recently profiled gene expression levels in matched tissues from patients with GCA (n=41) and GNCA (n= 94) from this high-risk population. [37] In agreement with previous studies we found a number of genes that were differentially expressed in GCA, but not GNCA, and vice versa. Added to this, differentially expressed genes reported in GCA were also dysregulated in a similar pattern in ESCC patients from this same population.[37] Collectively, this data may suggest etiological differences in the gastric carcinogenesis pathway, and in the exposures important for the development of GCA or GNCA in this high risk population. Differential roles for Fas signaling or specifically Fas-mediated apoptosis or proliferation may also be important in these gastric tumor subgroups. However further studies are required to clarify the role of Fas-signaling in gastric carcinogenesis in cardiac versus non-cardiac tumors.

The strongest gene-based association observed for overall risk of GC ($P = 0.0038$) as well as risk of GNCA ($P = 0.0127$) in our study population was observed for *MAP2K4*, with a marginal non-significant association ($P = 0.0529$) for GCA. *MAP2K1* was also significantly associated with risk of GC ($P = 0.0185$) and GCA ($P = 0.0283$) in our population, while *MAPK8* was associated with GCA ($P = 0.0436$), GNCA ($P = 0.0077$), and GC risk overall ($P = 0.0041$). *MAP kinase (MAPK)*-related gene products frequently integrate signaling outputs of different signal transduction circuits including Fas-mediated apoptosis in a cell.[38-41] *MAP2K4*, which encodes a map kinase kinase of JNK (JNKK1) and p38, is classically associated with growth arrest and apoptosis in cells and has been reported to be a metastasis suppressor involved in multiple cancer types. [38, 39] *MAP2K1* encodes MEK1, which functions in the MAPK/ERK cascade. MEK1 can target peroxisome proliferator-activated receptor gamma (PPARG), a nuclear receptor that promotes differentiation and apoptosis, while activation of MEK1 in Jurkat T lymphocytes attenuates Fas-mediated apoptosis. [39] *MAPK8* encodes the c_JUN N-terminal protein kinase JNK1, which is activated by JNKK1 (or the *MAP2K4* product) and regulates the activity of c-Jun and c-Myc as well as the proapoptotic Bcl-2 family protein. [41] In addition, exonic genetic variation in MAPK has been observed in a majority of GC cell lines. [42]

The second strongest gene-based association observed with overall GC risk was for *FAF1* ($P = 0.0039$) an interaction partner of Fas, which was also significantly associated with risk of both GCA ($P = 0.0265$) and GNCA ($P = 0.0412$) in our population. Initially postulated to be a tumor suppressor, [22] FAF1 have functions in several biological processes including Fas-induced apoptosis, NF-κB signaling, ubiquitination, proteasomal degradation, canonical Wnt signaling and neuronal cell survival. [22, 43-45] we identified thirteen significant *FAF1* SNPs ($P<0.05$) in strong LD (mean max $r^2 = 0.96$), representing three independent signals associated with reduced risk of GC. Given that FAF1 protein is an important mediator of apoptosis, it is plausible that one or more of these SNPs could alter expression of FAF1 or modify protein interactions that might alter apoptosis. Also, reduced FAF1 protein has been reported in a high percentage of human gastric carcinomas, most prominantly in carcinomas

containing signet ring cells. [46] A significant decrease in FAF1 mRNA expression was observed for Caucasian patients with cleft palate who were homozygous for the major T allele (TT genotype) for rs3827730 (P=0.0015). [47] Although rs3827730 was not significant after correcting for multiple testing comparisons, the T allele of *FAF1* rs3827730 was significantly associated with reduced risk of GC (per allele OR, 0.89, 95% CI, 0.80-0.99, P=0.026) and GCA (per allele OR: 0.88; 95% CI: 0.77-0.99; P = 0.039), but not GNCA, in the present study.

We also observed gene-based associations for *CFLAR* (P = 0.015), *CASP10* (P= 0.011), and *CASP8* (P= 0.013), which cluster on chromosome 2q32-q33, with overall risk of GC in our population. Furthermore, these genes were significantly associated with risk of GCA (*CFLAR* P= 0.020, *CASP10*, P = 0.015 and *CASP8*, P = 0.004), but not GNCA. CFLAR, CASP10 and CASP8 proteins regulate the extrinsic apoptosis pathway. *CFLAR*, which encodes the cellular FLICE-like inhibitory protein or c-FLIP, acts as an inhibitor of Fas-mediated apoptosis, [16, 17] and while bound to RIP2 can also mediate activation of NF-κB and/or non-apoptotic signals including cell proliferation. Both *CASP8* and *CASP10* are highly expressed (even co-expressed) in gastric adenocarcinomas, irrespective of histological subtypes and depth of invasion. [48] *CFLAR* mRNA and c-FLIP protein are also frequently elevated in gastric adenocarcinomas of Chinese patients. [49] Using a meta-analysis of GWAS data from the study populations evaluated here and other population of Chinese ethnicity, we recently reported a strong association of five SNPs which map to 2q33 and the *CASP8/ALS2CR12/TRAK2* gene region with risk of esophageal squamous cell carcinoma (ESCC). [50] However, neither *CASP8* rs10931936 (P = 0.8), which was included in the current study, nor the four remaining variants were shown to be associated with risk of GC in this population (data not shown), suggesting the latter association may be specific for ESCC.

IKBKB encodes a catalytically active-protein called IkappaB-kinase (IKKB) that is responsible (as part of a larger complex including Ikappa-kinase (IKKA)) for the dissociation of the inhibitor of NF-κB and its subsequent activation. [51] In this study, we observed a significant gene-based association for *IKBKB* with risk of GC (P=0.048) and GNCA (P = 0.048), but not GCA. *CHUK*, which encodes IKKA was not associated with risk of GC. *IKBKB* rs6029748 was identified as the most significant SNP in *IKBKB* in our study, and was associated with protection against GNCA (per allele OR: 0.86; 95%CI: 0.75-0.97, P= 0.018) as well as GC overall (per allele OR: 0.90; 95%CI: 0.82-0.98, P = 0.018). IKKB represents a key protein in the regulation of apoptosis in epithelial cells as well as in the reponse of gastrointestinal mucosa to external stimuli. [51] While the effects of loss of *IKBKB* on cancer risk appears to be tissue-specific, conditional knockout of *IKBKB* in the normal gastric epithelium of mice showed decreased mRNA expression of *CFLAR*, accelerated *Helicobacter*-dependent gastric apoptosis, proliferation, and the development of dysplasia. [51] However, little is known about the biological relevance of genetic variation in *IKBKB* and how this might influence the activity or protein interactions, as well as downstream NF-κB/IKKB-related processes such as apoptosis and inflammation.

Lastly, significant gene-based associations were observed for *PAK2* and *CASP8AP2* with risk of GC (P = 0.048 and P = 0.020, respectively), but not with risk of GCA or GNCA per

se, a result which may reflect limited power. *PAK2* encodes a Group 1 serine/threonine protein kinase (also called PAK2) and is the only member of the PAK family that is directly activated by CASP3, resulting in the morphological and biochemical changes of apoptosis. [52] *CASP8AP2* encodes a pro-apoptotic protein called FLICE-Associated Huge (FLASH) that acts as a downstream mediator (together with FAF-1) in the activation of CASP8 in Fas-mediated apoptosis and NF-κB activation. [53] Limited evidence indicates that somatic mutations in *CASP8AP2* are rare in gastric carcinomas, but increased expression of FLASH has been detected in 70% of gastric carcinoma tissues compared to normal mucosa, suggesting that FLASH may play an important role in gastric carcinogenesis. [53]

In our study population, cases were more likely to use tobacco and to drink alcohol than controls, however these exposures are not major risk factors for GC in our Chinese populations [4, 10] and neither smoking nor alcohol drinking confounded our genotypic findings. This study had several strengths and several limitations. Our examination of a large number of SNPs associated with Fas signaling is a strength, in addition to our comprehensive assessment of both gene- and overall pathway associations. Examination of many SNPs does, however, create a concern over multiple testing. The large number of cases studied also allowed us to assess all of these risks with reasonable power. Despite the large size of our study, further studies are needed to replicate these findings. Another limitation of this study is that we were not able to examine SNP associations by *H.pylori* (*Hp*) status. Infection with *H. pylori* is prevalent in this high-risk region of north central China, presumably due to undeveloped living conditions. [54] Thus, a very high prevalence of *Hp*-positive status in both cases and controls in this study limited our ability to evaluate this pathway in *Hp*-negative subjects. Finally, the generalizability of our findings to other ethnic populations remains to be determined.

In conclusion, our evidence suggests an important role for genetic variation in the Fas signaling pathway on risk of GC, and in particular GCA, in this high-risk Chinese population. This association appears to be driven mainly by genetic variation in MAP2K4, FAF1, MAPK8, CASP10, CASP8, CFLAR, MAP2K1, CASP8AP2, PAK2 and *IKBKB* genes. Polymorphisms in these genes may result in altered expression, signaling, and/or interactions with other proteins that lead to changes in the apoptotic/proliferation phenotype and thus GC risk. Further investigation into the association of this pathway with risk of GC is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NOVELTY & IMPACT

Although the incidence of gastric cancer (GC) is declining globally, it remains the 2nd leading cause of cancer death worldwide, and it has a poor prognosis. *Helicobacter pylori* is acknowledged as the primary risk factor for GC. Evidence from genome-wide associations and other studies suggests genetics plays a role in the etiology of GC, particularly in high risk regions of the world such as China. Also, the deregulation of Fas signaling is a likely early and necessary alteration in the development of GC. Here we report a further analysis of data from a GC genome-wide association study conducted in ethnic Chinese. Specifically, we investigated the etiologic role of 53 genes in the Fas signaling pathway through a comprehensive evaluation of pathway-, gene- and SNP-based associations with GC, including both cardia and noncardia subsites. Results suggest an important role for genetic variation in the Fas signaling pathway on risk of GC, particularly cardia, in this high risk Chinese population. The identification of predisposing genetic factors associated with development of GC may ultimately lead to improved prognostic and therapeutic strategies.

Table 1

Population characteristics in combined and individual Chinese population GWAS studies

	Combined				Shanxi				NIT			
Characteristic	**GNCA**	**GCA**	**Total GC**	**controls**	**GNCA**	**GCA**	**Total GC**	**controls**	**GCNA**	**GCA**	**Total GC**	**controls**
Total, n	**632**	**1126**	**1758**	**2111**	**531**	**864**	**1395**	**1660**	**101**	**262**	**363**	**451**
Male, n (%)	458 (72.5%)	885 (78.5%)	1342 (76.3%)	1434 (67.9%)	400 (75.3%)	731 (84.6%)	1131 (81.1%)	1226 (73.9%)	58 (57.4%)	153 (58.4%)	211 (58.1%)	208 (46.1%)
Age (SD)	54.6 (10)	57.2 (9.0)	56.3 (9.5)	55.98 (9.5)	55.4 (10.4)	59.3 (8.4)	57.8 (9.4)	57.8 (9.2)	50.6 (6.5)	51.3 (7.1)	50.4 (7.0)	49.5 (7.3)
Alcohol, Yes, n (%)	122 (19.3%)	210 (18.7%)	332 (18.9%)	298 (14.11%)	120 (22.6%)	203 (23.5%)	323 (23.2%)	287 (17.3%)	21 (21.9%)	8 (3.1%)	10 (2.8%)	13 (2.4%)
Smoking, Yes, n (%)	397 (62.8%)	735 (45.3%)	1132 (64.4%)	1218 (57.7%)	357 (67.2%)	628 (72.7%)	990 (70.9%)	1076 (64.8%)	36 (35.6%)	109 (41.6%)	146 (40.3%)	142 (31.5%)
Family history UGI, Yes, n (%)	130 (20.6%)	296 (26.2%)	426 (24.2%)	478 (22.6%)	96 (18.1%)	204 (23.7%)	298 (21.4%)	338 (20.4%)	36 (35.6%)	93 (35.5%)	129 (35.5%)	140 (31.0%)

Abbreviations: GC, gastric cancer; GCA, gastric cardia adenocarcinoma; GCNA, gastric non-cardia adenocarcinoma; UGI, upper gastrointestinal; SD, standard deviation; Shanxi, Shanxi Upper Gastrointestinal Cancer Genetics Project; NIT, Linxian General Population Nutrition Intervention Trial.

Table 2

Pathway-, gene- and most significant SNP-based *P*-values for Fas-signaling pathway genes and risk of GC in China

Pathway p^*	Gene Abb[tn]	Gene name	Cytogenetic Locus	Gene p^g P^g	No. of SNPs	Most Significant SNP	Associated SNP P^w
0.00055	*MAP2K4*	*Mitogen-activated protein kinase kinase 4*	17p11.2	0.0038	16	rs97889 3	0.000 5
	FAF1	*FAS (TNFRSF6) associated factor 1*	10q24.1	0.0039	28	rs18465 2	0.0003
	MAPK8	*Mitogen-activated protein kinase 8*	10q11.22	0.0041	5	10508902	0.0015
	CASP10	*Caspase 10, apoptosis-related cysteine peptidase*	2q33 q34	0.0 0	5	rs1 0085 7	0.0038
	CASP8	*Caspase 8, apoptosis-related cysteine peptidase*	2 33-q34	0.013	9	rs376 82	0.00247
	CFLAR	*Capase 8 and FADD-like apoptosis regulato*	2q3 q34	0.0149	7	rs1 2008	0.00382
	MAP2K1	*Mitogen-activated protein kinase kinase*	5q22 1-22.3	0.0185	9	rs12050 32	0.00430
	CASP8AP2	*Caspase 8 associated protein 2*	6 15	0.0200	8	rs11967579	0.00409
	PAK2	*p21 protein (Cdc42/Rac)-activated kinase 2*	3q 9	0.0476	11	rs6583176	0.00877
	IKBKB	*Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta*	8p11.2	0.0480	6	rs5029748	0.0182
	PARP1	*Poly (ADP-ribose) polymerase 1*	1q41-q42	0.0650	13	rs1805410	0.0 863
	UBE2I	*Ubiquitin-conjugating enzyme E2I*	16p13.3	0.065	4	rs8063	0.027 73
	PAK1	*p21 protein (Cdc42/Rac)-activated kinase 1*	11q13-q14	0.0 08	11	2725830	0.0174
	NFKB2	*Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2*	10q24	0.1 21	3	rs 0568 0	0.0 716
	RB1	*Retinoblastoma 1*	13q14.2	0.112	3	990814	0.03271
	PRKDC	*Protein kinase, DNA-activated, catalytic polypeptide*	q11	0.1 63	11	rs2213178	0.01744
	RAF1	*V-raf-1 murine leukemia viral oncogene homolog 1*	3p25	0.12 5	18	rs904453	0.02647
	DFFB	*DNA fragmentation factor, 40kD, beta polypeptide*	p36 3	0.1334	12	rs10797348	0.01571
	CASP6	*Caspase 6, apoptosis-related cysteine peptidase*	4q25	0.1466	7	rs3181187	0.03006
	CASP2	*Caspase 2, apoptosis-related cysteine peptidase*	7q34-q35	0.1663	3	rs10500136	0.07204
	TRAF1	*TNF receptor-associated factor 1*	9q33-q34	0.1988	5	rsl0985097	0.07332
	TRAF2	*TNF receptor-associated factor 2*	9q34	0.2183	7	rs7019752	0.09501
	ARHGDIB	*Rho GDP dissociation inhibitor (GDI) beta*	12pl2.3	0.2253	19	rsl0505784	0.02073
	MAP3K5	*Mitogen-activated protein kinase kinase kinase 5*	6q22.33	0.2931	33	rs9402838	0.01781
	CASP7	*Caspase 7, apoptosis-related cysteine peptidase*	10q7	0.3112	19	rslll96449	0.03379

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Pathway p^*	Gene Abb[tn]	Gene name	Cytogenetic Locus	Gene p^g P^g	No. of SNPs	Most Significant SNP	Associated SNP P^n
	BID	*BH3 interacting domain death agonist*	22qll.1	0.3137	17	rs382012	0.02841
	MAP3K1	*Mitogen-activated protein kinase kinase kinase 1*	5qll.2	0.3242	17	rs832585	0.05083
	MAP3K14	*Mitogen-activated protein kinase kinase kinase 14*	17q21	0.3411	10	rs7222752	0.06109
	APAF1	*Apoptotic peptidase activating factor 1*	12q23.1	0.3806	16	rs2288714	0.07195
	DIABLO	*Diablo, IAP-binding mitochondrial protein*	2q24.31	0.4121	2	rs12870	0.23107
	LMNB2	*Lamin B2*	19pl3.3	0.4436	6	rs3729525	0.14384
	PTPN13	*Protein tyrosine phosphatase, non-receptor type 13*	4q21.3	0.4500	22	rs998902	0.08045
	CASP9	*Caspase 9, apoptosis-related cysteine peptidase*	1p36.21	0.4541	9	rs2042370	0.14994
	CASP3	*Caspase 3, apoptosis-related cysteine peptidase*	4q34	0.4695	2	rs2720376	0.27082
	BIRC3	*Baculoviral IAP repeat containing 3*	11q22	0.5177	2	rs2846848	0.33858
	BIRC5	*Baculoviral IAP repeat containing 5*	17q25	0.5409	11	rs1042241	0.11041
	Fas	*Fas (TNFRSF6) associated via death domain*	10q24.1	0.5511	21	rs2276524	0.10180
	BIRC2	*Baculoviral IAP repeat containing 2*	11q22	0.5561	2	rs10895290	0.34886
	CRADD	*CASP2 and RIPK1 domain containing adaptor with death domain*	12q21.33-q23.1	0.5777	54	rs1858606	0.09334
	LMNA	*Lamin A/C*	1q22	0.6312	4	rs915179	0.23595
	FADD	*Fas (TNFRSF6)-associated via death domain*	11q13.3	0.6400	4	rs4818[illegible]5	0.27200
	CYCS	*Cytochrome C*	7p15.3	0.6612	[illegible]	rs2857[illegible]8	0.28806
	RIPK2	*Receptor-interacting serine-threonine kinase 2*	8q21	0.6922	10	rs39765	0.19500
	SUM01	*SMT3 suppressor of mif two 3 homolog 1 (S. cerevisiae)*	2q33	0.7013	2	rs7599810	0.52176
	MAP3K3	*Mitogen activated protein kinase 3*	17p11.2	0.7641	3	rs8061772	0.43900
	DFFA	*DNA fragmentation factor, 45 Da, alpha polypeptide*	Ip36.3-p36.2	0.7834	5	rs2781233	0.34981
	LMNB1	*Lamin B1*	5q23.2	0.7837	16	rs3828699	0.24698
	CHUK	*Conserved helix-loop-helix ubiquitous kinase*	10q24-q25	0.8072	5	rs7073610	0.36423
	NFKB1	*nuclear factor of kappa light polypeptide gene enhancer in B-cells 1*	4q24	0.8118	15	rs3774937	0.22925
	JUN	*Jun proto-oncogene*	1p32-p31	0.8298	4	rs2760494	0.38830
	FasLG	*Fas (TNFRSF6) ligand*	1q23	0.8412	7	rs2859228	0.30806
	SMPD2	*Sphingomyelin phosphodiesterase 2, neutral membrane*	6q21	0.8893	4	rs1476387	0.49245
	DAXX	*Death-domain associated protein*	6p21.3	0.9446	4	rs3130267	0.72723

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The pathway P-value (P^*) for all 53 genes is indicated. Gene-based *P*-values (P^g) are shown in order of lowest to highest *P*-value. The most significant SNP (nonminal *P*-value (P^n)) in each gene is indicated. Genes with $P^g < 0.05$, are highlighted in grey. Abbreviations (Abb^{th}): GC, gastric carcinoma; SNP, single nucleotide polymorphism.

Table 2

Pathway- and gene-based *P*-values for Fas signaling pathway genes and risk of GC, GCA and GNCA in China

GC				GCA			GNCA		
Pathway *p**	Gene Abbr	No. of SNPs	Gene *Pg*	Pathway *P**	No. of SNPs	Gene *Pg*	Pathway *P**	No. of SNPs	Gene *Pg*
0.00055	*MAP2K4*	16	0.0031	0.00634	16	0.0529	0.08054	16	0.0127
	FAF1	28	0.0039		28	0.0265		28	0.0412
	MAPK8	5	0.0041		5	0.0136		5	0.0077
	CASP10	5	0.0110		5	0.0151		5	0.1817
	CASP8	9	0.0130		9	0.0043		9	0.5739
	CFLAR	5	0.0149		5	0.0200		5	0.3181
	MAP2K1	9	0.0185		9	0.0203		9	0.4356
	CASP8AP2	8	0.0203		8	0.0719		8	0.1397
	PAK2	11	0.0476		11	0.1252		11	0.1777
	IKBKB	6	0.0480		6	0.2121		6	0.0478
	PARP1	13	0.0650		13	0.0471		13	0.2756
	UBE2I	4	0.0653		4	0.1868		4	0.0698
	PAK1	11	0.0909		11	0.1295		11	0.5396
	NFKB2	3	0.1021		3	0.1113		3	0.5882
	RB1	8	0.1129		8	0.2421		8	0.0871
	PRKDC	11	0.1163		11	0.0924		11	0.6748
	RAF1	18	0.1315		18	0.1114		18	0.5446
	DFFB	12	0.1334		12	0.0370		12	0.7200
	CASP6	7	0.1466		7	0.2131		7	0.0377
	CASP2	3	0.1603		3	0.2600		3	0.3542
	TRAF1	5	0.1988		5	0.0953		5	0.1052
	TRAF2	7	0.2183		7	0.1596		7	0.6689
	ARHGDIB	19	0.2253		19	0.1406		19	0.3283
	MAP3K5	33	0.2931		33	0.2215		33	0.5812
	CASP7	19	0.3112		19	0.5309		19	0.0542
	BID	17	0.3137		17	0.2084		17	0.7540
	MAP3K1	17	0.3212		17	0.3184		17	0.7026
	MAP3K14	10	0.3411		10	0.4773		10	0.1382
	APAF1	16	0.3866		16	0.3815		16	0.5208
	DIABLO	2	0.4121		2	0.5218		2	0.6363
	LMNB2	6	0.4365		6	0.7392		6	0.2731
	PTPN13	22	0.4500		20	0.3580		22	0.6403
	CASP9	9	0.4541		9	0.0542		9	0.8562
	CASP3	2	0.4695		2	0.2405		2	0.6957
	BIRC3	2	0.5177		2	0.5874		2	0.7775

GC				GCA			GNCA		
Pathway *P**	Gene Abb[tn]	No. of SNPs	Gene *Pg*	Pathway *P**	No. of SNPs	Gene *Pg*	Pathway *P**	No. of SNPs	Gene *Pg*
	BIRC5	11	0.5409		11	0.7644		11	0.4939
	Fas	22	0.5511		22	0.2186		19	0.8238
	BIRC2	2	0.5561		2	0.5361		2	0.8044
	CRADD	54	0.5707		54	0.1875		54	0.7499
	LMNA	4	0.6212		4	0.3230		4	0.0897
	FADD	4	0.6406		4	0.7635		4	0.6411
	CYCS	4	0.6612		4	0.5477		4	0.6315
	RIPK2	10	0.6922		10	0.8194		10	0.0497
	SUMO1	2	0.7013		2	0.8105		2	0.6782
	MAPK3	3	0.7641		3	0.8107		3	0.8729
	DFFA	5	0.7834		5	0.6841		5	0.1002
	LMNB1	16	0.7937		16	0.8502		16	0.0926
	CHUK	5	0.8072		5	0.9269		5	0.7569
	NFKB1	15	0.8118		15	0.7686		15	0.4729
	JUN	4	0.8298		4	0.7242		4	0.9575
	FasLG	7	0.8412		7	0.9368		7	0.5939
	SMPD2	4	0.8803		4	0.6006		4	0.9842
	DAXX	4	0.9446		4	0.8978		4	0.7273

Gene-based *P*-values (P^g) are shown in order of lowest to highest *P*-value for GC in the combined population. Pathway *P*-value (*P**) for all 53 genes in overall GC, GCA and GNCA are indicated. Genes with $P_g<0.05$ for GC are bolded. Color bars indicate genes commonly or differentially associated with risk of GCA and/or GNCA. Abbreviations (Abb^{tn}): GC, gastric cancer; GCA, gastric cardia adenocarcinoma; GNCA, gastric noncardia adenocarcinoma; SNP, single nucleotide polymorphism.