



Insulin-like growth factor binding protein-3 (IGFBP-3) genetic variant and the risk of esophageal squamous cell carcinoma in a Chinese population

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ABSTRACT. Insulin-like growth factor binding protein-3 (IGFBP-3) exerts anti-proliferative or pro-apoptotic effects through IGF-dependent as well as IGF-independent mechanisms *in vitro*. The purpose of this study was to examine the association between genetic variants in *IGFBP-3* (rs2270628) and the risk of esophageal squamous cell carcinoma (ESCC) in a Chinese Han population. Five hundred ESCC cases and 500 cancer-free controls of the Chinese Han population were

involved in this study. The *IGFBP-3* single-nucleotide polymorphism (SNP) rs2270628 was genotyped and the estimated adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for its association with the risk of ESCC were determined using unconditional logistic regression analysis. Compared with the rs2270628 CC genotype, TT genotype was associated with a significantly increased ESCC risk with OR (95%CI) of 2.07 (1.05-4.09), but CT genotype was not (OR = 1.25, 95%CI =0.94-1.66). *IGFBP-3* SNP rs2270628 may contribute to the risk of ESCC in the Chinese Han population.

Key words: Esophageal squamous cell carcinoma; IGFBP-3; Genetic polymorphism; Molecular epidemiology

INTRODUCTION

Esophageal cancer, an aggressive carcinoma, is the 6th most common cause of cancer-related deaths worldwide (Jemal et al., 2011); it was the 4th leading cause of cancer deaths and the 5th most commonly diagnosed cancer in China in 2009 (Chen et al., 2013). Esophageal cancer includes 2 major histological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC is the predominant histological type of esophageal cancer in China. The major risk factors for ESCC are not well understood, but are thought to include poor nutritional status, low intake of fruit and vegetables, and drinking beverages at high temperatures (Islami et al., 2009a,b; Wu et al., 2009). Although the environment and exposure are both important factors in the development of esophageal carcinogenesis, genetic factors also play an important role (Hiyama et al., 2007). Previous studies have suggested that genetic polymorphisms contribute to the mechanisms involved in esophageal carcinogenesis (Xing et al., 2003). A growing number of genes implicated in alcohol metabolism, folate metabolism, carcinogen metabolism, DNA repair, and cell cycle regulation may be associated with esophageal carcinogenesis (Hiyama et al., 2007).

The insulin-like growth factor (IGF) system is composed of a family of interacting ligands, receptors, and IGF-binding proteins (IGFBPs). IGF-I is a mitogen that plays a pivotal role in regulating cell proliferation, differentiation, and apoptosis (Khandwala et al., 2000). The major binding protein of IGF-I, IGFBP-3, exerts anti-proliferative or pro-apoptotic effects through IGF-dependent as well as IGF-independent mechanisms *in vitro* (Baxter, 2000, 2001; Firth and Baxter, 2002). Epidemiological studies have indicated that high levels of IGF-I and low levels of IGFBP-3 are associated with an increased risk of several common cancers, including prostate, breast, lung, and colorectal cancers (Yu and Rohan, 2000; Renehan et al., 2004; Gallagher and LeRoith, 2010). Results regarding the association between genetic variants of *IGFBP-3* and common cancers have been inconsistent. A previous study reported that IGFBP-3 expression was inversely correlated with the clinical pathological classification of ESCC (Zhao et al., 2012). Various researchers have studied the association between genetic polymorphisms of *IGFBP-3* and common cancers, one of which, the rs2270628 single-nucleotide polymorphism (SNP) of *IGFBP-3*, was associated with an increased risk of ovarian cancer (Terry et al., 2009; Pearce et al., 2011), but was not associated with EAC (McElholm et al., 2010).

The association between genetic polymorphisms of *IGFBP-3* and the risk of ESCC has not been evaluated in previous studies. Therefore, we investigated the association between the *IGFBP-3* rs2270628 SNP and the risk of ESCC in a hospital-based case-control study in a Chinese Han population.

MATERIAL AND METHODS

Study population

Eligible ESCC cases in this study were consecutively recruited between December 2010 and July 2012 from LuHe Hospital, Tangshan People's Hospital and Cixian People's Hospital, China. Once ESCC cases were diagnosed using histopathology methods, they were immediately contacted by the interviewer at the hospital. All patients in the study were of Chinese Han ethnicity, had lived locally for at least 5 years, were newly diagnosed with ESCC, and were in a stable medical condition as determined by their physician. A total of 500 ESCC cases consented to participate in the study and provide blood samples, giving a response rate of 93.5% (500/535). Eligible controls included Chinese Han in-patients and cancer-free individuals who had lived in the area for at least 5 years and reported no history of cancer. Controls were matched to the cases according to age at the time of enrollment (within 5 years) and gender. A total of 580 potential cancer-free controls were approached and 500 (86.2%) completed the interview and donated blood samples.

A standard epidemiologic questionnaire was used to determine demographic and life-style characteristics, such as gender, age, tobacco smoking, and alcohol consumption. Individuals who smoked once per day for over 1 year were defined as smokers, and smoking history was recorded as never or ever. Individuals who consumed 3 or more alcoholic drinks per week for over 6 months were considered to be alcohol drinkers. After the interview, a 5-mL venous blood sample was collected from each participant. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Hebei Medical University. Written informed consent was obtained from all participants.

Genotyping

Genomic DNA was extracted from the buffy-coat fraction of the blood samples using the DP318-08 Blood DNA Mini Kit (Tiangen Biotech Co. Ltd.; Beijing, China). The rs2270628 SNP was genotyped using the TaqMan real-time polymerase chain reaction (PCR) method with a 7900 HT Fast Real-time PCR system (Applied Biosystems; Foster City, CA, USA). The primers for amplifying the *IGFBP-3* fragment were 5'-TCA CTG TGG AAA TTG AGA TTG T-3' (sense) and 5'-AGA AAA GCA GAA TGC AAT TAT ATT GTG-3' (antisense). PCR was accomplished using 2.5 μ L Premix ExTaq (Jikang Biotechnology Co., Ltd.; Shanghai, China), with 0.125 μ L sense and antisense primers and 0.2 μ L probe. Reaction conditions involved an initial denaturation step for 2 min at 50°C, followed by 42 cycles at 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min. Endpoint analysis was conducted using the Allelic Discrimination Program of the SDS Image Analysis Software (Version 2.4). To ensure high genotyping accuracy, strict quality control procedures were implemented. Approximately 5% of samples underwent repeated genotyping, and the results were concordant.

Statistical analysis

Differences between ESCC cases and control subjects in the frequency distributions of the selected demographic variables, risk factors, and each of the alleles and genotypes of the SNP were evaluated using the χ^2 test. Unconditional logistic regression analyses were used to calculate odds ratios (ORs) to estimate the risk of ESCC and 95% confidence intervals (95% CIs). Multivariate adjustments were made when appropriate for age and gender in further stratification analysis of genotype data. The Hardy-Weinberg equilibrium for genotype distribution in controls was tested using a goodness-of-fit χ^2 test. Statistical significance was established as $P < 0.05$, and all tests were 2-sided and performed using SPSS 16.0 (SPSS; Chicago, IL, USA).

RESULTS

Population characteristics

Characteristics of the 500 confirmed ESCC cases and 500 cancer-free controls are summarized in Table 1. Approximately 80% of subjects in each group were male. The mean (standard deviation) age was 58.3 ± 8.6 years in patients, and 59.6 ± 8.5 years in controls. There were no significant differences in age and gender between the cases and controls ($P = 0.96$ and $P = 0.527$, respectively). However, ESCC cases were more likely to be smokers (61.2 vs 29.2%, $P < 0.001$) or drinkers (26.2 vs 15.4%, $P < 0.001$) than the controls. Among smokers, there was no significant difference between groups in the number of cigarettes smoked.

Table 1. Distribution of select characteristics in esophageal cancer cases and controls.

	Group		P value ^a
	Case (N = 500)	Control (N = 500)	
Gender (N, %)			0.527
Male	404 (80.8)	396 (79.2)	
Female	96 (19.2)	104 (20.8)	
Age (years) (N, %)			0.96
<50	74 (14.8)	70 (14.0)	
~50	170 (34.0)	174 (34.8)	
~60	172 (34.4)	168 (33.6)	
~70	84 (16.8)	88 (17.6)	
Smoking status (N, %)			<0.001
Non-smoker	194 (38.8)	354 (70.8)	
Smoker	306 (61.2)	146 (29.2)	
Pack-years smoked (N, %)			0.065
<30	137 (44.8)	52 (35.6)	
≥30	169 (55.2)	94 (64.4)	
Alcohol drinking (N, %)			<0.001
Non alcohol drinker	369 (73.8)	423 (84.6)	
Alcohol drinker	131 (26.2)	77 (15.4)	

^a2-sided χ^2 test.

IGFBP-3 genotype distributions and association with ESCC risk

Genotype distributions of *IGFBP-3* rs2270628 in the cases and controls are shown in Table 2. Observed genotype frequencies for the polymorphisms were in Hardy-Weinberg equilibrium in the control group ($P = 0.77$). The genotype frequencies were 64.2% CC, 30.6%

CT, and 5.2% TT in cancer cases and 70.4% CC, 26.8% CT, and 2.8% TT in controls. Logistic regression analyses further revealed that TT homozygotes showed a significant 2.07-fold increased risk of ESCC (95%CI = 1.05-4.09, P = 0.035).

Table 2. Genotype frequencies of *IGFBP-3* among cases and controls and their association with esophageal cancers.

<i>IGFBP-3</i> genotype	Control (N = 500)		Case (N = 500)		OR (95%CI) ^a	P value
	N (%)	N (%)	N (%)	N (%)		
CC	352 (70.4)		321 (64.2)			
CT	134 (26.8)		153 (30.6)		1.25 (0.94-1.66)	0.120
TT	14 (2.8)		26 (5.2)		2.07 (1.05-4.09)	0.035

OR = odds ratio; 95%CI = 95% confidence interval. ^aData were calculated by unconditional logistic regression, with the CC genotype as the reference group and adjusted for gender, age, and smoking and drinking status.

Association of ESCC risk and *IGFBP-3* polymorphisms by smoking status

Stratification analysis was conducted to evaluate the association between the *IGFBP-3* SNP rs22706228 and ESCC risk by smoking status. As shown in Table 3, compared with carriers of the CC genotype, those with the CT or TT genotypes showed a 37% increased risk in non-smokers (OR = 1.37, 95%CI = 1.01-2.00, P < 0.001). Carriers of the CT or TT genotypes showed a significant 5.93-fold increased risk in smokers (OR = 5.93, 95%CI = 3.78-9.31, P < 0.001).

Table 3. Risk of esophageal cancer association with *IGFBP-3* genotype by smoking status.

Smoking status	<i>IGFBP-3</i> genotype			
	CC ^a	OR (95%CI) ^b	CT+TT ^a	OR (95%CI) ^b
Non-smoker	124/247	1.00 (reference)	70/107	1.37 (1.01-2.00)
Smoker	197/105	4.16 (2.90-5.96)	109/41	5.93 (3.78-9.31)
<30 pack-years	92/35	5.82 (3.62-9.34)	45/17	5.61 (3.00-10.45)
≥30 pack-years	105/70	3.11 (2.07-4.69)	64/24	1.88 (1.07-3.59)

OR = odds ratio; 95%CI = 95% confidence interval. ^aNumber of cases/number of controls. ^bData were calculated by logistic regression, with the CC genotype as the reference group and adjusted for age, gender, and drinking status.

Association of ESCC risk and *IGFBP-3* polymorphisms by drinking status

The same stratification analysis was executed to evaluate the association between the *IGFBP-3* SNP rs22706228 and ESCC risk by drinking status. As shown in Table 4, compared with carriers of the CC genotype, carriers of the CT or TT genotypes showed a 39% increased risk in non-drinkers (OR = 1.39, 95%CI = 1.03-1.88, P < 0.05). Carriers of the CT or TT genotypes showed a 99% increased risk in drinkers (OR = 1.99, 95%CI = 1.17-3.40, P < 0.05).

Table 4. Risk of esophageal cancer association with *IGFBP-3* genotype by drinking status.

Drinking status	<i>IGFBP-3</i> genotype			
	CC ^a	OR (95%CI) ^b	CT+TT ^a	OR (95%CI) ^b
Non-drinker	234/300	1.00 (reference)	135/123	1.39 (1.03-1.88)
Drinker	87/52	1.80 (1.20-2.72)	44/25	1.99 (1.17-3.40)

OR = odds ratio; 95%CI = 95% confidence interval. ^aNumber of cases/number of controls. ^bData were calculated by logistic regression, with the CC genotype as the reference group and adjusted for age, gender, and smoking status.

DISCUSSION

This is the first study to investigate the association between the *IGFBP-3* SNP rs2270628 and risk of ESCC. This hospital-based case-control study in a Chinese Han population indicated that the rs2270628 T allele was associated with a significantly increased risk of ESCC.

IGFBP-3 is located on chromosome 7p13 and contains 5 exons. The protein binds more than 90% of circulating IGF-I and forms a ternary complex with an acid-labile subunit. *IGFBP-3* regulates normal and malignant cell growth through mechanisms that are dependent or independent of IGF-I binding (Baxter, 2000, 2001; Khandwala et al., 2000; Firth and Baxter, 2002). Previous studies have demonstrated that *IGFBP-3* has anti-proliferative and pro-apoptotic effects, which are mediated through a specific cell surface receptor (Firth and Baxter, 2002). Epidemiological studies have shown that high circulating levels of *IGFBP-3* were associated with a low risk of development of several common cancers, including prostate, colorectal, breast, and lung cancers, but these associations were modest and varied between different cancer sites (Renehan et al., 2004). In addition, a study conducted in the US reported that colorectal cancer patients with the *IGFBP-3* G2133C variant allele had lower circulating *IGFBP-3* levels and an increased risk of colorectal cancer (Le Marchand et al., 2005). Another study reported that the percentage of positive *IGFBP-3* staining in ESCC tissues was significantly lower than that in non-malignant tissues; *IGFBP-3* expression was inversely correlated with the clinical pathological classification of ESCC, and patients with low levels of *IGFBP-3* expression showed reduced overall survival (Zhao et al., 2012). These inverse associations between *IGFBP-3* levels and risk of ESCC, breast cancer, and colorectal cancer indicate that *IGFBP-3* plays a protective role against cancer.

In a twin study, both genetic and lifestyle factors affected the levels of IGF-I and *IGFBP-3*, while genetic variation accounted for 60% of *IGFBP-3* levels in serum (Harrela et al., 1996; Holmes et al., 2002); however, the specific loci involved were not determined. Polymorphisms that alter gene expression or protein function may result in a relative increase or decrease in circulating *IGFBP-3* levels and may influence carcinogenesis. Recent studies have examined *IGFBP-3* SNPs, but the number of studies on SNP rs2270628 is very limited. Su et al. (2010) found that circulating IGF-I levels were not associated with proliferative benign breast disease risk, although the minor allele of the *IGFBP-3* SNP rs2270628 was associated with higher IGF-I levels. In a cross-sectional study of 1121 control subjects, Tamimi et al. (2007) concluded that none of the genetic variations within *IGFBP-3* were significantly associated with mammographic density. The Breast and Prostate Cohort Consortium genotyped *IGF-I*, *IGFBP-1*, and *IGFBP-3* SNPs in 6912 breast cancer cases and 8819 matched controls and observed no statistical associations between rs2270628 and prostate cancer risk (Gu et al., 2010; Schumacher et al., 2010; Tsilidis et al., 2013). In a pooled analysis, Terry et al. (2009) identified that *IGFBP-3* SNP rs2270628 was associated with IGF-I plasma levels as well as with an increased risk of ovarian cancer in UK and New England (USA) populations. Similar results were reported in other studies (Pearce et al., 2011; Malaguarnera et al., 2012). No association was observed between rs2270628 and endometrial cancer risk (McGrath et al., 2011). A population-based study demonstrated that the *IGFBP-3* SNP rs2270628 was not associated with EAC (McElholm et al., 2010). However, in the current study in a Chinese Han population, we found that rs2270628 was associated with a significantly increased risk of ESCC. The discrepancies in the results may be related to different hereditary backgrounds and

etiologies. For example, the frequency of the rs2270628 T allele in the Chinese Han population is 0.15, but is 0.19 in Caucasians (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>). Another possibility may be related to the different etiologies of the primary tumors, because the epidemiology and pathogenesis of ESCC and EAC are different (Anonymous, 2008).

Although our finding of an association between *IGFBP-3* rs2270628 and ESCC is novel, the evidence is preliminary and further studies are needed to confirm our results. Interestingly, compared with subjects with the CC genotype, those with the T allele showed a significantly increased risk of ESCC in non-smokers and smokers. This may be because cigarette smoking is more of a promoter of carcinogenesis than a mutagenic initiator (Sharp et al., 2001; Znaor et al., 2003; Castellsagué et al., 2004; Freedman et al., 2007; Holmes and Vaughan, 2007; Ishiguro et al., 2009; Wu et al., 2006; 2009). Similarly, carriers of the T allele had a significantly increased risk of ESCC in non-drinkers and drinkers compared with subjects with the CC genotype, indicating that alcohol metabolism may be associated with esophageal carcinogenesis (Hiyama et al., 2007).

Limitations of this study include the following. First, the sample size of this study was small. Second, the results were not adjusted for other probable risk factors for ESCC (e.g., dietary habits and lifestyle factors) because this information was not available. Third, because the control group was from a hospital cohort, it may not reflect the true genotype distribution of the Chinese Han population. In addition, our study lacked information on the phenotype of serum levels of IGFBP-3, limiting further analysis of the genotype-phenotype correlation.

In conclusion, our study demonstrated that *IGFBP-3* SNP rs2270628 may contribute to the risk of ESCC in the Chinese Han population.

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