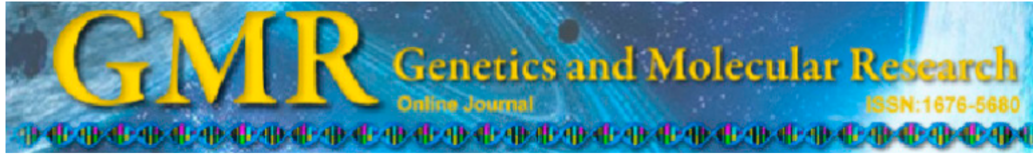

Influence of the c.1517G>C genetic variant in the *XRCC1* gene on pancreatic cancer susceptibility in a Chinese population

Z.M. Zhao*, C.G. Li*, M.G. Hu, Y.X. Gao and R. Liu

Department of Surgical Oncology, The Chinese PLA General Hospital, Beijing, China

*These authors contributed equally to this study.
Corresponding author: Z.M. Zhao
E-mail: zhi_ming_zhao@sina.com

Genet. Mol. Res. 13 (2): 4466-4472 (2014)
Received May 15, 2013
Accepted January 14, 2014
Published June 16, 2014
DOI http://dx.doi.org/10.4238/2014.June.16.5

ABSTRACT. We investigated the influence of the c.1517G>C genetic variant in the X-ray repair complementing group 1 gene (*XRCC1*) on pancreatic cancer (PC) susceptibility in Chinese patients. A total of 390 PC patients and 392 controls were enrolled in this case-control study. The genotypes of c.1517G>C genetic variants were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Our findings suggested that the allele and genotype frequencies in PC patients were significantly different from those in cancer-free controls. The CC genotype was associated with an increased risk of PC compared to the wild-type GG genotype (odds ratio = 2.43, 95% confidence interval 1.43-4.13, χ^2 = 11.19, P = 0.001). The C allele may contribute to the development of PC (C *vs* G, odds ratio = 1.32, 95% confidence interval 1.06-1.64, χ^2 = 6.25, P = 0.012). Results from this study indicate that the c.1517G>C genetic variant of the *XRCC1* gene is significantly associated with PC

 ©FUNPEC-RP www.funpecrp.com.br

susceptibility in the Chinese population.

Key words: Pancreatic cancer; *XRCC1* gene; Cancer susceptibility; Genetic variant

INTRODUCTION

Pancreatic cancer (PC) is one of the leading causes of cancer death worldwide. While the incidence of PC is increasing, the 5-year survival rate remains below 5% (Jemal et al., 2007; Tanaka et al., 2011; Nakao et al., 2012). Possible risk factors for PC include advanced age, alcohol consumption, tobacco smoking, overweight, high body mass index, diabetes mellitus, and a family history of PC (Lowenfels and Maisonneuve, 2003; Li et al., 2006; Lowenfels and Maisonneuve, 2006; Larsson et al., 2007; Luo et al., 2007; Nakao et al., 2012). Previous reports have identified a positive association between genetic variants and PC risk (Lin et al., 2001; Duell et al., 2002; Li et al., 2006, 2007; Nakao et al., 2012). However, the exact mechanism of PC remains poorly understood. Several studies have reported that the human X-ray repair complementing group 1 gene (*XRCC1*) is an important candidate gene that influences PC risk (Duell et al., 2002; Jiao et al., 2006; Li et al., 2006, 2007; Wang et al., 2006; McWilliams et al., 2008; Shen et al., 2011; Nakao et al., 2012; Jiang et al., 2013; Yan et al., 2013; Chen et al., 2014); the potential associations between *XRCC1* genetic variants and PC risk have been analyzed (Duell et al., 2002; Li et al., 2006, 2007; McWilliams et al., 2008; Nakao et al., 2012). Some of these genetic variants, such as Arg194Trp, Arg280His, and Arg399Gln, were significantly associated with PC (Duell et al., 2002; Li et al., 2006, 2007; Nakao et al., 2012). However, there have been no similar studies examining the association between the c.1517G>C genetic variant in the *XRCC1* gene and PC risk. Therefore, we investigated this genetic variant to determine its influence on PC susceptibility.

MATERIAL AND METHODS

Study subjects

A total of 390 patients with diagnosed and histologically confirmed primary PC and 392 cancer-free controls were recruited for participation in the study from the Chinese PLA General Hospital between January 2008 and January 2013. All subjects were genetically unrelated Chinese individuals of Han ethnicity and lived in Beijing, China. Controls were frequency-matched to PC patients for age and gender. Subjects with a previous medical history of cancer or other medical diseases were excluded. Clinical characteristics for the populations studied are summarized in Table 1, including age, gender, smoking status, alcohol consumption, diabetes mellitus, body mass index (BMI), and family history of PC. The study was approved by the ethical review committee at the Chinese PLA General Hospital. All study participants signed written informed consent.

Genotyping

Genomic DNA was isolated from peripheral blood samples using the DNA Blood

 ©FUNPEC-RP www.funpecrp.com.br

Mini kit (QIAGEN; Hilden, Germany) according to manufacturer instructions. Primers for polymerase chain reaction (PCR) were designed using the Primer Premier 5.0 software. Table 2 shows the primer sequences, annealing temperature, amplification fragment size, and region. The PCR reaction was carried out in a 20 μL mixture, containing 50 ng template DNA, 1X buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.25 μM primers, 2.0 mM $MgCl_2$, 0.25 mM dNTPs, and 0.5 U *Taq* DNA polymerase (Promega; Madison, WI, USA). PCR amplification was achieved using 1 cycle at 94°C for 5 min, followed by 32 cycles at 94°C for 30 s, 58.4°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The c.1517G>C genetic variant genotyping was determined using the PCR-restriction fragment length polymorphism (PCR-RFLP) method. PCR amplified products were digested with 5 U *Hae*III restriction enzyme (MBI Fermentas; St. Leon-Rot, Germany) at 37°C for 10 h and electrophoresed on an agarose gel, and then visualized under ultraviolet light. To confirm the PCR-RFLP genotyping results, approximately 15% of the subjects were randomly selected for DNA sequencing (ABI3730xl DNA Analyzer; Applied Biosystems; Foster City, CA).

Statistical analysis

The chi-squared (χ^2) test was used to assess Hardy-Weinberg equilibrium (HWE) in genotype frequencies and the differences in clinical characteristics of the studied populations. Unconditional logistic regression analysis was utilized to estimate the odds ratios (ORs) with their 95% confidence intervals (CIs) to determine the influence of having the c.1517G>C genetic variant on PC susceptibility. A P value < 0.05 was considered to be statistically significant. All statistical analyses were analyzed using SPSS 15.0 (SPSS Inc.; Chicago, IL, USA).

RESULTS

Subjects characteristics

A total of 782 subjects were enrolled in this case-control study, including 390 PC cases and 392 cancer-free controls. Table 1 shows the subject characteristics. Our data suggest that the PC patients were comparable to the cancer-free controls in terms of the distribution of gender, age, alcohol consumption, smoking status, diabetes mellitus, BMI, and family history of PC (all P > 0.05, Table 1). The distributions of genotype in PC patients and cancer-free controls did not significantly deviate from HWE (all P values > 0.05, Table 3).

Identification of *XRCC1* genetic variant

Through PCR-RFLP and DNA sequencing methods, we identified the c.1517G>C genetic variant in the *XRCC1* gene. The results of sequence analyses suggested that this genetic variant represents a non-synonymous mutation caused by G to C mutations in exon 14 of the *XRCC1* gene. This change results in a glycine (Gly) to alanine (Ala) amino acid replacement (p.Gly506Ala, reference sequences GenBank IDs: NC_000019.9, NM_006297.2, and NP_006288.2). Amplified PCR products were digested with the *Hae*III restriction enzyme and 3 genotypes were detected, GG (231 base pairs, bp), GC (231, 163, and 68 bp), and CC (163 and 68 bp) (Table 2). Genotype and allele frequencies of PC patients and cancer-free controls

are shown in Table 3. The G-allele and GG-genotype were predominant in the studied subjects (Table 3). The allele frequencies in PC patients (G, 67.44%; C, 32.56%) were significantly different from those of cancer-free controls (G, 73.21%; C, 26.79%; $\chi^2 = 6.26$, P = 0.012). The genotype frequencies between the PC patients and cancer-free controls were significantly different ($\chi^2 = 11.87$, P = 0.003, Table 3).

Table 1. Characteristics of pancreatic cancer cases and healthy controls.

Characteristics	Cases (N = 390)	Controls (N = 392)	χ^2 values	P values*
Gender (N)			1.13	0.288
Male	306 (78.46)	295 (75.26)		
Female	84 (21.54)	97 (24.74)		
Age (years)			1.81	0.178
Mean ± SD	59.22 ± 13.39	58.67 ± 14.61		
<55	138 (35.38)	157 (40.05)		
≥55	252 (64.62)	235 (59.95)		
Alcohol consumption			0.43	0.510
Never	307 (78.72)	316 (80.61)		
Ever	83 (21.28)	76 (19.39)		
Smoking status			0.28	0.596
Never	293 (75.13)	288 (73.47)		
Ever	97 (24.87)	104 (26.53)		
Diabetes mellitus (N)			2.09	0.148
Yes	105 (26.92)	124 (31.63)		
No	285 (73.08)	268 (68.37)		
BMI			0.27	0.606
<23	225 (57.69)	219 (55.87)		
≥23	165 (42.31)	173 (44.13)		
Family history of PC (N)			2.82	0.093
Yes	72 (18.46)	55 (14.03)		
No	318 (81.54)	337 (85.97)		

PC = pancreatic cancer; BMI = body mass index; *P values calculated by chi-square (χ^2) test.

Table 2. PCR primers and PCR-RFLP method for investigating the c.1517G>C genetic variant of the *XRCC1* gene.

Primer sequences	Annealing temperature (°C)	Amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
5'-CCCAGCTGAGAACTGAGAAGAG-3' 5'-TGCTCACTCTCTACTCCTCTTGG-3'	58.4	231	Exon14	*Hae*III	GG: 231 GC: 231, 163, 68 CC: 163, 68

PCR = polymerase chain reaction; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

Table 3. Genotype and allele frequencies of the c.1517G>C genetic variant of the *XRCC1* gene in pancreatic cancer (PC) patients and healthy subjects.

Groups	Genotype frequencies (%)			Allele frequencies (%)		χ^2	P
	GG	GC	CC	G	C		
PC patients (N = 390)	187 (47.95)	152 (38.97)	51 (13.08)	526 (67.44)	254 (32.56)	4.94	0.084
Healthy subjects (N = 392)	205 (52.29)	164 (41.84)	23 (5.87)	574 (73.21)	210 (26.79)	1.74	0.418
Total (N = 782)	392 (50.13)	316 (40.41)	74 (9.46)	1100 (70.33)	464 (29.67)	0.79	0.675
	$\chi^2 = 11.87$, P = 0.003			$\chi^2 = 6.26$, P = 0.012			

 ©FUNPEC-RP www.funpecrp.com.br

Influence of the *XRCC1* genetic variant on PC susceptibility

Table 4 shows the influence of the c.1517G>C genetic variant in the *XRCC1* gene on PC susceptibility. A significantly increased risk of PC was observed in the homozygote comparison (CC *vs* GG: OR = 2.43, 95%CI = 1.43-4.13, χ^2 = 11.19, P = 0.001), recessive model (CC *vs* GC/GG: OR = 2.41, 95%CI = 1.44-4.04, χ^2 = 11.85, P = 0.001), and allele contrast (C *vs* G: OR = 1.32, 95%CI = 1.06-1.64, χ^2 = 6.25, P = 0.012). We detected no statistical association between this genetic variant and PC risk in the heterozygote comparison (GC *vs* GG: OR = 1.02, 95%CI = 0.76-1.37, χ^2 = 0.01, P = 1.367) and the dominant model (CC/GC *vs* GG: OR = 1.19, 95%CI = 0.90-1.58, χ^2 = 1.48, P = 0.224).

Table 4. Association between the c.1517G>C genetic variant of the *XRCC1* gene and pancreatic cancer (PC) risk.

Comparisons	OR (95%CI)	χ^2 value	P values*
CC *vs* GG (homozygote comparison)	2.43 (1.43-4.13)	11.19	0.001
GC *vs* GG (heterozygote comparison)	1.02 (0.76-1.37)	0.01	1.367
CC/GC *vs* GG (dominant model)	1.19 (0.90-1.58)	1.48	0.224
CC *vs* GC/GG (recessive model)	2.41 (1.44-4.04)	11.85	0.001
C *vs* G (allele contrast)	1.32 (1.06-1.64)	6.25	0.012

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

DISCUSSION

Various studies have shown that PC is a common solid cancer caused by complex interactions between genetic and environmental factors (Lin et al., 2001; Duell et al., 2002; Li et al., 2006, 2007; Rizzato et al., 2011; Nakao et al., 2012; Tong et al., 2012; Zhang et al., 2012). It is well known that genetic factors play key roles in PC pathogenesis (Lin et al., 2001; Lowenfels and Maisonneuve, 2003; Duell et al., 2008; Landi, 2009; Chu et al., 2010; Nitsche et al., 2011). It has also been suggested that the *XRCC1* gene is one of the most important candidate genes influencing the susceptibility to PC (Duell et al., 2002; Li et al., 2006, 2007; McWilliams et al., 2008; Nakao et al., 2012), and its genetic polymorphisms are involved in modulating PC risk (Duell et al., 2002; Hung et al., 2005; Li et al., 2006; Mandal et al., 2010). Nakao et al. observed that *XRCC1* Arg399Gln was significantly associated with PC risk in a Japanese population (Nakao et al., 2012). Li et al. (2006) reported that *XRCC1* Arg194Trp affects the clinical prognosis of patients with PC. McWilliams et al. reported no significant differences in PC risk for the Arg194Trp, Arg280His, and Arg399Gln genetic variants in the *XRCC1* gene (McWilliams et al., 2008). However, these observations are conflicting, limiting the understanding of PC risk. In this case-control study, we first evaluated the influence of c.1517G>C genetic variant in the *XRCC1* gene on PC risk in a Chinese Han population through association analysis. We found that the distributions of genotype and allele frequencies in PC patients were significant different from those of cancer-free controls (P < 0.05, Table 3). The CC genotype was significantly associated with an increased risk of PC compared with the GG genotype and GC/GG carriers (P < 0.01, Table 4). The C allele and CC genotype of c.1517G>C genetic variant may contribute to an increased risk of PC. Our data indicate that c.1517G>C genetic variant in the *XRCC1* gene is significantly associated with PC risk in the

 ©FUNPEC-RP www.funpecrp.com.br

Chinese Han population, and may be used as a molecular marker for detecting susceptibility to PC. Further functional studies in larger populations are necessary to confirm our findings and to explain the molecular mechanisms influencing genetic variants on PC risk.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Chen H, Tang C, Liu M, Zhou B, et al. (2014). Association of XRCC1 gene single nucleotide polymorphisms and susceptibility to pancreatic cancer in Chinese. *Tumour Biol.* 35: 27-32.

Chu D, Kohlmann W and Adler DG (2010). Identification and screening of individuals at increased risk for pancreatic cancer with emphasis on known environmental and genetic factors and hereditary syndromes. *JOP* 11: 203-212.

Duell EJ, Holly EA, Bracci PM, Wiencke JK, et al. (2002). A population-based study of the Arg399Gln polymorphism in X-ray repair cross-complementing group 1 (XRCC1) and risk of pancreatic adenocarcinoma. *Cancer Res.* 62: 4630-4636.

Duell EJ, Bracci PM, Moore JH, Burk RD, et al. (2008). Detecting pathway-based gene-gene and gene-environment interactions in pancreatic cancer. *Cancer Epidemiol. Biomarkers Prev.* 17: 1470-1479.

Hung RJ, Hall J, Brennan P and Boffetta P (2005). Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am. J. Epidemiol.* 162: 925-942.

Jemal A, Siegel R, Ward E, Murray T, et al. (2007). Cancer statistics, 2007. *CA Cancer J. Clin.* 57: 43-66.

Jiang H, Wu D, Ma D, Lin G, et al. (2013). Association between X-ray repair cross-complementation group 1 rs25487 polymorphism and pancreatic cancer risk. *Tumour Biol.* 34: 3417-3421.

Jiao L, Bondy ML, Hassan MM, Wolff RA, et al. (2006). Selected polymorphisms of DNA repair genes and risk of pancreatic cancer. *Cancer Detect. Prev.* 30: 284-291.

Landi S (2009). Genetic predisposition and environmental risk factors to pancreatic cancer: A review of the literature. *Mutat. Res.* 681: 299-307.

Larsson SC, Orsini N and Wolk A (2007). Body mass index and pancreatic cancer risk: A meta-analysis of prospective studies. *Int. J. Cancer* 120: 1993-1998.

Li D, Frazier M, Evans DB, Hess KR, et al. (2006). Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J. Clin. Oncol.* 24: 1720-1728.

Li D, Li Y, Jiao L, Chang DZ, et al. (2007). Effects of base excision repair gene polymorphisms on pancreatic cancer survival. *Int. J. Cancer* 120: 1748-1754.

Lin Y, Tamakoshi A, Kawamura T, Inaba Y, et al. (2001). An epidemiological overview of environmental and genetic risk factors of pancreatic cancer. *Asian Pac. J. Cancer Prev.* 2: 271-280.

Lowenfels AB and Maisonneuve P (2003). Environmental factors and risk of pancreatic cancer. *Pancreatology* 3: 1-7.

Lowenfels AB and Maisonneuve P (2006). Epidemiology and risk factors for pancreatic cancer. *Best Pract. Res. Clin. Gastroenterol.* 20: 197-209.

Luo J, Iwasaki M, Inoue M, Sasazuki S, et al. (2007). Body mass index, physical activity and the risk of pancreatic cancer in relation to smoking status and history of diabetes: a large-scale population-based cohort study in Japan - the JPHC study. *Cancer Causes Control* 18: 603-612.

Mandal RK, Mittal T, Kapoor R and Mittal RD (2010). NER and BER repair gene polymorphisms in a healthy north Indian cohort and comparison with different ethnic groups worldwide. *Asian Pac. J. Cancer Prev.* 11: 1601-1604.

McWilliams RR, Bamlet WR, Cunningham JM, Goode EL, et al. (2008). Polymorphisms in DNA repair genes, smoking, and pancreatic adenocarcinoma risk. *Cancer Res.* 68: 4928-4935.

Nakao M, Hosono S, Ito H, Watanabe M, et al. (2012). Selected polymorphisms of base excision repair genes and pancreatic cancer risk in Japanese. *J. Epidemiol.* 22: 477-483.

Nitsche C, Simon P, Weiss FU, Fluhr G, et al. (2011). Environmental risk factors for chronic pancreatitis and pancreatic cancer. *Dig. Dis.* 29: 235-242.

Rizzato C, Campa D, Giese N, Werner J, et al. (2011). Pancreatic cancer susceptibility loci and their role in survival. *PLoS One* 6: e27921.

Shen WD, Chen HL and Liu PF (2011). XRCC1 polymorphisms and pancreatic cancer: A meta-analysis. *Chin. J. Cancer Res.* 23: 165-170.

 ©FUNPEC-RP www.funpecrp.com.br

Tanaka M, Okazaki T, Suzuki H, Abbruzzese JL, et al. (2011). Association of multi-drug resistance gene polymorphisms with pancreatic cancer outcome. *Cancer* 117: 744-751.
Tong D, Zhou Y, Chen W, Deng Y, et al. (2012). T cell immunoglobulin- and mucin-domain-containing molecule 3 gene polymorphisms and susceptibility to pancreatic cancer. *Mol. Biol. Rep.* 39: 9941-9946.
Wang L, Lin DX, Lu XH, Miao XP, et al. (2006). Polymorphisms of the DNA repair genes XRCC1 and XPC: relationship to pancreatic cancer risk. *Wei Sheng Yan. Jiu.* 35: 534-536.
Yan D, Wang XY, Li HJ, Xu XJ, et al. (2013). Relationship between single nucleotide polymorphisms and its haplotype of X-ray repair cross complementing group 1 and susceptibility of pancreatic carcinoma. *Zhonghua Zhong. Liu Za Zhi.* 35: 472-477.
Zhang Z, Wang J, Shen B, Peng C, et al. (2012). The ABCC4 gene is a promising target for pancreatic cancer therapy. *Gene* 491: 194-199.