Interaction of polymorphisms in xeroderma pigmentosum group C with cigarette smoking and pancreatic cancer risk

XIAO-HUI LIANG[1*], DONG YAN[2*], JIA-XING ZHAO[2], WEI DING[2], XIN-JIAN XU[3] and XI-YAN WANG[4]

[1]Department of Hypertension, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830054; [2]Department of Hepatopancreatobiliary Surgery, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011; [3]Department of Pancreatic Surgery, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830054; [4]Xinjiang Research Institute of Cancer Prevention and Control, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011, P.R. China

Received January 15, 2018; Accepted June 8, 2018

DOI: 10.3892/ol.2018.9350

Abstract. The aim of the present study was to evaluate the association between xeroderma pigmentosum group C (XPC) polymorphisms and pancreatic cancer (PC) risk. A total of 7 XPC tagging SNPs (tag-SNPs) were selected from the International HapMap Project Databases (rs2228001A/C, rs2470353G/C, rs2228000C/T, rs3731114C/G, rs3729587G/C, rs2607775C/G and rs3731055G/A) and were genotyped in 205 patients with PC and 230 non-cancer control subjects using a SNaPshot assay. The C allelic gene frequency of rs2470353 was higher in patients with PC compared with that in the control group (P=0.003). Compared with the GG gene type, PC risk was increased in subjects with GC and GC+CC gene types (P=0.012 and P=0.006, respectively). PC risk increased 3.505-fold for the subjects who were heavy smokers (tobacco, ≥25 packets/year) with the GC+CC gene type (P=0.008). The G allelic gene frequency of rs2607775 was higher in PC patients compared with that in the control group (P=0.003). Compared with the CC gene type, PC risk increased in subjects with CG and CG+GG gene types (P=0.013 and P=0.005, respectively). Furthermore, PC risk increased 3.950-fold in subjects who were heavy smokers (tobacco, ≥25 packets/year) with the CG+GG gene type (P=0.001). Haplotype analysis further revealed that the CCC haplotype of rs2228000, rs3731114 and rs3729587 increased PC risk (odds ratio, 1.610; 95% confidence interval, 1.035-2.481; P=0.034). The present study revealed that XPC gene polymorphisms could increase the risk of PC in the study population, particularly among heavy smokers.

Correspondence to: Professor Xi-Yan Wang, Xinjiang Research Institute of Cancer Prevention and Control, Affiliated Tumor Hospital of Xinjiang Medical University, 789 Suzhou East Street, Urumqi, Xinjiang 830011, P.R. China
E-mail: wxyforum@163.com

*Contributed equally

Key words: pancreatic cancer, xeroderma pigmentosum group C, polymorphism, DNA repair, genetic susceptibility

Introduction

Although it has a low morbidity rate, pancreatic cancer (PC) is one of the most fatal malignant tumor types, with the highest mortality rate worldwide (1). Nearly all patients with PC succumb to the disease within 1-2 years (2). Following pancreaticoduodenectomy (Whipple procedure), the 5-year survival rate is 25-30% for node-negative (3) and 10% for node-positive (4) diseases. The majority of patients are diagnosed at an advanced stage, owing to a lack of effective diagnostic techniques for PC diagnosis at the early stages of disease (5). The traditional tumor serum markers, carcinoembryonic antigen and carbohydrate antigen 19-9, are neither sensitive nor specific for screening patients with PC (6,7). A number of environmental factors, including alcohol consumption, smoking history, body mass index, diabetes history and family history of PC have been demonstrated to be high-risk factors for PC (8,9). However, certain individuals exposed to these risk factors do not develop PC, which suggests that genetic factors may also influence cancer progression.

The xeroderma pigmentosum group C (XPC) gene is located at chromosome 3p25 (10); it contains 16 exons and 15 introns, and encodes a protein of 940 amino acids (11). The encoded protein is an indispensable component in the early stages of global genome nucleotide excision repair (NER), particularly in the damage recognition and initiation of NER (12); it is involved in initiating protein complex formation and repair of these complexes (13,14). There are >687 single nucleotide polymorphisms (SNPs) in the XPC gene, with >100 SNPs in the coding regions (http://www.ncbi.nlm.nih.gov/projects/SNP). However, to date, only a small number of correlation analysis studies have focused on XPC polymorphisms and PC risk. There are three polymorphisms most frequently detected in the XPC gene: poly AT insertion/deletion on intron 9 (PAT), A to C substitution in exon 15 (Lys939Gln, rs2228001) and C to T substitution in exon 9 (Ala499Val, rs2228000). The PAT polymorphism has been demonstrated to confer an increased risk of PC (15,16);

however, an association with PC risk has not been observed for rs2228001 and rs2228000 (17,18).

The use of tag-SNPs markedly improves the effectiveness of candidate gene and disease correlation analyses (19). Therefore, in the present study, to understand the association between XPC polymorphisms and PC susceptibility, XPC gene tag-SNPs and functional SNPs were investigated in patients with pathologically proven PC.

Materials and methods

Study subjects. A total of 205 patients with PC, with an age range between 24 and 87 years (mean age, 63.69±11.40 years), who were treated at The Affiliated Cancer Hospital and The First Affiliated Hospital of Xinjiang Medical University (Urumqi, Xinjiang, China) between December 2007 and December 2015 were enrolled in the present study. All patients had pathologically proven PC. Among these patients, 131 cases underwent pancreaticoduodenectomy, 19 cases underwent iodine-125 seed implantation and palliative surgery (biopsy obtained during surgery), 51 cases underwent distal (combined with the spleen) pancreatectomy and 4 cases underwent fine-needle aspiration biopsy under computed tomography scan guidance.

In addition, a total of 230 non-cancer subjects, with an age range between 26 and 88 years (mean age, 63.69±11.86 years), who were admitted to The First Affiliated Hospital of Xinjiang Medical University during the same period, were recruited as a control group. These subjects had no previous history of pancreatic disease and had not been diagnosed with any malignant cancer.

All subjects recruited to this study signed informed consent forms and the study protocol was approved by the Ethical Committee of The First Affiliated Hospital of Xinjiang Medical University.

'Drinking' was defined as consuming alcohol more than once a week, continuously over a 6-month period in a lifetime. 'Smoking' was defined as accumulative smoking of >100 cigarettes in a lifetime. The accumulative smoking amount (packets/year) indicated the smoking status according to the following formula: Accumulative smoking amount (packets/year)=mean number of cigarettes per day/20 times the number of years of smoking. The median of the accumulative smoking amount was used as the cut-off point to define mild and heavy smokers (20).

Blood collection and DNA extraction. Peripheral blood (3 ml) was collected from each participant, placed in an EDTA tube and stored at -80˚C within 30 min. Genomic DNA was extracted from blood samples using a DNA blood extraction kit (BioTeke, Beijing, China) according to the manufacturer's protocol.

SNP selection and genotyping. SNPs were selected from the HapMap database (https://www.genome.gov/10001688/international-hapmap-project/ HapMap Data Rel 24/Phase II, Nov08, on NCBI B36 assembly, dbSNP b126), which provided the genotype data collected from Han Chinese individuals living in Beijing. SNPs in the XPC gene were selected by combined analysis of functional SNPs and tag-SNPs from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and HapMap databases. The minor allele frequencies were >5%, and the linkage disequilibrium (LD) coefficient r^2 values were >0.8. A total of 7 tag-SNPs were located, 2 of which were in the 5'-untranslated region (5'UTR) (rs2607775 and rs3731055), 3 of which were in introns (intron 5, rs3729587; intron 6, rs3731114; and intron 12, rs2470353) and another 2 of which were in exons (exon 9, rs2228000; and exon 15, rs2228001).

The 7 SNPs were detected using a SNaPshot assay (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with PCR primers designed with Primer 5 software (21) The primers of the SNPs were as follows: rs2228001 forward, 5'-CTGTAGTGGGGCAGCAGCAACT-3' and reverse, 5'-AGAGGAGGGGACCAGCTCTCAA-3'; rs2470353 forward, 5'-TGCTGGGCAGGAAGAGGTACAC-3 and reverse, 5'-GACCTGGGCCTGTTTGGCTACT-3'; rs2228000 forward, 5'-CCCACTTTTCCTCCTGCTCACA-3' and reverse, 5'-AGGACAAAGGCTGGGTCCAAGA-3'; rs3731114 forward, 5'-ACCCGCCTGCCTCTGTCCTA-3' and reverse, 5'-TGCCAGACTGGTGGGGAGAC-3'; rs3729587 forward, 5'-GAAACTTGCCATGGCCACAGAG-3' and reverse, 5'-AAGGGGTCCATGAGGACACACA-3'; rs2607775 forward, 5'-GTTTCCGAGCCATGTTGCTTGT-3' and reverse, 5'-CTTTCCTGCTTCCCGCAGTTTT-3'; and rs3731055 forward, 5'-TCCGGAGATTGACGTTGCTCTT-3' and reverse 5'-CTCAGGGCCTACGGCAAAATTC-3'. Results were analyzed using GeneMapper 4.0 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). For quality control, genotyping was performed in a double-blinded manner with 5% randomly duplicated samples. Hence, reproducibility was 100%.

Bioinformatics analysis. The functions of the XPC SNPs were predicted using the SNPinfo Web Server (https://snpinfo.niehs.nih.gov/). XPC expression and survival analysis in PC was evaluated using The Cancer Genome Atlas data by the online analysis tool UALCAN (http://ualcan.path.uab.edu/analysis.html) (22).

Statistical analysis. SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. A goodness-of-fit χ^2 test was used to assess the Hardy-Weinberg equilibrium. Allele frequencies were assessed by χ^2 test. Using unconditional logistic regression with adjustment for age and sex, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the relative risks of PC associated with SNP genotypes. HaploView version 4.2 (Broad Institute, Cambridge, MA, USA) was used to generate the LD plot and to assess the association between haplotypes and PC. Comparisons of all variables between cases and control subjects were performed using the online tool on the website http://ualcan.path.uab.edu/. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The demographic characteristics of subjects and related risk factors are presented in Table I. According to χ^2 tests, no significant differences in age, sex, drinking status, body mass index, diabetes history, smoking history or family history of cancer were identified between the case and control groups ($P>0.05$). Heavy smokers (tobacco

Table I. General characteristics of the pancreatic cancer cases (n=205) and controls (n=230).

Characteristics	Cases, n (%)	Controls, n (%)	χ^2	P-value
Age, years			0.600	0.896
≤49	31 (15.1)	36 (15.7)		
50-59	33 (16.1)	36 (15.7)		
60-69	65 (31.7)	66 (28.6)		
≥70	76 (37.1)	92 (40.0)		
Sex			0.003	0.953
Male	126 (61.5)	142 (61.7)		
Female	79 (38.5)	88 (38.3)		
Diabetes			2.330	0.127
No	135 (65.9)	167 (72.6)		
Yes	70 (34.1)	63 (27.4)		
BMI, kg/m^2			6.026	0.110
<18.5	84 (41.0)	69 (30.0)		
18.5-23.9	71 (34.6)	93 (40.4)		
24-27.9	33 (16.1)	48 (20.9)		
≥28	17 (8.3)	20 (8.7)		
Smoking			1.909	0.167
Non-smoker	105 (51.2)	133 (57.8)		
Smoker	100 (48.8)	97 (42.2)		
Packets/year smoked[a]			5.520	0.019
<25	68 (68.0)	80 (82.5)		
≥25	32 (32.0)	17 (17.5)		
Drinking			0.011	0.917
Seldom	156 (76.1)	176 (76.5)		
Often	49 (23.9)	54 (23.5)		
Family history of cancer			2.812	0.094
No	179 (87.3)	212 (92.2)		
Yes	26 (12.7)	18 (7.8)		

[a]Calculated as percentage of smokers. P-values were calculated from two-sided χ^2 tests. BMI, body mass index.

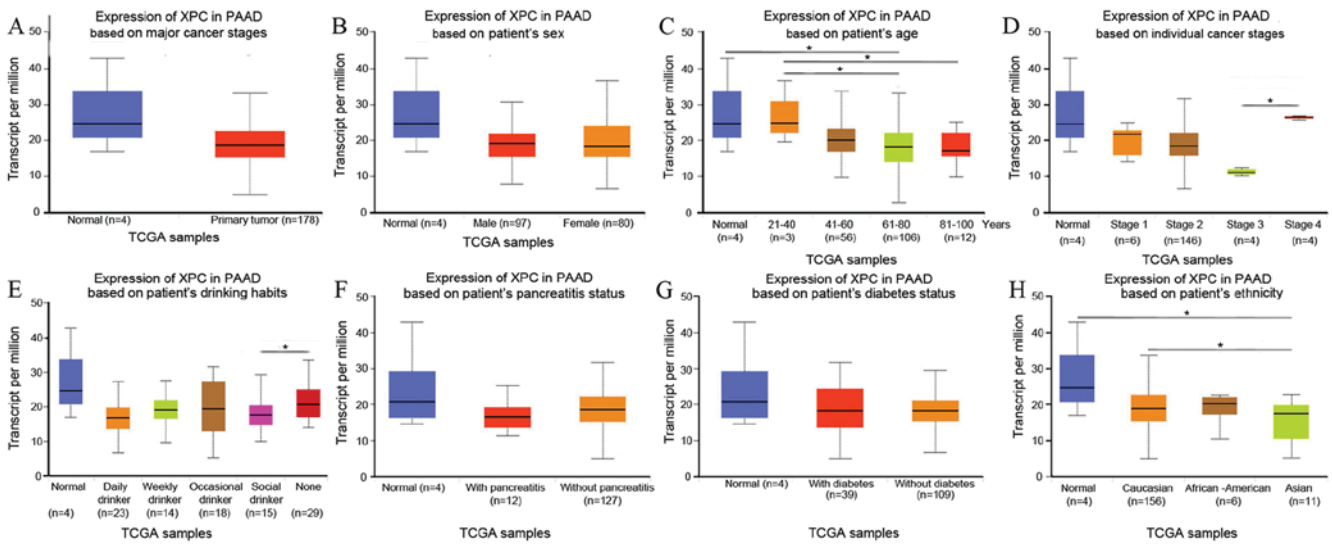

Figure 1. (A) XPC expression in PAAD patients, (B) sex, (C) age, (D) cancer stage, (E) drinking habit, (F) chronic pancreatitis status, (G) diabetes status, and (H) patient ethnicity was analyzed using the online tool UALCAN (18). *P<0.05. XPC, xeroderma pigmentosum group C; PAAD, pancreatic adenocarcinoma; TCGA, The Cancer Genome Atlas.

Table II. Characteristics of the 7 tag-SNPs in the XPC gene.

SNP	Chromosome position	Location	Alleles	MAF		HWE P-value		P-value
				Case	Control	Case	Control	
rs2228001	14187449	Extron 15	A/C	0.351	0.365	0.600	0.633	0.667
rs2470353	14190268	Intron 12	G/C	0.124	0.065	0.595	0.981	0.003
rs2228000	14199887	Extron 9	C/T	0.305	0.300	0.728	0.300	0.876
rs3731114	14206622	Intron 6	C/G	0.205	0.217	0.146	0.409	0.652
rs3729587	14208625	Intron 5	G/C	0.334	0.302	0.780	0.754	0.312
rs2607775	14220095	5'UTR	C/G	0.163	0.096	0.197	0.149	0.003
rs3731055	14220439	5'UTR	G/A	0.232	0.241	0.697	0.348	0.740

P-values were calculated from two-sided χ^2 tests. [a]P<0.05. SNP, single nucleotide polymorphism; XPC, xeroderma pigmentosum group C; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; UTR, untranslated region.

≥25 packets/year) in the case group accounted for 32.0%, which was a significantly higher value compared with that in the control group (17.5%) (P=0.019).

Association analysis of PC susceptibility. The distribution of allelic gene frequency in the 7 tag-SNP loci conformed to the Hardy-Weinberg equilibrium in the case and control groups (P>0.05; Table II). The C allelic gene frequency of rs2470353 in patients with pathologically proven PC was significantly increased compared with that in the control group (P=0.003). Compared with the GG gene type, PC risk was significantly increased in subjects with the variant allele C (GC and GC+CC; P=0.012 and P=0.006, respectively). The G allelic gene frequency of rs2607775 was significantly increased in patients with PC compared with that in the control group (P=0.003). Compared with the CC gene type, PC risk was significantly increased in subjects with the variant allele G (CG and CG+GG; P=0.013 and P=0.005; Table III). The distribution of gene type and allelic gene frequency in the other 5 tag-SNP loci were not significantly different between the case and control groups (P>0.05; Tables II and III).

Function prediction and expression analysis. The XPC SNP functions were predicted using the SNPinfo Web Server (https://snpinfo.niehs.nih.gov/). rs2470353 was identified to be located in the region of intron 12, but was not predicted to be a functional SNP. rs2607775 was located in the transcription factor binding site (TFBS) of the 5'UTR of XPC. Therefore, rs2607775 was predicted to influence XPC expression.

Since only blood samples were collected in the present study, XPC expression in pancreatic adenocarcinoma (PAAD) was analyzed using the online tool UALCAN (http://ualcan.path.uab.edu/analysis.html) (22). XPC expression was decreased in PAAD patients (P>0.05; Fig. 1A) irrespective of sex (Fig. 1B), age (Fig. 1C), cancer stage (Fig. 1D), drinking habit (Fig. 1E), chronic pancreatitis status (Fig. 1F), diabetes status (Fig. 1G) and ethnicity (Fig. 1H), with few exceptions. Notably, XPC expression was demonstrated to be significantly decreased in Asian patients with PAAD (P=0.02; Fig. 1H). Although a general trend of decreased XPC expression was observed in patients with PAAD, the majority of these differences were not statistically significant, potentially due to the small number of control samples (n=4) and the inevitably large individual differences in expression.

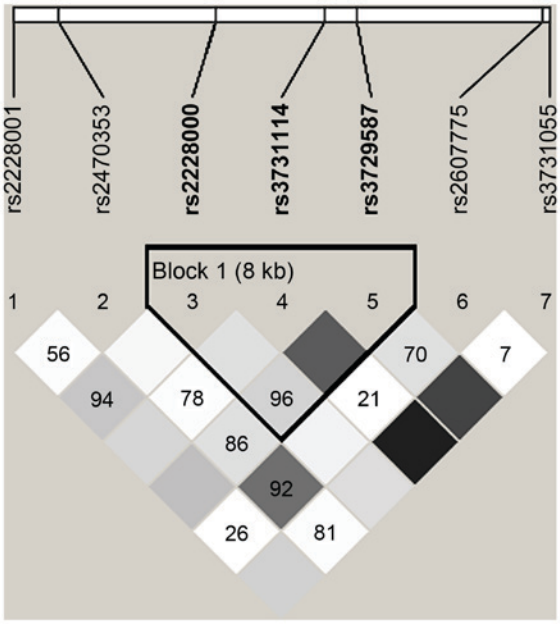

Figure 2. Linkage disequilibrium plot of all polymorphic sites in the XPC gene. The upper part of the figure shows 7 sites of tag-SNP in the XPC gene, while the number in the lower part is a value of 100xD' (linkage disequilibrium parameter). The standard color scheme of Haploview was used to display the strength of LD: black indicates strong LD, grey intermediate, whereas white denotes no LD. r^2 values are shown within the boxes. LD, linkage distribution; XPC, xeroderma pigmentosum group C; SNP, single nucleotide polymorphism.

LD and haplotype association analysis. In Fig. 2, the range of the area surrounded by black lines indicates that 3 tag-SNPs of the XPC gene were contained in a haplotype and in a state of linkage disequilibrium. Block 1 comprised rs2228000, rs3731114 and rs3729587 (Fig. 2). The frequencies of the haplotypes CCC and TCG were higher in patients with cancer compared with those in the non-cancer controls, and the CCC haplotype for Block 1 significantly increased the risk of PC (OR, 1.610; 95% CI, 1.035-2.481; P=0.034; Table IV).

Table III. Association between polymorphisms of XPC genes and pancreatic cancer.

				χ^2 test		Logistic regression	
SNP	Genotype	Case, n	Control, n	OR (95% CI)	P-value	OR (95% CI)[a]	P-value[a]
rs2228001	A/A	88	91	1.000		1.000	
	A/C	90	110	0.846 (0.565-1.268)	0.418	0.824 (0.547-1.239)	0.352
	C/C	27	29	0.963 (0.528-1.755)	0.901	1.009 (0.548-1.857)	0.977
	A/C+C/C	117	139	0.870 (0.594-1.276)	0.477	0.860 (0.585-1.265)	0.444
rs2470353	G/G	158	201	1.000		1.000	
	G/C	43	28	1.954 (1.162-3.285)	0.011	1.942 (1.154-3.267)	0.012[b]
	C/C	4	1	5.089 (0.563-45.980)	0.108	5.253 (0.577-47.822)	0.141
	G/C+C/C	47	29	2.062 (1.241-3.425)	0.005	2.053 (1.235-3.412)	0.006[b]
rs2228000	C/C	98	116	1.000		1.000	
	C/T	89	90	1.171 (0.786-1.742)	0.438	1.164 (0.780-1.737)	0.457
	T/T	18	24	0.888 (0.455-1.731)	0.727	0.914 (0.463-1.803)	0.795
	C/T+T/T	107	114	1.111 (0.762-1.619)	0.584	1.113 (0.762-1.626)	0.579
rs3731114	C/C	133	143	1.000		1.000	
	C/G	60	74	0.872 (0.576-1.319)	0.516	0.848 (0.557-1.291)	0.442
	G/G	12	13	0.992 (0.437-2.252)	0.986	1.006 (0.441-2.292)	0.989
	C/G+G/G	72	87	0.890 (0.602-1.316)	0.559	0.885 (0.596-1.313)	0.543
rs3729587	G/G	90	111	1.000		1.000	
	G/C	93	99	1.159 (0.779-1.723)	0.467	1.132 (0.759-1.689)	0.543
	C/C	22	20	1.357 (0.697-2.641)	0.369	1.392 (0.711-2.726)	0.335
	G/C+C/C	115	119	1.192 (0.817-1.740)	0.363	1.184 (0.810-1.732)	0.383
rs2607775	C/C	146	190	1.000		1.000	
	C/G	51	36	1.844 (1.143-2.974)	0.011	1.839 (1.139-2.970)	0.013[b]
	G/G	8	4	2.603 (0.769-8.811)	0.112	2.500 (0.733-8.522)	0.143
	C/G+G/G	59	40	1.920 (1.217-3.028)	0.005	1.914 (1.212-3.024)	0.005[b]
rs3731055	G/G	122	135	1.000		1.000	
	G/A	71	79	0.995 (0.664-1.489)	0.979	0.981 (0.655-1.471)	0.928
	A/A	12	16	0.830 (0.378-1.824)	0.642	0.830 (0.377-1.829)	0.644
	G/A+A/A	83	95	0.967 (0.659-1.418)	0.863	0.965 (0.657-1.417)	0.856

[a]P-value, OR and 95% CI were calculated by unconditional logistic regression analysis adjusted for age and sex. [b]P<0.05. SNP, single nucleotide polymorphism; XPC, xeroderma pigmentosum group C; CI, confidence interval.

Interaction analysis of smoking and XPC gene polymorphism. Compared with non-smoking subjects with wild-type GG gene in the rs2470353 locus, PC risk did not significantly increase in smoking subjects with the GC gene type (P>0.05). PC risk increased by 3.505-fold in heavy smokers (tobacco ≥25 packets/year) with the variant allele C (OR=4.505, 95% CI=1.418-15.007, P=0.008; Table V). Compared with non-smoking subjects with wild gene type CC in the rs2607775 locus, PC risk did not increase in smoking subjects with the CC gene type (P>0.05). PC risk increased by 3.950-fold in heavy smokers (tobacco ≥25 packets/year) with the variant allele G (CG+GG) (OR, 4.950; 95% CI, 1.758-13.924; P=0.001; Table VI).

Discussion

Previous studies have reported XPC polymorphisms to be associated with cancer risk. There are three polymorphisms most frequently detected in the XPC gene: Poly AT insertion/deletion on intron 9 (PAT), A to C substitution in exon 15 (Lys939Gln, rs2228001) and C to T substitution in exon 9 (Ala499Val, rs2228000) (23). Epidemiological studies have demonstrated that the $PAT^{+/+}$ genotype results in a 1.85-fold increase in the risk of squamous cell carcinoma of the head and neck (24) and a 1.6-fold increase in the risk of lung cancer (25). Meta-analysis revealed that the exon 15 Lys939Gln (rs2228001 A>C) C/C gene type is associated with increased risk of lung cancer and esophageal cancer (26,27). The XPC Ala499Val (rs2228000, C>T) polymorphism is associated with the risk of endometrial, colorectal and liver cancer, as well as other malignant cancer types (28-30). However, the association between other XPC polymorphisms and bladder cancer remains controversial (31-33).

In the present study, the associations between genetic polymorphisms of XPC and PC risk were investigated using a tag-SNP method. The results revealed that variant alleles

Table IV. XPC haplotype of rs2228000, rs3731114 and rs3729587 frequencies and associations with pancreatic cancer risk.

Haplotype	Freq	Cases (freq)	Controls (freq)	OR (95% CI)	P-value
CGC	0.208	0.201	0.217	0.928 (0.667-1.285)	0.646
CCC	0.106	0.129	0.082	1.610 (1.035-2.481)	0.034[a]
TCG	0.299	0.301	0.297	1.021 (0.765-1.367)	0.887
CCG	0.384	0.365	0.401	0.863 (0.651-1.127)	0.276

P-values are calculated by χ^2 test. The case/control omnibus test is a H-1 degree of freedom test, if there are H haplotypes. [a]P<0.05. XPC, xeroderma pigmentosum group C; OR, odd ratio; CI, confidence interval; Freq, frequency.

Table V. Risk of XPC genotypes at rs2470353 with pancreatic cancer by smoking status.

Genotype	Smoking status, pack-years	Cases, n	Controls, n	OR (95% CI)	P-value
GG	Non-smoker	83	115	1.000	
	<25	56	73	1.041 (0.598-1.635)	0.724
	≥25	19	13	2.071 (0.967-4.431)	0.082
GC+CC	Non-smoker	22	18	1.683 (0.846-3.345)	0.096
	<25	12	7	2.366 (0.891-6.278)	0.076
	≥25	13	4	4.505 (1.418-15.007)	0.008[a]

P-values were calculated by unconditional logistic regression analysis adjusted for age and sex. [a]P<0.05. XPC, xeroderma pigmentosum group C; OR, odds ratio; CI, confidence interval.

Table VI. Risk of XPC genotypes at rs2607775 with pancreatic cancer by smoking status.

Genotype	Smoking status, pack-years	Cases, n	Controls, n	OR (95% CI)	P-value
CC	Non-smoker	77	106	1.000	
	<25	55	72	1.047 (0.661-1.658)	0.831
	≥25	14	12	1.503 (0.668-3.376)	0.288
CG+GG	Non-smoker	28	27	1.414 (0.771-2.595)	0.270
	<25	13	8	2.251 (0.891-5.759)	0.089
	≥25	18	5	4.950 (1.758-13.924)	0.001[a]

P-values were calculated by unconditional logistic regression analysis adjusted for age and sex. [a]P<0.05. XPC, xeroderma pigmentosum group C; OR, odds ratio; CI, confidence interval.

at two loci were associated with increased PC risk, even though the rs2470353 locus was located in the intron area and the rs2607775 locus was located in the 5'UTR (P<0.05). The other five tag-SNP loci, including in exon 9 (rs2228000) and exon 15 (rs222800), did not exhibit significant differences in the distribution of gene type or allelic gene frequency between the case and control groups (P>0.05; Tables III and IV).

G/C polymorphisms at or near the exonic boundaries in intron 12 of the XPC gene may affect mRNA translation through exon skipping and/or aberrant mRNA folding (34-37). SNPs in the 5'UTR may affect XPC gene expression via promoter modulation (34), resulting in reduced DNA repair capacity (DRC) and increased risk of PC. Unfortunately, although rs2470353 is located in the region of intron 12, it is not a predicted functional SNP. rs2607775 is located on the TFBS of the 5'UTR of XPC, and is therefore predicted to influence XPC expression (38,39). Furthermore, XPC expression in PAAD was analyzed using UALCAN (22) in the present study. XPC expression was identified to be decreased in patients with PAAD irrespective of sex, age, cancer stage, drinking habits, chronic pancreatitis status, diabetes status, or race, with few exceptions. The most notable finding was that XPC expression was decreased significantly in Asian patients with PAAD. However, due to the small number of normal control samples, the majority of these differences were not statistically significant.

SNPs in coding regions, as well as non-coding regions of the XPC gene, that are in LD with each other as part of a

given haplotype may act in a collective manner to influence the phenotype. The results of the present study revealed that the CCC haplotype of rs2228000, rs3731114 and rs3729587 exhibited a higher frequency in patients with PC, compared with that in the control group, indicating that the CCC haplotype may result in an increased risk of PC. However, the exact mechanism of this remains unclear.

Smoking is recognized as a traditional risk factor for PC (9,40). The present study also revealed that smoking in the XPC rs2470353 (GC+CC) and/or rs2607775 (CG+GG) subjects significantly increased PC risk. In subjects with an accumulative smoking amount of ≥25 packets/year, PC risk increased 3.505-fold with rs2470353 (GC+CC) (OR, 4.505; 95% CI, 1.418-15.007; P=0.008) and 3.950-fold with rs2607775 (CG+GG) (OR, 4.950; 95% CI, 1.758-13.924; P=0.001), indicating that the combination of mutations and smoking may serve an important role in PC progression. Smoking causes genetic damage and/or cell mutations (41) that may not be repaired by the NER pathway (13,14), since genetic damage may not be recognized by XPC with the rs2470353 (GC+CC) and/or rs2607775 (CG+GG) mutant gene type.

The present study has initially indicated that the XPC gene rs2470353 and rs2607775 loci polymorphisms are associated with PC risk. The haplotype CCC of rs2228000, rs3731114 and rs3729587 was also identified to be associated with increased PC risk. However, additional SNP loci have been continuously selected for future study, particularly the functional loci of the exon regions and loci at splicing regions. In addition, the chromosome hereditary variation of the XPC gene and its association with PC could be further verified by transcription analysis, in order to evaluate the effects on regulation and splicing of the XPC gene.

Acknowledgements

Not applicable.

Funding

This study was supported by research grants from the National Natural Science Foundation of China (grant no. 30960433).

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The XPC expression and survival analysis in PC was evaluated using the online analysis tool UALCAN (http://ualcan.path.uab.edu/analysis.html) (21).

Authors' contributions

XHL, DY and XYW conceived and designed the experiments. DY, WD and XYW participated in clinical data collection. XHL and DYA performed the experiments. XHL, DY and JXZ analyzed the data. XHL, DY and JXZ contributed to the interpretation of results obtained and manuscript construction. DY and XYW analyzed and interpreted the patients' data regarding the clinical characteristics. XHL and DY wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All samples were obtained with the informed consent of the participants prior to their inclusion in the study, according to Helsinki Declaration principles and with approval of the Ethical Committee of The First Affiliated Hospital of Xinjiang Medical University.

Patient consent for publication

All patients provided informed consent for the publication of any associated data.

Competing interests

The authors declare that they have no conflicts of interest.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin 65: 5-29, 2015.
2. Chakraborty S, Baine MJ, Sasson AR and Batra SK: Current status of molecular markers for early detection of sporadic pancreatic cancer. Biochim Biophys Acta 1815: 44-64, 2011.
3. Trede M, Schwall G and Saeger HD: Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg 211: 447-458, 1990.
4. Kang MJ, Jang JY, Chang YR, Kwon W, Jung W and Kim SW: Revisiting the concept of lymph node metastases of pancreatic head cancer: Number of metastatic lymph nodes and lymph node ratio according to N stage. Ann Surg Oncol 21: 1545-1551, 2014.
5. Ryan DP, Hong TS and Bardeesy N: Pancreatic adenocarcinoma. N Eng J Med 371: 1039-1049, 2014.
6. DiMagno EP, Reber HA and Tempero MA: AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. Gastroenterol 117: 1464-1484, 1999.
7. Lamerz R: Role of tumour markers, cytogenetics. Ann Oncol 10 Suppl 4: 145-149, 1999.
8. Pandol S, Gukovskaya A, Edderkaoui M, Dawson D, Eibl G and Lugea A: Epidemiology, risk factors, and the promotion of pancreatic cancer: Role of the stellate cell. J Gastroenterol Hepatol 27 (Suppl 2): S127-S134, 2012.
9. Krejs GJ: Pancreatic cancer: Epidemiology and risk factors. Dig Dis 28: 355-358, 2010.
10. Dou K, Xu Q and Han X: The association between XPC Lys939Gln gene polymorphism and urinary bladder cancer susceptibility: A systematic review and meta-analysis. Diagn Pathol 8: 112, 2013.
11. Li L, Peterson C and Legerski R: Sequence of the mouse XPC cDNA and genomic structure of the human XPC gene. Nucleic Acids Res 24: 1026-1028, 1996.
12. Melis JP, Luijten M, Mullenders LH and van Steeg H: The role of XPC: Implications in cancer and oxidative DNA damage. Mutat Res 728: 107-117, 2011.
13. Khan SG, Muniz-Medina V, Shahlavi T, Baker CC, Inui H, Ueda T, Emmert S, Schneider TD and Kraemer KH: The human XPC DNA repair gene: Arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. Nucleic Acids Res 30: 3624-3631, 2002.
14. Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D and Hoeijmakers JH: Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol Cell 2: 223-232, 1998.
15. Duell EJ, Bracci PM, Moore JH, Burk RD, Kelsey KT and Holly EA: Detecting pathway-based gene-gene and gene-environment interactions in pancreatic cancer. Cancer Epidemiol Biomarkers Prev 17: 1470-1479, 2008.
16. Wang L, Lin DX, Lu XH, Miao XP and Li H: Polymorphisms of the DNA repair genes XRCC1 and XPC: Relationship to pancreatic cancer risk. Wei Sheng Yan Jiu 35: 534-536, 2006 (In Chinese).

17. Zhao F, Shang Y, Zeng C, Gao D and Li K: Association of single nucleotide polymorphisms of DNA repair genes in NER pathway and susceptibility to pancreatic cancer. Int J Clin Exp Pathol 8: 11579-11586, 2015.
18. McWilliams RR, Bamlet WR, Cunningham JM, Goode EL, de Andrade M, Boardman LA and Petersen GM: Polymorphisms in DNA repair genes, smoking, and pancreatic adenocarcinoma risk. Cancer Res 68: 4928-4935, 2008.
19. Tabor HK Risch NJ and Myers RM: Candidate-gene approaches for studying complex genetic traits: Practical considerations. Nat Rev Genet 3: 391-397, 2002.
20. Hirooka N, Kadowaki T, Sekikawa A, Ueshima H, Choo J, Miura K, Okamura T, Fujiyoshi A, Kadowaki S, Kadota A, *et al*: Influence of cigarette smoking on coronary artery and aortic calcium among random samples from populations of middle-aged Japanese and Korean men. J Epidemiol Community Health 67: 119-124, 2013.
21. Lalitha S: Primer Premier 5. Biotech Software Intern Rep 1: 6, 2000.
22. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S: UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19: 649-658, 2017.
23. Francisco G, Menezes PR, Eluf-Neto J and Chammas R: XPC polymorphisms play a role in tissue-specific carcinogenesis: A meta-analysis. Eur J Hum Genet 16: 724-734, 2008.
24. Shen H, Sturgis EM, Khan SG, Qiao Y, Shahlavi T, Eicher SA, Xu Y, Wang X, Strom SS, Spitz MR, *et al*: An intronic poly (AT) polymorphism of the DNA repair gene XPC and risk of squamous cell carcinoma of the head and neck: A case-control study. Cancer Res 61: 3321-3325, 2001.
25. Marín MS, López-Cima MF, García-Castro L, Pascual T, Marrón MG and Tardón A: Poly (AT) polymorphism in intron 11 of the XPC DNA repair gene enhances the risk of lung cancer. Cancer Epidemiol Biomarkers Prev 13: 1788-1793, 2004.
26. Jin B, Dong Y, Zhang X, Wang H and Han B: Association of XPC polymorphisms and lung cancer risk: A meta-analysis. PLoS One 9: e93937, 2014.
27. Guo W, Zhou RM, Wan LL, Wang N, Li Y, Zhang XJ and Dong XJ: Polymorphisms of the DNA repair gene xeroderma pigmentosum groups A and C and risk of esophageal squamous cell carcinoma in a population of high incidence region of North China. J Cancer Res Clin Oncol 134: 263-270, 2008.
28. Wang Y, Li Z, Liu N and Zhang G: Association between CCND1 and XPC polymorphisms and bladder cancer risk: A meta-analysis based on 15 case-control studies. Tumour Biol 35: 3155-3165, 2014.
29. Paszkowska-Szczur K, Scott RJ, Górski B, Cybulski C, Kurzawski G, Dymerska D, Gupta S, van de Wetering T, Masojć B, Kashyap A, *et al*: Polymorphisms in nucleotide excision repair genes and susceptibility to colorectal cancer in the Polish population. Mol Biol Rep 42: 755-764, 2015.
30. Paszkowska-Szczur K, Scott RJ, Serrano-Fernandez P, Mirecka A, Gapska P, Górski B, Cybulski C, Maleszka R, Sulikowski M, Nagay L, *et al*: Xeroderma pigmentosum genes and melanoma risk. Int J Cancer 133: 1094-1100, 2013.
31. Sak SC, Barrett JH, Paul AB, Bishop DT and Kiltie AE: The polyAT, intronic IVS11-6 and Lys939Gln XPC polymorphisms are not associated with transitional cell carcinoma of the bladder. Br J Cancer 92: 2262-2265, 2005.
32. Sanyal S, Festa F, Sakano S, Zhang Z, Steineck G, Norming U, Wijkström H, Larsson P, Kumar R and Hemminki K: Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 25: 729-734, 2004.
33. Sak SC, Barrett JH, Paul AB, Bishop DT and Kiltie AE: Comprehensive analysis of 22 XPC polymorphisms and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 15: 2537-2541, 2006.
34. Cheng AJ, Mao YM and Cui RZ: The effect of gene polymorphism in promoter and intron 1 on human ApoA I expression. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 23: 610-613, 2006 (In Chinese).
35. Duan ZX, Zhu PF, Dong H, Gu W, Yang C, Liu Q, Wang ZG and Jiang JX: Functional significance of the TLR4/11367 polymorphism identified in Chinese Han population. Shock 28: 160-164, 2007.
36. Kinslow CJ, El-Zein RA, Hill CE, Wickliffe JK and Abdel-Rahman SZ: Single nucleotide polymorphisms 5' upstream the coding region of the NEIL2 gene influence gene transcription levels and alter levels of genetic damage. Genes Chromosomes Cancer 47: 923-932, 2008.
37. Law AJ, Kleinman JE, Weinberger DR and Weickert CS: Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. Hum Mol Genet 16: 129-141, 2007.
38. Qiao B, Ansari AH, Scott GB, Sak SC, Chambers PA, Elliott F, Teo MT, Bentley J, Churchman M, Hall J, *et al*: In vitro functional effects of XPC gene rare variants from bladder cancer patients. Carcinogenesis 32: 516-521, 2011.
39. Bai Y, Xu L, Yang X, Hu Z, Yuan J, Wang F, Shao M, Yuan W, Qian J, Ma H, *et al*: Sequence variations in DNA repair gene XPC is associated with lung cancer risk in a Chinese population: A case-control study. BMC Cancer 7: 81, 2007.
40. Maisonneuve P and Lowenfels AB: Epidemiology of pancreatic cancer: An update. Dig Dis 28: 645-656, 2010.
41. Lee YJ, Shim HS, Kang YA, Hong SJ, Kim HK, Kim H, Kim SK, Choi SH, Kim JH and Cho BC: Dose effect of cigarette smoking on frequency and spectrum of epidermal growth factor receptor gene mutations in Korean patients with non-small cell lung cancer. J Cancer Res Clin Oncol 136: 1937-1944, 2010.