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Original Article

Associations of BNIP3 and DAPK1 gene polymorphisms with disease susceptibility, clinicopathologic features, anxiety, and depression in gastric cancer patients

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Abstract: The purpose of this study is to explore the associations of BNIP3 and DAPK1 polymorphisms with disease susceptibility, clinicopathologic characteristics, depression, and anxiety in gastric cancer (GC) patients. In this study, 150 GC patients and 100 healthy controls were recruited. 1000 Genomes database and Haploview 4.0 software were used to select tag SNPs. Improved multiplex ligase detection reaction was used for genotyping. Data were analyzed using Chi-square test (χ^2 test) and univariate and multivariate logistic regression. The results demonstrated that the rs10781582 of BNIP3 in the dominant model was associated with a reduced risk of GC in the younger group (P_{BH} = 0.015), and the minor allele G of rs1329600 at DAPK1 was associated with reduced risk of GC (P_{BH} = 0.018). In the stratified analysis, the rs3793742 and rs10781582 of BNIP3 in the dominant model were associated with gender and age of GC patients, respectively (rs3793742: P_{BH} = 0.033; rs10781582: P_{BH} = 0.030). The rs10781582 of BNIP3 in the dominant model was correlated with depression in GC patients (P_{BH} = 0.003). However, no association was found between BNIP3 and DAPK1 polymorphisms and differentiation degree, TNM stage, lymph node metastases, visceral metastasis, and anxiety. In summary, polymorphisms of BNIP3 and DAPK1 were associated with a protective effect against GC. So far, this is the first study to explore the association between BNIP3 and DAPK1 gene polymorphism and GC risk, which may provide new insight about biologic mechanisms of GC pathogenesis.

Keywords: BNIP3, DAPK1, polymorphism, gastric cancer

Introduction

GC (Gastric cancer) is the fifth most commonly diagnosed cancer and the third-leading cause of cancer death throughout the world [1]. It is a malignant tumor with high heterogeneity, which has different histologic and molecular subtypes [2]. Despite progress in surgery together with neoadjuvant chemotherapy and radiotherapy, patients with advanced GC still have a poor prognosis [3]. Over 70% of patients are in the advanced stage at diagnosis, with a median overall survival (OS) of approximately 11 months and five-year survival rate of about 25-30% [4-6]. The poor outcomes are mainly due to the failure of early diagnosis. Although we have a better understanding of environmental factors and epigenetics of GC, early detection and effective therapy remain a challenge [7]. For instance, many environmental factors (e.g., *H. pylori* infection, asbestos) have a profound effect on oncogenesis [8, 9], and associations between aberrant methylation of SFRP1, FAT4, and SOX11 genes and GC risk have been reported [10, 11]. The environmental factors and methylation of the above genes may provide evidence for early detection, but more efforts must be made to screen high-risk individuals and make early diagnosis to improve outcomes.

The occurrence of GC is a process induced by various factors. In addition to environmental and epigenetic factors, genetics may play a crucial part in the occurrence and development of GC [8, 9, 12]. Polymorphism, change

generated at the genetic level, refers to 2 or more discrete genotypes or alleles simultaneously or frequently occurring in the same population. Polymorphism occurs in non-coding regions and gene regions without significant regulatory functions with frequency ≥ 1%. However, mutation occurs in protein-coding regions or regulatory regions with frequency < 1%, leading to genetic differences between individuals, thus causing the presence of the same or different phenotypes. Polymorphisms have various forms: copy number variation (CNV), repeating patterns of DNA, and single nucleotide polymorphism (SNP) [13, 14]. SNP, a change in the position of a single base pair, is the most common form of polymorphism in the human genome, and exploration of SNPs is a crucial strategy to study the association between genes and diseases.

It is reported that susceptibility to GC may be associated with gene SNPs, especially in the genes encoding interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and one carbon metabolism methylenetetrahydrofolate reductase (MTHFR), which play a crucial part in regulating DNA methylation and epigenetics [15]. Polymorphisms of the above genes may be proven to be biomarkers for early diagnosis of GC, but more efforts are warranted to identify novel polymorphism markers. It is well-known that Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and Death-associated protein kinase (DAPK) are apoptosis-related genes. As far as we know, no study has explored the association of BNIP3 polymorphism with disease risk (including GC). By contrast, associations between polymorphisms of DAPK1 and susceptibility to various cancers (excluding GC) were found. For example, DAPK1 rs10124291, rs721936, rs3128477 SNPs were associated with lung cancer risk [16], and DAPK1 rs11141901, rs1041326, rs1045042 SNPs were correlated with susceptibility to breast cancer [17]. However, the association between BNIP3 and DAPK1 SNPs and GC risk has not yet been reported.

Although BNIP3 and DAPK1 are apoptosis-related genes, studies demonstrated that they were associated with various psychiatric disorders. It is reported that BNIP3 was related to anti-depressive effects [18] and served as an antistress factor in mice brain [19]. A previous study demonstrated that DAPK1 was associated with antidepressant-like effects [20]. Furthermore, there were associations between DAPK1 polymorphisms and Alzheimer's disease (AD) [21]. Inspired by these findings, we sought to determine the correlations between BNIP3 and DAPK1 polymorphisms and patients' depression and anxiety.

Based on previous studies, we hypothesized that BNIP3 and DAPK1 SNPs contribute to susceptibility of GC. This study is the first to explore the correlation between polymorphism of BNIP3 and DAPK1 and risk of GC. We further evaluated the associations of BNIP3 and DAPK1 SNPs with patients' clinicopathologic characteristics (age, sex, tumor differentiation, tumor stage, lymph node metastases, visceral metastasis, depression and anxiety).

Materials and methods

Subjects

In this study, 150 GC patients and 100 healthy controls were recruited to explore whether polymorphisms of BNIP3 and DAPK1 influenced GC susceptibility. The 150 GC patients, confirmed by histopathology and endoscopy, were recruited from the First Affiliated Hospital of Anhui Medical University, and the 100 controls, free of GC and other major diseases, were selected from those without a family history of GC. Controls were paired by age and gender with GC patients. All subjects were unrelated Han Chinese on a genetic level. All GC patients were asked to complete a questionnaire on the demographic characteristics (age, gender), clinical characteristics (tumor differentiation, WHO classification, TNM stage, tumor metastasis, cigarette smoking, and alcohol drinking). Informed consent was signed by all subjects. Furthermore, our study got approval from the ethical committee of Anhui Medical University.

Depression and anxiety assessment

In this investigation, we evaluated depression of GC patients with 24-item Hamilton Depression Rating Scale (HAMD) and anxiety with 14-item Hamilton Anxiety Rating Scale (HAMA). If HAMD score was ≥ 8, patients got a diagnosis of depression [22]. If HAMA score ≥ 7, patients got a diagnosis of anxiety [23]. Patients with

baseline score of HAMD < 8 were grouped into a non-depression group, whereas patients with baseline score of HAMD ≥ 8 were grouped into a with-depression group. Patients with baseline score of HAMA < 7 were grouped into without-anxiety group, whereas patients with baseline score of HAMA ≥ 7 were grouped into with-anxiety group.

Tag SNP selection and genotyping

The 1000 Genomes database and Haploview 4.0 software were used for the selection of tag SNP. All SNPs of BNIP3 and DAPK1 were screened by the 1000 Genomes database and tag SNPs were screened out by linkage disequilibrium (LD) chart of Haploview 4.0 software. The criteria for tag SNPs were r^2 greater than 0.8 and minor allele frequency (MAF) greater than 0.1, and tag SNPs within 5000 bp upstream of the gene were selected. Eventually, 2 tag SNPs (rs3793742, rs1078-1582) were selected from BNIP3 and 2 tag SNPs (rs1329600, rs7875475) were selected from DAPK1.

We extracted genomic DNA from all participates' peripheral blood utilizing the QIAGEN kit (QIAGEN, Hilden, Germany). The isolated genomic DNA was stored at -80°C before use. Improved multiplex ligase detection reaction (iMLDR) was applied to the genotyping of tag SNPs.

Statistical analysis

Normal and skewed distribution data were depicted as mean ± standard deviation (SD) and median (P_{25}-P_{75}), respectively. Differences of normal and skewed distribution data were compared by *t*-test and Mann-Whitney *U* test, respectively. Qualitative data were depicted as number (percentage) and compared by Chi-square (χ^2) test. χ^2 test was also applied to determine whether frequencies of genotypes conformed to Hardy-Weinberg equilibrium (HWE). We used dominant and recessive models to analyze the genotype distribution. Univariate logistic regression was applied to estimate crude odds ratios (ORs) and 95% confidence intervals (CIs). Multivariate logistic regression was used to correct for confounders (age, sex, cigarette smoking, and alcohol drinking). Error of the first kind and multiple comparisons problems were controlled by false discovery rate (FDR) and represented as Bonjamini-Hochberg *P* value (P_{BH}). Additionally, the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) was applied to haplotype analysis, and the frequency of haplotype was greater than or equal to 0.03. GC patients were grouped according to gender, mean age, differentiation degree, WHO classification, TNM stage, lymph node metastasis, visceral metastasis, depression and anxiety (male = 1, female = 2; ≤ mean = 1, > mean = 2; poorly-differentiated = 1, well/moderately differentiated = 2; poorly differentiated adenocarcinoma = 1, papillary adenocarcinoma and other types = 2; stage I-III = 1, stage IV = 2; patients with lymph node metastasis = 1, patients without lymph node metastasis = 2; patients with visceral metastasis = 1, patients without visceral metastasis = 2; patients without depression = 1, patients with depression = 2; patients without anxiety = 1, patients with anxiety = 2). $P < 0.05$ was considered significant.

Results

Baseline characteristics of cases and controls and HWE

150 GC patients and 100 controls were enrolled. Among patients, the mean age was 61.22 years and 114 (76.00%) cases were male. Among controls, the mean age was 60.09 years and 73 cases (73.00%) were male. The distributions of gender and age between patients and controls were not significantly different ($P > 0.05$). The number of GC patients with age ≤ mean and > mean was 71 (47.33%) and 79 (52.67%), those with tumor differentiation of moderate to well and poor was 114 (76%) and 36 (24.00%), and those with poorly differentiated adenocarcinoma and papillary adenocarcinoma and other types was 104 (69.33%) and 46 (30.67%). Patients with TNM stage of I-III and IV was 53 (35.33%) and 97 (64.67%), with lymph node metastases of positive and negative was 100 (66.67%) and 50 (33.33%), with visceral metastases of positive and negative was 54 (36.00%) and 96 (64.00%), with depression positive and negative was 46 (30.67%) and 104 (69.33%), with anxiety positive and negative was 93 (62.00%) and 57 (38.00%), respectively. **Table 1** displays the genotype frequencies of BNIP3 and DAPK1 gene polymorphisms and the results of

Table 1. Genotype frequencies of polymorphisms in BNIP3 and DAPK1 genes and results of Hardy-Weinberg equilibrium

Gene	SNP (minor allele)	Case group (n = 150)				Control group (n = 100)			
		Wild type	Heterozygous	Homozygous mutants	HWE *P*-value	Wild type	Heterozygous	Homozygous mutants	HWE *P*-value
BNIP3	rs3793742 (T)	59 (39.3)	61 (40.7)	30 (20.0)	0.058	34 (34.0)	51 (51.0)	15 (15.0)	0.561
	rs10781582 (A)	71 (47.3)	58 (38.7)	21 (14.0)	0.111	40 (40.0)	50 (50.0)	10 (10.0)	0.323
DAPK1	rs1329600 (G)	105 (70.0)	41 (27.3)	4 (2.7)	0.999	72 (72.0)	27 (27.0)	1 (1.0)	0.374
	rs7875475 (T)	114 (76.0)	36 (24.0)	0 (0)	0.095	79 (79.0)	17 (17.0)	4 (4.0)	0.026

SNP, single nucleotide polymorphism; *HWE*, Hardy-Weinberg equilibrium.

HWE. Whereas, of 4 tag SNPs, 1 SNP (rs7875475) was not in HWE ($P < 0.05$) in the control group, and it was excluded from further analysis.

Association of BNIP3 and DAPK1 gene polymorphisms with GC susceptibility

The associations between genotypes and GC susceptibility under dominant and recessive models are displayed in **Table 2**. Results demonstrated that BNIP3 and DAPK1 gene polymorphisms were not associated with GC susceptibility. After stratifying by gender and age, the associations were further explored. After gender stratification, no association was found in males and females (Table S1). After age stratification, univariate logistic analysis and multivariate logistic analysis demonstrated that rs10781582 of BNIP3 in the dominant model was correlated with reduced risk of GC in the younger group (age ≤ mean) ($P = 0.004$, $P_{adj} = 0.005$), and this association still existed after FDR correction ($P_{BH} = 0.015$) (**Table 3**; **Figure 1A**). **Table 4** showed the associations between SNP allele and GC susceptibility. The findings demonstrated that the minor allele G of rs1329600 at DAPK1 was correlated with reduced risk of GC ($P_{BH} = 0.018$) (**Figure 1B**). We stratified subjects by gender and age to further explore the association between SNP allele of BNIP3 and DAPK1 genes and GC susceptibility. After gender stratification, no association was found between alleles and risk of GC in males and females (Table S2). After age stratification, the minor allele G of rs10781582 at DAPK1 was related to reduced risk of GC in the younger subjects ($P = 0.019$). However, this association did not exist after FDR correction ($P_{BH} = 0.057$) (Table S3). We found no statistically significant result between SNPs and GC susceptibility by haplotype analysis (Table S4).

Associations of BNIP3 and DAPK1 gene polymorphisms with clinicopathologic features of GC patients

We next identified the correlations of BNIP3 and DAPK1 polymorphisms with clinicopathologic features among GC patients. Distributions of BNIP3 and DAPK1 genotypes in GC patients according to the clinicopathologic features are shown in **Table 5**. The results demonstrated that rs3793742 of BNIP3 in the dominant model was associated with gender of GC patients, and the frequency of TT + CT variants was higher in female patients compared with male patients ($P_{adj} = 0.011$, $P_{BH} = 0.033$) (Table S5; **Figure 2A**). rs10781582 of BNIP3 in the dominant model was associated with age of GC patients, and the frequency of AA + AT variants was higher in the older group (age > mean) compared with the younger group (age ≤ mean) ($P_{adj} = 0.010$, $P_{BH} = 0.030$) (Table S5; **Figure 2B**). However, no association was found between BNIP3 and DAPK1 polymorphisms and differentiation degree, WHO classification, TNM stage, lymph node metastases, or visceral metastasis (Table S5).

Associations of BNIP3 and DAPK1 gene polymorphisms with depression, anxiety

All the 150 GC patients had baseline HAMD and HAMA scores, of which 46 patients (30.67%) got a diagnosis of depression, and 93 patients (62.00%) got a diagnosis of anxiety. The relationships of BNIP3 and DAPK1 gene polymorphisms with depression are showed in **Table 6**. The rs10781582 of BNIP3 in the dominant model was correlated with depression ($P = 4.18\times10^{-4}$, $P_{adj} = 0.001$, $P_{BH} = 0.003$) (**Figure 2C**). However, no correlation was found between BNIP3 and DAPK1 polymorphisms and anxiety (Table S6).

Table 2. Associations of BNIP3 and DAPK1 SNPs with susceptibility to GC under different inherited models

Gene	SNP	Dominant model					Recessive model				
		Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]	Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]
BNIP3	rs3793742	0.795 (0.469-1.347)	0.393	0.797 (0.468-1.359)	0.405	0.608	0.706 (0.358-1.392)	0.315	0.672 (0.337-1.338)	0.257	0.378
	rs10781582	0.742 (0.444-1.239)	0.253	0.730 (0.436-1.222)	0.231	0.608	0.683 (0.307-1.519)	0.349	0.682 (0.306-1.520)	0.349	0.378
DAPK1	rs1329600	1.102 (0.630-1.927)	0.733	1.107 (0.632-1.939)	0.722	0.722	0.369 (0.041-3.348)	0.375	0.370 (0.041-3.368)	0.378	0.378

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted by gender and age; [b]Adjusted for multiple testing by FDR correction.

Table 3. Age-specific associations of BNIP3 and DAPK1 polymorphisms with susceptibility to GC under different inherited models

Age	SNP	Dominant model					Recessive model				
		Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]	Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]
≤ Mean	BNIP3										
	rs3793742	1.382 (0.627-2.811)	0.459	1.332 (0.628-2.822)	0.455	0.683	0.529 (0.29-1.339)	0.179	0.527 (0.208-1.335)	0.177	0.531
	rs10781582	0.329 (0.153-0.708)	0.004	0.332 (0.154-0.714)	0.005	0.015	1.221 (0.412-3.622)	0.719	1.195 (0.401-3.563)	0.749	0.749
	DAPK1										
	rs1329600	1.043 (0.471-2.306)	0.918	1.045 (0.472-2.314)	0.913	0.913	0.673 (0.059-7.640)	0.750	0.668 (0.059-7.598)	0.745	0.749
> Mean	BNIP3										
	rs3793742	0.472 (0.219-1.018)	0.055	0.459 (0.208-1.012)	0.053	0.159	0.950 (0.347-2.598)	0.920	0.923 (0.325-2.619)	0.880	0.880
	rs10781582	1.519 (0.745-3.095)	0.250	1.520 (0.739-3.128)	0.256	0.384	0.339 (0.092-1.254)	0.105	0.339 (0.091-1.260)	0.106	0.212
	DAPK1										
	rs1329600	1.178 (0.534-2.597)	0.685	1.175 (0.533-2.593)	0.689	0.689	-	-	-	-	-

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; Mean = 60.766; [a]Adjusted by gender; [b]Adjusted for multiple testing by FDR correction.

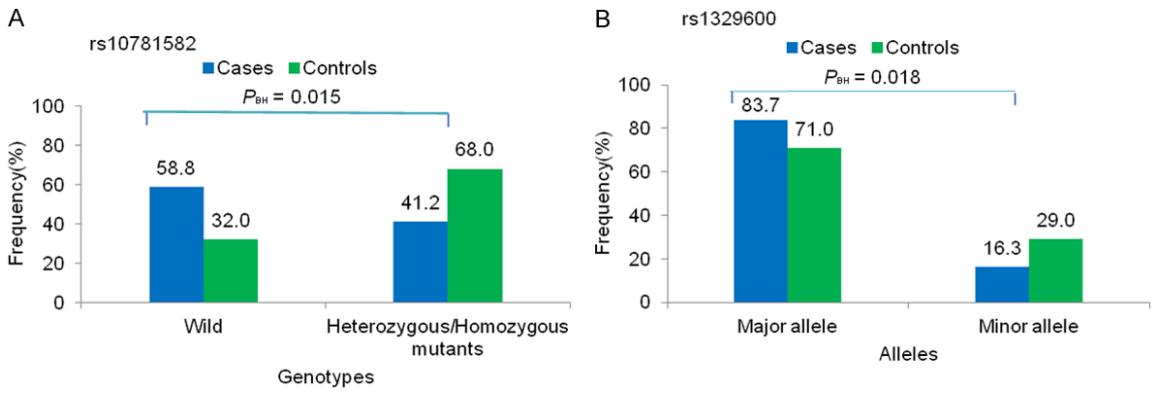

Figure 1. Frequency distribution of BNIP3 and DAPK1 gene polymorphism in cases and controls. A. Frequency distribution of rs10781582 of BNIP3 genotypes in younger cases and controls (age ≤ mean). B. Frequency distribution of rs1329600 of DAPK1 alleles in cases and controls.

Table 4. Allele frequencies of BNIP3 and DAPK1 polymorphisms in GC patients and controls

Gene	SNP (minor allele/major allele)	GC (n = 150)	Control (n = 100)	*OR (95% CI)*	*P*-value	P_{BH} [a]
BNIP3	rs3793742 (T/C)	121/179	81/119	0.993 (0.690-1.430)	0.970	0.970
	rs10781582 (A/T)	100/200	70/130	0.929 (0.637-1.354)	0.700	0.970
DAPK1	rs1329600 (G/A)	49/251	58/142	0.478 (0.281-0.812)	0.006	0.018

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted for multiple testing by FDR correction.

Discussion

This study revealed that rs10781582 of BNIP3 in the dominant model was correlated with reduced risk of disease in GC patients whose age was less than or equal to the mean, and the minor allele G of rs1329600 at DAPK1 was also correlated with reduced risk of GC. In terms of clinicopathologic features, the rs3793742 and rs10781582 polymorphisms of BNIP3 in the dominant model were associated with gender and age, respectively. The rs10781582 polymorphism of BNIP3 in the dominant model was associated with depression in GC patients. Nevertheless, no associations were found between BNIP3 and DAPK1 polymorphisms and differentiation degree, WHO classification, TNM stage, lymph node metastases, and visceral metastasis. This study is the first to explore the association between BNIP3 and DAPK1 polymorphism and GC risk and patients' clinicopathologic features.

Accumulating evidence demonstrates that SNPs of many genes are associated with GC susceptibility, such as Thrombospondin-2 (*THBS2*), Thrombospondin-4 (*THBS4*), microRNA-149 (miR-149), de novo methyltransferase 3B (DNMT3B) and TP73 antisense RNA 1 (TP73-AS1) [24-27]. Unfortunately, as far as we know, few studies have explored the association of BNIP3 polymorphism with disease risk (including GC). By contrast, many studies have identified the association of DAPK1 polymorphism with susceptibility of various diseases (excluding GC). For example, the rs4878104 polymorphism of DAPK1 influenced susceptibility to late-onset Alzheimer's disease (LOAD) [21], the rs2075533 SNP of DAPK1 was associated with lung cancer [16], and an association was found between rs11141901 of DAPK1 and enhanced risk of breast cancer [17]. Nevertheless, no study has explored whether BNIP3 and DAPK1 polymorphisms were associated with risk of GC. In the present study, the results demonstrated that the rs10781582 polymorphism of BNIP3 in the dominant model was correlated with reduced risk of GC among younger individuals (age ≤ mean), but not among older participants (age > mean). Higher-level exposure to carcinogenic substances and a weaker immune system in older people may be responsible for this age difference [28]. The functions of BNIP3 gene in the

Table 5. Distribution of genotype frequencies of polymorphisms in BNIP3 and DAPK1 genes according to GC patients' demographic and clinicopathologic characteristics (n = 150)

		rs3793742			rs10781582			rs1329600			rs7875475		
		C/C	C/T	T/T	T/T	A/T	A/A	A/A	A/G	G/G	C/C	C/T	T/T
Gender	Male	51 (44.74)	44 (38.60)	19 (16.67)	50 (43.86)	47 (41.23)	17 (14.91)	77 (67.54)	33 (28.95)	4 (3.51)	89 (78.07)	25 (21.93)	0 (0)
	Female	8 (22.22)	17 (47.22)	11 (30.56)	21 (58.33)	11 (30.56)	4 (11.11)	28 (77.78)	8 (22.22)	0 (0)	25 (69.44)	11 (30.56)	0 (0)
Age (years)	≤ Mean	24 (33.80)	28 (39.44)	19 (26.76)	42 (59.15)	21 (29.58)	8 (11.27)	49 (69.01)	20 (28.17)	2 (2.82)	55 (77.46)	16 (22.54)	0 (0)
	> Mean	35 (44.30)	33 (41.77)	11 (13.92)	29 (36.71)	37 (46.84)	13 (16.45)	56 (70.89)	21 (26.58)	2 (2.53)	59 (74.68)	20 (25.32)	0 (0)
Differentiation degree	Moderate to well	41 (35.96)	51 (44.74)	22 (19.30)	53 (46.49)	47 (41.23)	14 (12.28)	80 (70.18)	32 (28.07)	2 (1.75)	91 (79.82)	23 (20.18)	0 (0)
	Poorly	18 (50.00)	10 (27.78)	8 (22.22)	18 (50.00)	11 (30.56)	7 (19.44)	25 (69.44)	9 (25.0)	2 (5.56)	23 (63.89)	13 (36.11)	0 (0)
WHO classification	Poorly differentiated adenocarcinoma	37 (35.58)	45 (43.27)	22 (21.15)	48 (46.15)	41 (39.42)	15 (14.42)	69 (66.35)	33 (31.73)	2 (1.92)	85 (81.73)	19 (18.27)	0 (0)
	Papillary adenocarcinoma and other types	22 (47.83)	16 (34.78)	8 (17.39)	23 (50.00)	17 (36.96)	6 (13.04)	36 (78.26)	8 (17.39)	2 (4.35)	29 (63.04)	17 (36.96)	0 (0)
TNM stage	I-III	20 (37.74)	19 (35.85)	14 (26.42)	23 (43.40)	26 (49.06)	4 (7.54)	38 (71.70)	15 (28.30)	0 (0)	37 (69.81)	16 (30.19)	0 (0)
	IV	39 (40.21)	42 (43.30)	16 (16.49)	48 (49.48)	32 (32.99)	17 (17.53)	67 (69.07)	26 (26.80)	4 (4.13)	77 (79.38)	20 (20.62)	0 (0)
Lymph node metastases	Positive	35 (35.00)	41 (41.00)	24 (24.00)	52 (52.00)	37 (37.00)	11 (11.00)	72 (72.00)	26 (26.00)	2 (2.00)	77 (77.00)	23 (23.00)	0 (0)
	Negative	24 (48.00)	20 (40.00)	6 (12.00)	19 (38.00)	21 (42.00)	10 (20.00)	33 (66.00)	15 (30.00)	2 (4.00)	37 (74.00)	13 (26.00)	0 (0)
Visceral metastasis	Positive	24 (44.44)	25 (46.30)	5 (9.26)	22 (40.74)	21 (38.89)	11 (20.37)	35 (64.81)	18 (33.33)	1 (1.85)	43 (79.63)	11 (20.37)	0 (0)
	Negative	35 (36.46)	36 (37.50)	25 (26.04)	49 (51.04)	37 (38.54)	10 (10.42)	70 (72.92)	23 (23.96)	3 (3.12)	71 (73.96)	25 (26.04)	0 (0)
Depression	Positive	17 (36.96)	18 (39.13)	11 (23.91)	32 (69.57)	11 (23.91)	3 (6.52)	34 (7.91)	11 (23.91)	1 (2.17)	36 (78.26)	10 (21.74)	0 (0)
	Negative	42 (40.38)	43 (41.35)	19 (18.27)	39 (37.50)	47 (45.19)	18 (17.31)	71 (68.27)	30 (28.85)	3 (2.88)	78 (75.00)	26 (25.00)	0 (0)
Anxiety	Positive	35 (37.63)	39 (41.94)	19 (20.43)	42 (45.16)	38 (40.86)	13 (13.98)	66 (70.97)	24 (25.81)	3 (3.22)	74 (79.57)	19 (20.43)	0 (0)
	Negative	24 (42.11)	22 (38.60)	11 (19.29)	29 (50.88)	20 (35.09)	8 (14.04)	39 (68.42)	17 (29.82)	1 (1.75)	40 (70.18)	17 (29.82)	0 (0)

GC, gastric cancer; Mean = 61.22; *BNIP3*: rs3793742, rs10781582; *DAPK1*: rs1329600, rs7875475.

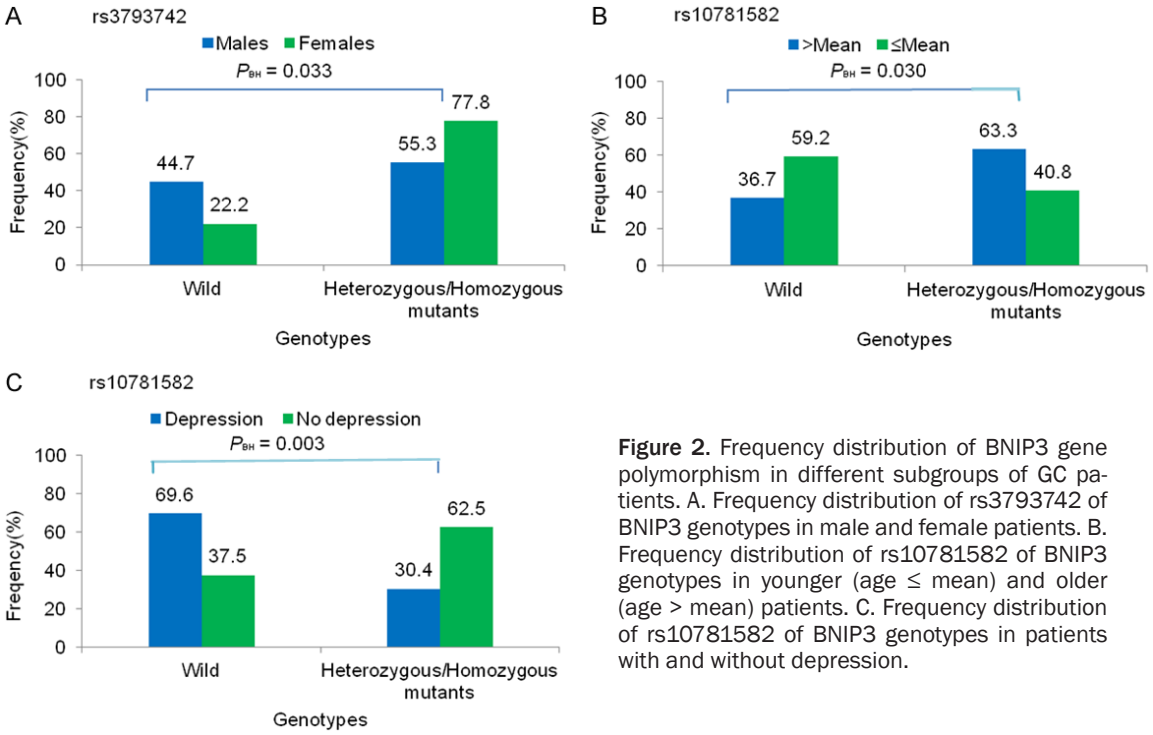

Figure 2. Frequency distribution of BNIP3 gene polymorphism in different subgroups of GC patients. A. Frequency distribution of rs3793742 of BNIP3 genotypes in male and female patients. B. Frequency distribution of rs10781582 of BNIP3 genotypes in younger (age ≤ mean) and older (age > mean) patients. C. Frequency distribution of rs10781582 of BNIP3 genotypes in patients with and without depression.

immune system have been reported, including promoting the generation of natural killer (NK) cell memory and enhancing host pathogen defense and longevity [29, 30]. The immune system declines with age [31]. BNIP3 may play a stronger part in the immune system of younger individuals compared with older ones. We speculated that rs10781582 polymorphism could regulate BNIP3 expression, thus changing its immune function in younger individuals and decreasing the risk of GC. Furthermore, evidence suggested that age plays a critical part in the increased risk for GC; the older individuals are more likely to suffer from GC; and its incidence and mortality rates progressively increase with age [8, 32, 33]. Alterations in apoptosis may contribute to this age-associated disease [34]. It is well known that BNIP3 is an apoptosis-related gene. We speculated that rs10781582 SNP could modulate the expression of BNIP3, thus changing its apoptosis-related function in younger people and reducing the risk of GC. However, the mechanisms underlying this age-specific susceptibility of GC need to be further explored. Similar to our study, the mutant genotypes (AA + GA) of myeloperoxidase (MPO) were correlated with a reduced risk of GC among younger individuals (age < 58 years), but not among older individuals [28]. In this study, the minor allele G of rs1329600 at DAPK1 was also correlated with a decreased risk of GC. It is well known that DAPK1 is a member of five serine/threonine (Ser/Thr) kinases family that have the role of suppressing tumor and mediating cellular processes (e.g., apoptosis and autophagy). The loss-of-function of DAPK1 is associated with varieties of cancer [35]. Research showed that gene polymorphisms could regulate its expression and induce diseases [36-38]. The possibility is that rs1329600 could upregulate the expression level of DAPK1, thus contributing to its protective effect against GC. Further research is warranted to elucidate the mechanism of BNIP3 and DAPK1 polymorphisms on GC risk.

The rs3793742 and rs10781582 of BNIP3 in the dominant model were associated with gender and age of GC patients, respectively. Nevertheless, no association was found between BNIP3 and DAPK1 gene polymorphisms and differentiation degree, TNM stage, lymph node metastases, or visceral metastasis in the present study. The gender disparity may be caused by several contributing factors, such as sex hormones, environmental factors (e.g., smoking, drinking), and H. pylori infection [39, 40]. It is reported that the pathogenic mecha-

Table 6. Associations of BNIP3 and DAPK1 polymorphisms with depression of GC patients under different inherited models

Gene	SNP	Dominant model					Recessive model				
		Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]	Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]
BNIP3	rs3793742	1.156 (0.565-2.363)	0.692	1.175 (0.55 7-2.476)	0.672	0.672	0.711 (0.307-1.648)	0.427	0.729 (0.306-1.732)	0.474	0.649
	rs10781582	0.263 (0.125-0.552)	4.18×10^{-4}	0.276 (0.128-0.595)	0.001	0.003	3.000 (0.838-10.746)	0.091	2.578 (0.705-9.431)	0.152	0.456
DAPK1	rs1329600	0.759 (0.349-1.651)	0.478	0.734 (0.330-1.632)	0.448	0.672	1.337 (0.135-13.203)	0.804	1.723 (0.166-17.899)	0.649	0.649

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted by gender, age, cigarette and alcohol; [b]Adjusted for multiple testing by FDR correction.

nism of GC may be different between age groups [41]; and moreover, the different molecular expression profile between age groups may account for the age-specific pathogenesis of GC [42]. The possibility is that BNIP3 polymorphism could modulate the expression of BNIP3, which contributes to the different expression of BNIP3 between younger and older patients, thus leading to the above age association. Additional investigations are warranted to better understand the mechanisms underlying these phenomena. There is a robust body of studies that described the associations of polymorphisms of other genes with the above clinicopathologic characteristics of GC patients. For example, there exist significant association between polymorphism of Matrix metalloproteinase-9 (MMP-9) and advanced stage GC patients, but not gender, age or lymph node metastases [43]. The TBX21-1993 polymorphism was closely correlated with lymph node metastasis and distant metastasis of GC patients, but not gender, age, differentiation degree, or TNM stage [44]. There were significant associations of PRKAA1 polymorphisms with tumor differentiation and TNM stage [45].

Depression and anxiety are common psychiatric issues in cancer patients. Cancer patients may be inclined to develop depression and anxiety after cancer diagnosis or during postdiagnosis treatment [46]. It is reported that GC patients did suffer from depression and anxiety [47, 48]. In this study, the results suggested that the rs10781582 polymorphism of BNIP3 was associated with depression in GC patients. Prior studies have explored the association of BNIP3 gene with depression. It is reported that BNIP3 encodes a mitochondrial protein which is related to anti-depressive effects [18]. Later, to elucidate the physiological functions of BNIP3, they explored whether BNIP3 has a correlation with the depression, utilizing learned helplessness (LH) mice. Results demonstrated that BNIP3 served as an antistress factor [19]. We speculated that rs10781582 could regulate BNIP3 expression (e.g., affecting coding process of mitochondrial protein), thus affecting GC patients' depression state. In terms of anxiety, the results of this study demonstrated no association between BNIP3 and DAPK1 polymorphisms and anxiety, and no investigation has studied the correlations between BNIP3 and DAPK1 genes or genes polymorphisms and anxiety in GC patients. However, one study suggested that FK506 binding protein 5 (FKBP5) gene polymorphism was involved in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis may play an important part in anxiety and depression of advanced GC patients suffering from long-term stress exposure [49]. Another study reported that the brain-derived neurotrophic factor (BDNF) Val66Met variant could influence anxiety coping response, and increase susceptibility to anxiety or depression in advanced GC patients [50]. Further studies are warranted to explore the association and mechanism of BNIP3 and DAPK1 polymorphisms with depression and anxiety of GC patients.

Some limitations existed in our study. First, the number of cases and controls are smaller compared with studies exploring associations between polymorphisms of other genes and susceptibility of GC, and a case-control study with limited subjects could lead to selection bias [51, 52], we assessed the statistical power of allele association analysis. The small difference of the positive rate between cases and controls that was applied to comparison resulted in lower statistical power. However, when the positive rate difference between cases and controls was 0.170, the statistical power of all three SNPs could exceed 0.80 (Table S7). Second, all participates, unrelated Chinese Han nationality, were recruited only from the First Affiliated Hospital of Anhui Medical University, which limited extrapolation of the present findings. Third, only two SNPs (rs3793742, rs10781582) in BNIP3 and two SNPs (rs1329600, rs7875475) in DAPK1 are assessed, and it is possible that we may have neglected some other important SNPs or the observed correlations may be due to other polymorphisms in linkage disequilibrium with the selected polymorphisms of BNIP3 or DAPK1. Finally, although we did adjust some confounding factors by multivariate analysis, it is possible that other confounders had not been controlled.

In conclusion, the rs10781582 of BNIP3 in the dominant model was associated with a protective effect against GC in the younger group, and a similar association was found in the minor allele G of rs1329600 of DAPK1. Furthermore, correlations were found between

BNIP3 polymorphisms and gender, age, and depression of GC patients. The results may provide new insights to explore more biological mechanisms for GC pathogenesis. Further investigation is underway to clarify the association between polymorphisms of BNIP3 and DAPK1 and susceptibility to GC.

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Disclosure of conflict of interest

None.

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Table S1. Gender-specific associations of BNIP3 and DAPK1 polymorphisms with susceptibility to gastric cancer under different inherited models

Gender	SNP	Dominant model					Recessive model				
		Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]	Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]
Female	BNIP3										
	rs3793742	1.750 (0.570-5.371)	0.328	1.671 (0.534-5.229)	0.378	0.378	0.649 (0.205-2.054)	0.462	0.650 (0.205-2.062)	0.464	0.711
	rs10781582	0.420 (0.151-1.170)	0.097	0.431 (0.154-1.210)	0.110	0.330	1.391 (0.315-6.148)	0.663	1.327 (0.297-1.059)	0.711	0.711
	DAPK1										
	rs1329600	0.486 (0.160-1.471)	0.201	0.503 (0.164-1.540)	0.229	0.344	-	-	-	-	-
Male	BNIP3										
	rs3793742	0.643 (0.350-1.182)	0.155	0.643 (0.350-1.182)	0.155	0.389	0.703 (0.299-1.652)	0.703	0.687 (0.291-1.622)	0.392	0.392
	rs10781582	0.893 (0.492-1.619)	0.709	0.880 (0.484-1.601)	0.675	0.675	0.511 (0.192-1.363)	0.180	0.516 (0.193-1.378)	0.187	0.374
	DAPK1										
	rs1329600	1.468 (0.758-2.844)	0.255	1.464 (0.756-2.837)	0.259	0.389	-	-	-	-	-

SNP, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted by age; [b]Adjusted for multiple testing by FDR correction.

Table S2. Gender-specific associations of alleles of BNIP3 and DAPK1 polymorphisms with gastric cancer susceptibility

Gene	SNP	Females					Males				
		GC (n = 36)	Control (n = 27)	*OR (95% CI)*	*P*-value	P_{BH}[a]	GC (n = 114)	Control (n = 73)	*OR (95% CI)*	*P*-value	P_{BH}[a]
BNIP3	rs3793742	39/33	24/30	1.477 (0.727-3.002)	0.280	0.280	82/146	57/89	0.877 (0.571-1.346)	0.548	0.697
	rs10781582	19/53	21/33	0.563 (0.264-1.202)	0.136	0.227	81/147	49/97	1.091 (0.704-1.690)	0.697	0.697
DAPK1	rs1329600	8/64	11/43	0.489 (0.182-1.314)	0.151	0.227	41/187	18/128	1.559 (0.857-2.835)	0.143	0.429

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted for multiple testing by FDR correction.

Table S3. Age-specific associations of alleles of BNIP3 and DAPK1 polymorphisms with gastric cancer susceptibility

Gene	SNP	≤ Mean					> Mean				
		GC (n = 68)	Control (n = 50)	*OR (95% CI)*	*P*-value	P_{BH}[a]	GC (n = 82)	Control (n = 50)	*OR (95% CI)*	*P*-value	P_{BH}[a]
BNIP3	rs3793742	62/74	37/63	1.427 (0.841-2.419)	0.186	0.279	59/105	44/56	0.715 (0.431-1.188)	0.195	0.293
	rs10781582	36/100	41/59	0.518 (0.299-0.899)	0.019	0.057	64/100	29/71	1.567 (0.919-2.672)	0.098	0.293
DAPK1	rs1329600	23/113	16/84	1.069 (0.532-2.147)	0.852	0.852	26/138	13/87	1.261 (0.442-1.521)	0.526	0.526

GC, gastric cancer; *SNP*, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; Mean = 60.77; [a]Adjusted for multiple testing by FDR correction.

Table S4. Haplotype association analysis of BNIP3 and DAPK1 polymorphisms with gastric cancer

Gene	Haplotype	GC (freq)	Control (freq)	χ^2 value	*OR (95% CI)*	*P*-value	P_{BH} [a]
BNIP3	CA	98.71 (0.329)	67.11 (0.336)	0.053	0.956 (0.653-1.399)	0.817	0.920
	CT	80.29 (0.268)	51.89 (0.259)	0.019	1.029 (0.685-1.546)	0.892	0.920
	TT	119.71 (0.399)	78.11 (0.391)	0.010	1.019 (0.706-1.471)	0.920	0.920
DAPK1	AC	215.02 (0.717)	146.00 (0.730)	0.104	0.936 (0.627-1.397)	0.747	0.920
	AT	35.98 (0.120)	25.00 (0.125)	0.029	0.954 (0.553-1.645)	0.866	0.920
	GC	48.98 (0.163)	29.00 (0.145)	0.304	1.151 (0.699-1.895)	0.581	0.920

GC, gastric cancer; *freq*, frequency; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted for multiple testing by FDR correction.

Table S5. Association between BNIP3 and DAPK1 polymorphisms and clinicopathologic features of gastric cancer

Variables	Gene	SNP	Dominant model			Recessive model		
			Adjusted[a] *OR (95% CI)*	*Adjusted P-value*	P_{BH} [b]	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH} [b]
Gender	BNIP3	rs3793742	4.735 (1.433-15.641)	0.011	0.033	0.592 (0.166-2.117)	0.420	0.840
		rs10781582	0.771 (0.262-2.273)	0.638	0.638	1.037 (0.197-5.456)	0.966	0.966
	DAPK1	rs1329600	0.421 (0.130-1.360)	0.148	0.222	-	-	-
Age (years)	BNIP3	rs3793742	0.698 (0.355-1.373)	0.297	0.446	2.109 (0.914-4.866)	0.080	0.240
		rs10781582	2.395 (1.233-4.650)	0.010	0.030	0.663 (0.256-1.718)	0.397	0.600
	DAPK1	rs1329600	0.866 (0.427-1.757)	0.690	0.690	1.292 (0.176-9.505)	0.802	0.802
Differentiation degree	BNIP3	rs3793742	0.498 (0.222-1.114)	0.090	0.270	0.749 (0.288-1.947)	0.553	0.553
		rs10781582	0.733 (0.330-1.626)	0.444	0.666	0.614 (0.215-1.751)	0.361	0.542
	DAPK1	rs1329600	1.169 (0.500-2.732)	0.719	0.719	0.258 (0.033-1.994)	0.194	0.542
WHO classification	BNIP3	rs3793742	0.541 (0.259-1.130)	0.102	0.287	1.259 (0.503-3.150)	0.623	0.746
		rs10781582	0.789 (0.382-1.629)	0.521	0.521	1.188 (0.418-3.374)	0.746	0.746
	DAPK1	rs1329600	0.578 (0.254-1.315)	0.191	0.287	0.388 (0.051-2.932)	0.359	0.746
TNM stage	BNIP3	rs3793742	0.827 (0.406-1.684)	0.600	0.610	1.882 (0.816-4.341)	0.138	0.138
		rs10781582	0.707 (0.350-1.429)	0.334	0.610	0.406 (0.127-1.301)	0.129	0.138
	DAPK1	rs1329600	1.216 (0.574-2.576)	0.610	0.610	-	-	-
Lymph node metastases	BNIP3	rs3793742	0.505 (0.241-1.057)	0.070	0.210	2.344 (0.854-6.434)	0.098	0.293
		rs10781582	1.619 (0.779-3.365)	0.197	0.295	0.524 (0.197-1.394)	0.195	0.293
	DAPK1	rs1329600	1.490 (0.693-3.204)	0.308	0.308	0.382 (0.049-2.953)	0.356	0.356
Visceral metastasis	BNIP3	rs3793742	1.457 (0.710-2.992)	0.305	0.446	0.289 (0.101-0.828)	0.021	0.063
		rs10781582	0.758 (0.372-1.545)	0.446	0.446	1.794 (0.686-4.691)	0.233	0.350
	DAPK1	rs1329600	0.633 (0.298-1.345)	0.235	0.446	0.752 (0.070-8.058)	0.814	0.814

SNP, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted by age, cigarette and alcohol; [b]Adjusted for multiple testing by FDR correction.

Table S6. Associations of BNIP3 and DAPK1 polymorphisms with anxiety of gastric cancer patients under different inherited models

Gene	SNP	Dominant model					Recessive model				
		Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]	Crude *OR (95% CI)*	Crude *P*-value	Adjusted[a] *OR (95% CI)*	Adjusted *P*-value	P_{BH}[b]
BNIP3	rs3793742	0.830 (0.423-1.626)	0.587	0.875 (0.433-1.767)	0.709	0.889	1.074 (0.469-2.459)	0.866	1.107 (0.471-2.602)	0.816	0.816
	rs10781582	0.795 (0.411-1.539)	0.496	0.911 (0.457-1.819)	0.792	0.889	0.995 (0.385-2.573)	0.992	0.803 (0.301-2.141)	0.661	0.816
DAPK1	rs1329600	1.128 (0.551-2.308)	0.741	1.054 (0.504-2.204)	0.889	0.889	1.867 (0.189-18.389)	0.593	2.514 (0.243-25.971)	0.439	0.816

SNP, single nucleotide polymorphism; *OR*, odds ratio; *CI*, confidence interval; [a]Adjusted by gender, age, cigarette and alcohol; [b]Adjusted for multiple testing by FDR correction.

Table S7. Statistical power of allele association analysis for different positive rate differences between case group and control group

Gene	SNPs	Case-control study			
		Current positive rate difference	Statistical power	Assumed positive rate difference	Statistical power
BNIP3	rs3793742	0.002	0.05	0.170	0.83
	rs10781582	0.017	0.06	0.170	0.86
DAPK1	rs1329600	0.127	0.67	0.170	0.92

SNP, single nucleotide polymorphism.