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Association of c.461G>A genetic variant of *OGG1* gene with pancreatic cancer susceptibility in Chinese

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ABSTRACT. This study aimed to evaluate the potential association of single nucleotide polymorphisms of the 8-oxoguanine DNA glycosylase gene (*OGG1*) with susceptibility to pancreatic cancer (PC). A total of 764 Chinese Han subjects were recruited in this study. The polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing methods were used to detect the genotype of c.461G>A genetic variant of *OGG1*. The genotype and allele frequencies were statistically different in PC patients compared with cancer-free controls. The AA genotype was statistically associated with increased PC susceptibility compared to GG wild genotype (AA *vs* GG, OR = 2.62, 95%CI = 1.48-4.63, χ^2 = 11.46, P = 0.001). Allele A could contribute to the increased risk of PC (A *vs* G, OR = 1.35, 95%CI = 1.08-1.69, χ^2 = 6.86, P = 0.009). Our data indicated that the c.461G>A genetic variant of the *OGG1* gene was associated with susceptibility to PC in a Chinese Han population.

Key words: Pancreatic cancer; *OGG1* gene; Risk factors; Single nucleotide polymorphisms; Cancer susceptibility

INTRODUCTION

Pancreatic cancer (PC) is one of the most common malignancies in the world (Jemal et al., 2007; Li et al., 2009; Tanaka et al., 2011; Nakao et al., 2012). It is a major and constantly growing health burden. The 5-year survival rate is less than 5% (Jemal et al., 2007; Tanaka et al., 2011; Nakao et al., 2012). The possible risk factors for PC include genetic variants, gender, age, alcohol consumption, smoking status, overweight, diabetes mellitus, body mass index, and family history of PC (Li et al., 2006; Lowenfels and Maisonneuve, 2006; Larsson et al., 2007; Luo et al., 2007; McWilliams et al., 2008; Li et al., 2009; Nakao et al., 2012). It is widely accepted that genetic factors play key roles in the pathogenesis of PC. Many studies have indicated that the 8-oxoguanine DNA glycosylase gene (*OGG1*) is an important candidate gene for influencing PC risk (Li et al., 2007; Duell et al., 2008; McWilliams et al., 2008; Li et al., 2009; Zhang et al., 2011; Nakao et al., 2012; Yan et al., 2014; Chen et al., 2014). Previous published studies investigated the potential association of single nucleotide polymorphisms (SNPs) in *OGG1* with PC risk, such as serine (Ser) 326 cysteine (Cys) and arginine (Arg) 299 glutamine (Gln) (Li et al., 2007; McWilliams et al., 2008; Li et al., 2009; Zhang et al., 2011; Nakao et al., 2012). However, to date, no similar studies have reported the potential association of c.461G>A genetic variant of *OGG1* with the risk factors of PC. Thus, this study aimed to determine the distribution of this genetic variant of the *OGG1* gene and to assess its effects on PC.

MATERIAL AND METHODS

Study population

A total of 764 Chinese Han subjects were recruited in this study from January 2009 to November 2012 from the Chinese PLA General Hospital. The PC patients had been diagnosed with histologically and pathologically confirmed primary PC. The cancer-free controls were frequency matched with the PC patients in terms of gender and age, excluding those with a history of PC and other medical diseases. All subjects were genetically unrelated Chinese Han individuals. The general characteristics of the subjects are summarized in Table 1, including gender, age, alcohol consumption, smoking status, diabetes mellitus, body mass index and family history of PC. The ethics committee of the Chinese PLA General Hospital approved the protocol of this study. Informed written consent was obtained from all individuals.

Genotyping

Genomic DNA was extracted from peripheral venous blood of all subjects using the Axygen DNA isolation kit (Axygen, Union City, CA, USA). The specific polymerase chain reaction (PCR) primers (F: 5'-GGTACCTAGGATCTGACCTGTGG-3'; R: 5'-AAGCCATGGTA GGTGACATCATC-3') were designed by the Primer Premier 5.0 software. PCR was performed in a total volume of 20 μL, containing 50 ng template DNA, 1X buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl), 0.25 μM primers, 2.0 mM $MgCl_2$ (Bioteke Corporation, Beijing, China), 0.25 mM dNTPs (Bioteke Corporation), and 0.5 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 32 s, 60.5°C for 32 s and 72°C for 32 s, and then a final extension at 72°C for

5 min. The c.461G>A genetic variant of *OGG1* was genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method. Following the supplier manual, the amplified PCR products (5 µL) were digested with 5 U *Aci*I restriction enzyme (MBI Fermentas, St. Leon-Rot, Germany) at 37°C for 10 h. The digested products were verified by agarose gel electrophoresis with ethidium bromide and visualization of bands under UV light. To confirm the genotyping results based on PCR-RFLP, approximately 10% of samples were randomly verified by DNA sequencing (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA, USA).

Table 1. Characteristics of the pancreatic cancer (PC) cases and controls.

Characteristics	PC cases (N)		Controls (N)		χ^2 values	P values*
	N = 382	%	N = 382	%		
Gender (N)					2.61	0.107
Male	245	64.14	266	69.63		
Female	137	35.86	116	30.37		
Age (years)					1.21	0.272
means ± SD	56.76 ± 15.43		57.22 ± 16.45			
< 55	154	40.31	169	44.24		
≥ 55	228	59.69	213	55.76		
Smoking status					1.36	0.243
Never	279	73.04	293	76.70		
Ever	103	26.96	89	23.30		
Alcohol consumption					1.25	0.263
Never	288	75.39	301	78.80		
Ever	94	24.61	81	21.20		
Body mass index					0.19	0.664
< 23	197	51.57	203	53.14		
≥ 23	185	48.43	179	46.86		
Diabetes mellitus (N)					0.88	0.347
Yes	112	29.32	124	32.46		
No	270	70.68	258	67.54		
Family history of PC (N)					0.54	0.462
Yes	49	12.83	56	14.66		
No	333	87.17	326	85.34		

*P values calculated by chi-square (χ^2) test.

Statistical analyses

All statistical analyses were performed using the SPSS 15.0 software (SPSS Inc.; Chicago, IL, USA). The chi-square (χ^2) test was utilized to assess the Hardy-Weinberg equilibrium in genotype and allele frequencies, and the differences in general clinical characteristics of the populations studied. The odds ratios (ORs) with their 95% confidence intervals (CIs) for the influence of the c.269C>A genetic variant on PC susceptibility were estimated using an unconditional logistic regression analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS

Subject characteristics

The general clinical characteristics of 382 PC patients and 382 cancer-free controls are given in Table 1. The PC patients and cancer-free controls were comparable with regard to the distribution of gender, age, alcohol consumption, smoking status, diabetes mellitus, body mass index, and family history of PC (all $P > 0.05$, Table 1).

Identification of *OGG1* genetic variants

Through PCR-RFLP and DNA sequencing, we detected the c.461G>A genetic variant of the *OGG1* gene in exon 3. On the basis of sequence analyses, we found that this genetic variant was a non-synonymous mutation, which caused a G to A mutation and resulted in arginine (Arg) to histidine (His) amino acid replacement (p.Arg154His, reference sequences GenBank IDs: NG_012106.1, NM_002542.5, and NP_002533.1). The PCR-amplified products were digested with *Aci*I restriction enzyme and divided into three genotypes: GG (159 and 83 bp), GA (242, 159 and 83 bp) and AA (242 bp). The genotype distributions in the PC patients and cancer-free controls were in accordance with Hardy-Weinberg equilibrium (all P > 0.05, Table 2). Table 2 shows the genotype and allele frequencies of this genetic variant in PC patients and cancer-free controls. Allele G and genotype GG were predominant in the subjects studied. The allele frequencies in PC patients (G, 68.98%; A, 31.02%) were significantly different from those of cancer-free controls (G, 75.00%; A, 25.00%; $\chi^2 = 6.87$, P = 0.009, Table 2). The genotype frequencies of PC patients were not consistent with healthy controls, with the differences being statistically significant ($\chi^2 = 11.68$, P = 0.003, Table 2).

Table 2. Genotype and allele frequencies of *OGG1* c.461G>A genetic polymorphism in pancreatic cancer (PC) patients and healthy controls.

Groups	Genotype frequencies (%)			Allele frequencies (%)		χ^2	P
	GG	GA	AA	G	A		
PC patients (N = 382)	190 (49.74)	147 (38.48)	45 (11.78)	527 (68.98)	237 (31.02)	3.88	0.1436
Healthy controls (N = 382)	210 (54.98)	153 (40.05)	19 (4.97)	573 (75.00)	191 (25.00)	1.77	0.4128
Total (N = 764)	400 (52.35)	300 (39.27)	64 (8.38)	1100 (71.99)	428 (28.01)	0.53	0.7672
	$\chi^2 = 11.68$, P = 0.003			$\chi^2 = 6.87$, P = 0.009			

*P values calculated by chi-square (χ^2) test.

Association between *OGG1* genetic variants and pancreatic cancer risk

Table 3 summarizes the potential association between the c.461G>A genetic variant and PC risk in the populations studied. There was a significantly increased risk of PC in the homozygote comparison (AA *vs* GG: OR = 2.62, 95%CI = 1.48-4.63, $\chi^2 = 11.46$, P = 0.001), recessive model (AA *vs* GA/GG: OR = 2.55, 95%CI = 1.46-4.45, $\chi^2 = 11.51$, P = 0.001) and allele contrast (A *vs* G: OR = 1.35, 95%CI = 1.08-1.69, $\chi^2 = 6.86$, P = 0.009). However, a significantly increased risk of PC was not found in the homozygote comparison (GA *vs* GG: OR = 1.06, 95%CI = 0.79-1.43, $\chi^2 = 0.15$, P = 0.694) and dominant model (AA/GA *vs* GG: OR = 1.23, 95%CI = 0.93-1.64, $\chi^2 = 2.10$, P = 0.148).

Table 3. Association between *OGG1* c.461G>A genetic polymorphism and pancreatic cancer (PC) risk.

Comparisons	OR (95%CI)	χ^2 value	P values*
AA *vs* GG (Homozygote comparison)	2.62 (1.48-4.63)	11.46	0.001
GA *vs* GG (Heterozygote comparison)	1.06 (0.79-1.43)	0.15	0.694
AA/GA *vs* GG (Dominant model)	1.23 (0.93-1.64)	2.10	0.148
AA *vs* GA/GG (Recessive model)	2.55 (1.46-4.45)	11.51	0.001
A *vs* G (Allele contrast)	1.35 (1.08-1.69)	6.86	0.009

OR = odds ratio; 95%CI = 95% confidence interval. *P values calculated by chi-square (χ^2) test.

DISCUSSION

PC is one of the leading causes of cancer death for both men and women worldwide. It arises from complex interactions between genetic and environmental factors. It is generally accepted that genetic factors play key functions in the development of PC (Lin et al., 2001; Lowenfels and Maisonneuve, 2003; Duell et al., 2008; Landi, 2009; Chu et al., 2010; Nitsche et al., 2011; Chen et al., 2014). Emerging studies indicate that *OGG1* is an important candidate gene for PC risk (Duell et al., 2008; McWilliams et al., 2008; Li et al., 2007, 2009; Zhang et al., 2011; Nakao et al., 2012; Chen et al., 2014). Recently, the potential association of *OGG1* genetic variants with the risk of PC has been evaluated, i.e., Arg299Gln and Ser326Cys (McWilliams et al., 2008; Li et al., 2007, 2009; Zhang et al., 2011; Nakao et al., 2012). The findings from these observations still remain conflicting rather than conclusive for the association with risk of PC. Zhang et al. (2011) reported a statistically significantly increased risk for the variant allele (326Cys) of Ser326Cys genetic variant compared with the wild-type allele (326Ser) (Ser/Cys or Cys/Cys *vs* Ser/Ser: OR = 1.57, 95%CI 1.04, 2.39). Zhang et al. (2011) indicated that the genetic variants of the *OGG1* gene could influence PC risk. Li et al. (2009), found a weak interaction of Ser326Cys polymorphism CC/CG genotype with diabetes in increased risk of PC. McWilliams et al. (2008) suggested that there are no significant differences in PC risk for Arg299Gln and Ser326Cys genetic variants. Nakao et al. (2012) found no significant association with PC risk for Ser326Cys genetic variant. In the present study, we detected the association of c.461G>A genetic variant with the risk of PC in a Chinese Han population for the first time. We found significant differences in the allele and genotype frequencies between PC patients and cancer-free controls for this SNP (Table 2). The genotype AA was significantly associated with increased risk of developing PC compared to wild genotype GG and GA/GG carriers (Table 3). Our data suggested that allele A and genotype AA could contribute to increasing the risk of PC (Table 3). The c.461G>A genetic variant of the *OGG1* gene was significantly associated with susceptibility to PC in Chinese Han people. Our findings provide more evidence of the role of the *OGG1* gene in the pathogenesis of PC, and thus, genetic variants of *OGG1* could be useful molecular biomarkers for evaluating the risk of PC. Further studies will be needed to confirm these findings in larger different populations and to explain the underling molecular mechanisms between *OGG1* genetic variants and the risk of PC.

Conflicts of interest

The authors declare no conflict of interest.

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