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Cancer Detect Prev. Author manuscript; available in PMC 2007 April 26.

Published in final edited form as:
Cancer Detect Prev. 2006 ; 30(3): 284–291.

Selected polymorphisms of DNA repair genes and risk of pancreatic cancer

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Abstract

Background—Genetic variants of DNA repair genes may contribute to pancreatic carcinogenesis. O^6-methylguanine-DNA methyltransferase (MGMT) is the major protein that removes alkylating DNA adducts, and apurinic/apyrimidinic endonuclease 1 (APE1) and X-ray repair cross-complementing group 1 (XRCC1) play important roles in the base excision repair pathway.

Methods—We investigated the association between polymorphisms of *MGMT* (Leu^{84}Phe and Ile^{143}Val), *APE1* (Asp^{148}Glu), and *XRCC1* (Arg^{194}Trp and Arg^{399}Gln) and risk of pancreatic cancer in a case-control study. Exposure information from 384 patients with primary pancreatic ductal adenocarcinoma and 357 cancer-free healthy controls were collected and genomic DNAs were genotyped for five markers. Controls were frequency matched to patients by age at enrollment (±5 years), gender, and race. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) by using unconditional logistic regression models.

Results—There was no significant main effect or interaction with smoking of these genetic variants on the risk of pancreatic cancer. However, the *XRCC1*^{194} polymorphism had a significant interaction with the *APE1*^{148} ($p = 0.005$) or *MGMT*^{84} polymorphism ($p = 0.02$) in modifying the risk of pancreatic cancer.

Conclusions—This study suggests that polymorphisms of genes involved in the repair of alkylating DNA adduct and DNA base damage may play a role in modulating the risk of pancreatic cancer. Larger studies are required to validate these preliminary findings. The mechanism of the combined genotype effects remains to be elucidated.

Keywords

Pancreatic cancer risk; Hardy-Weinberg equilibrium; Smoking status; Risk factors; Exposure information; *MGMT*^{84} polymorphism; MGMT; APE1; XRCC1; Single nucleotide polymorphism; Alkylating DNA adduct; Single-strand break; Base excision repair; Gene-environment interaction; Gene-gene interaction

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Sources of support: National Institutes of Health grants RO3CA84581 (to D.L.), RO1CA98380 (to D.L.), and P20CA101936 (to J.L.A.).

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1. Introduction

Cellular DNA is consistently subjected to damage induced by exogenous and endogenous agents. Unresolved DNA damage can cause gene mutation and subsequent malignant transformation. Multiple repair mechanisms have evolved in humans to minimize the consequences of DNA damage, preserving genomic integrity [1]. Pancreatic cancer is one of the malignancies associated with exposure to environmental carcinogens [2], including cigarette smoke. Different types of DNA adducts have been detected in the human pancreas [3,4]. It is biologically plausible that deficient DNA repair play an important role in pancreatic carcinogenesis and common polymorphisms of DNA repair genes may confer altered susceptibility to human pancreatic cancer.

Although there is no direct evidence from human studies, it has been suspected that *N*-nitroso compounds, either from endogenous or exogenous sources [5], are among the etiological agents in pancreatic cancer development based on rich evidence from animal studies. Metabolically activated *N*-nitroso carcinogens can induce DNA single-strand breaks (SSBs) and can react with cellular DNA to form a broad spectrum of alkylating DNA adducts including the highly mutagenic lesion O^6-methyldeoxyguanine [6].

Alkylating DNA adducts are repaired by a direct damage-reversal mechanism [7] as well as by the base excision repair (BER) mechanism [9]. For example, O^6-methylguanine-DNA methyltransferase (MGMT) can remove the methyl group from the O^6-methyldeoxyguanine in a suicidal manner. Other methylated bases can be removed by methylpurine glycosylase, which creates the abasic or apurinic-apyrimidinic (AP) site. The AP site is incised by AP endonuclease and followed by either short patch repair or long patch repair. In short patch repair, an incised AP site is occupied by poly(ADP-ribose) polymerase and then DNA polymerase beta (Polβ) adds one nucleotide into the repair gap and simultaneously removes the 5′-sugar phosphate. Finally, the DNA ligase III/X-ray repair cross-complementing group 1(XRCC1) complex accomplishes repair by sealing the SSBs in DNA ends [10,11]. In human cells, the most abundant AP endonuclease is the AP endonuclease 1 (APE1) protein [8,9]. XRCC1 has been shown to interact both physically and functionally with APE1, Polβ, and ligase III [12]. XRCC1 mutants display sensitivity to alkylating agents and have exhibited elevated levels of sister chromatid exchange in Chinese hamster ovary cell lines [13].

Many studies have investigated DNA repair gene polymorphisms and the risk of a variety of human cancers [14]. However, few were on pancreatic cancer. We selected five coding region non-synonymous single nucleotide polymorphisms (SNPs) of three DNA repair genes: *MGMT* exon 3 $Leu^{84}Phe$ (C to T, rs#12917) and exon 5 $Ile^{143}Val$ (A to G, rs#2308321), *APE1* exon 5 $Asp^{148}Glu$ (T to A, rs#3136820); *XRCC1* exon 6 $Arg^{194}Trp$ (C to T, rs#1799782) and exon 10 $Arg^{399}Gln$ (G to A, rs#25487), and investigated their associations with the risk of pancreatic cancer in a hospital-based case-control study. We examined the main effect of these genetic variants and their interaction with smoking on risk of pancreatic cancer, given the fact that smoking can induce a variety of DNA damages in human genome but only portions of smokers develop pancreatic cancer. Furthermore, we examined the combined effects of these SNPs because these three genes are functionally related in the same DNA repair pathway. Finally, we performed exploratory haplotype analysis of the *MGMT* and *XRCC1* polymorphisms.

2. Materials and methods

2.1. Study design and study population

The study design and data collection methods have been described previously [15]. Briefly, in this hospital-based study, both patients and controls were consecutively enrolled at The

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University of Texas M.D. Anderson Cancer Center from January 2000 to January 2005. All study participants were U.S. residents and could communicate in English. Potential patients were identified in the Gastrointestinal Cancer Center of M.D. Anderson. Eligible patients were all diagnosed with a pathologically confirmed primary pancreatic ductal adenocarcinoma (International Classification of Diseases for Oncology code C25.3; World Health Organization, 2000). Potential controls were patient companions, i.e. individuals without cancer but visiting M.D. Anderson along with a cancer patient who was diagnosed with any non-pancreatic cancer. Eligible controls were identified by using a brief screening questionnaire colleting information on demographics, cancer history, state of residence, relationships to patients, and willingness to participate in a research project. Eligible controls were selected from friends and family members who were not genetically related to their accompanying cancer patients (usually spouses and in-laws). Patients and controls with prior cancer history (except for non-melanoma cancer of the skin) were not eligible for the present study. A written informed consent was obtained from each study participant for interview and a blood sample.

During the study period, a total of 452 eligible patients (representing 77.8% of those being approached) and 376 controls (representing 78.0% of those being approached) were consented. Among them, 15.0% of the patients and 5.1% of the controls were excluded from the study because they either failed to donate a blood specimen or to complete the interview. As a result, a total of 384 patients and 357 controls were ascertained for the present study and the patients and controls were frequency matched by age at enrollment (5-year interval), gender, and race. The research protocol was approved by the M.D. Anderson Cancer Center Institutional Review Board.

2.2. Data collection

Specifically trained personnel conducted the in-person interviews using a structured risk factor questionnaire to collect information on demographic characteristics and exposure information. Ever- and never-smokers were classified according to whether they had smoked 100 cigarettes in their lifetime. Former smokers were defined as those who had quit smoking for at least one year before recruitment. Recent quitters (quit less than a year) were considered as current smokers. Cumulative smoking was calculated in pack-years, i.e. the number of packs smoked per day multiplied by the number of years of smoking. The same interviewers performed interviews on both patients and controls. No proxy interviews were conducted.

2.3. Laboratory analyses

A blood sample was collected in heparinized vacutainers (BD Biosciences, Franklin Lakes, NJ) from each participant. Peripheral blood mononuclear cells were separated from freshly drawn blood by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation and were stored at –80 °C. DNA was extracted with the use of a FlexiGene DNA kit (Qiagen, Valencia, CA) following the manufacturer's instructions, and the aliquot was stored at 4 °C. The *MGMT*^{143} polymorphism was determined by using the polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) method [16]. The SSCP results were verified by direct DNA sequencing of 1% of the samples. The *XRCC1*^{399} polymorphism was determined using a PCR-restriction fragment length polymorphism method as described previously [17]. Other genotyping assays were conducted by a commercial service (BioServe Biotechnologies Ltd., Laurel, MD) using a PCR-based allele-specific genotyping assay with the Masscode technology. For quality control, positive allele controls were run in every experiment. In addition, 5% random sample repeats were included in each batch of samples. Final calls on genotypes were made in the most conservative manner, and any ambiguous calls (mostly caused by allele dropout as a result of poor DNA quality or quantity) were recorded as missing. The missing rate was less than 6% for each

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polymorphic site and the discrepant call rate was less than 0.1% for the repeats. The discrepancy was resolved by additional genotyping.

2.4. Statistical analysis

All statistical analyses were performed by using the Stata 9 software (StataCorp, College Station, TX). All tests were two-tailed, and $p \leq 0.05$ was considered the level of statistical significance. Pearson's χ^2 test (or the Fisher's exact test when the expected number in any cell was <5) was used to examine differences in risk factor and genotype distributions between patients and controls. Hardy-Weinberg equilibrium was tested by using the goodness-of-fit χ^2 test separately in patients and in controls. Genotype-specific relative risks were estimated as odds ratios (ORs) and their associated 95% confidence intervals (CIs) by using unconditional logistic regression models. Matching factors, age at enrollment (coded in years: <50 as the referent, 50-59, 60-69, and ≥70), gender (male and female as the referent), and race (non-Hispanic white as the referent, Hispanic, African American, and others) were included in the multivariate models. In all OR estimations, the reference group was consisted of individuals who were homozygous for the common alleles and the comparison group was consisted of individuals with either the heterozygous or homozygous mutant genotype. Gene-environment interactions were examined by using the stratified analyses. The variables of interest included age at enrollment (<62 years versus ≥62 years, 62 years is the median age of all study participants), gender, and smoking status (never-smoker versus ever-smoker). Former and current smokers were grouped as ever-smokers because risk associated with polymorphisms was similar in these two groups. Gene-gene interactions were evaluated by generating cross-product terms between each two SNPs in the logistic regression models. Two-by-four tables were constructed to evaluate the scale of interaction (i.e. the departure from an additive or multiplicative model) [18]. The statistical significance of the interaction term was tested by using a likelihood ratio test, with the full model containing the interaction term, the main effect of the genotypes, and the reduced model lacking the interaction term. The allelic association between the two markers of the *MGMT* or the *XRCC1* gene was evaluated by using the SNPAlyze software (Dynacom Co. Ltd., Mobara, Japan). Haplotypes were reconstructed from the genotype data by using the PHASE program (Version 2) [19]. Pearson's χ^2 test was used to test the difference of the haplotype distribution between the patients and the controls.

3. Results

3.1. Risk factors and genotype distributions in the study participants

Table 1 shows that the patients and the controls were comparable in terms of the distribution of age, gender, and race. Patients were more likely to be ever-smokers than controls were (61.2% versus 53.5%, $p = 0.03$). A larger proportion of the patients smoked more than 20 pack-years than controls did (40.1% versus 27.5%, $p = 0.001$). Table 2 shows that the genotype distributions of the five markers were essentially equivalent between the patients and the controls. The minor allele frequency in the controls was 0.48 for *APE1*^{148}, 0.12 for both *MGMT*^{84} and *MGMT*^{143}, 0.06 for *XRCC1*^{194} and 0.38 for *XRCC1*^{399}. Genotype distributions in the patients and in the controls were all in Hardy-Weinberg equilibrium (all p values ≥ 0.10).

3.2. Stratified analyses by risk factors

No significant differential effect of each polymorphism on the risk of pancreatic cancer was observed in subgroup analysis by age, gender, and race. A weak insignificant association between the *XRCC1*^{194}Trp allele and risk of pancreatic cancer was only observed among non-Hispanic whites with an age- and gender-adjusted OR of 1.64 (95% CI, 0.98-2.73, $p = 0.06$), but not seen among other racial groups. Some differential effects of genotypes were observed by smoking status. Relative to the carriage of the *MGMT* Leu^{84}Leu genotype, the carriage of at least one copy of the *MGMT*^{84} Phe allele was associated with an increased risk of pancreatic

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cancer (OR, 2.62; 95% CI, 1.20-5.72) in light smokers (<20 pack-years), but not in never-smokers and heavy smokers. Relative to the carriage of the *MGMT* Ile^{143}Ile genotype, the carriage of at least one copy of the *MGMT*^{143} Val allele showed a significant protective effect (OR, 0.48; 95% CI, 0.26-0.90) among heavy smokers (≥20 pack-years), but not among never-smokers and light smokers. The interaction between smoking pack-year and the *MGMT*^{143} polymorphism was of borderline significance ($p = 0.05$). Relative to the *XRCC1* Arg^{399}Arg genotype, the carriage of at least one copy of the *XRCC1* ^{399}Gln allele was associated with an increased risk (OR, 2.06; 95% CI, 1.01-4.18) among smokers who had daily cigarette consumption less than one pack, but not among never-smokers and smokers who consumed one pack or more per day ($p = 0.17$ for interaction) (data not shown).

3.3. Gene-gene interaction in modifying pancreatic cancer risk

Combined genotype effect was evaluated between each two polymorphisms of the same gene and of different genes. OR was adjusted for age, gender, race, and pack-year of smoking (0, <20, and ≥20). Table 3 shows the interaction of the *XRCC1*^{194} polymorphism with other polymorphisms in modifying the risk of pancreatic cancer risk. Compared with those carrying both the *XRCC1* Arg^{194}Arg and *APE1* Asp^{148}Asp wild type, individuals carrying at least one copy of the *XRCC1*^{194}Trp allele and *APE1* Asp^{148}Asp had a significantly increased risk in developing pancreatic cancer (OR, 4.98; 95% CI, 1.61-15.4; $p = 0.005$). However, the risk diminished among those carrying at least one copy of the *XRCC1* ^{194}Trp allele and at least one copy of the *APE1* ^{148}Glu allele (OR, 0.85; 95% CI, 0.47-1.53; $p = 0.63$) ($p = 0.005$ for interaction). The interaction between the *XRCC1*^{194} and the *MGMT*^{84} polymorphism was also statistically significant ($p = 0.02$). Compared with those carrying the *XRCC1* Arg^{194}Arg and the *MGMT* Leu^{84}Leu, individuals carrying at least one copy of the *XRCC1*^{194} and *MGMT*^{84} variant allele had a two-fold increase in risk of pancreatic cancer (OR, 3.04; 95% CI, 1.14-7.87; $p = 0.02$). There were no joint effects of the *XRCC1*^{194} and other polymorphisms and other combined genotype effects.

3.4. MGMT and XRCC1 haplotype analyses

We performed a linkage disequilibrium analysis to test the allelic association between the two markers for the *MGMT* and the *XRCC1* gene separately. The two *MGMT* SNPs were in weak linkage disequilibrium ($D' = –0.3971$; $p = 0.04$). Individuals carrying the *MGMT* ^{84}Phe allele also had the *MGMT* ^{143}Ile allele. Three haplotypes were inferred: ^{84}Phe-^{143}Ile (77.0%), ^{84}Phe-^{143}Val (11.5%), and ^{84}Leu-^{143}Ile (11.5%). The *XRCC1* ^{194}Arg and ^{399}Gln alleles were in linkage disequilibrium ($D' = –0.496$; $p < 0.0001$). Four haplotypes were inferred: ^{194}Arg-^{399}Arg (56.4%), ^{194}Arg-^{399}Gln (37.1%), ^{194}Trp-^{399}Arg (4.8%), and ^{194}Trp-^{399}Gln (1.7%). The haplotype frequencies did not differ significantly between patients and controls overall ($p = 0.67$ for *MGMT* and $p = 0.38$ for *XRCC1*) and in stratum specified by smoking status (ever-smokers versus never-smokers) (data not shown).

4. Discussion

In this hospital-based study, we investigated five polymorphisms of three genes that are involved in the repair of DNA base damages in association with risk of pancreatic cancer. There was no significant main effect of each genetic variant on the risk of developing pancreatic cancer. Stratified analyses showed insignificant gene-smoking interactions and significant gene-gene interactions. The *XRCC1* Arg^{194}Trp polymorphism modified risk of pancreatic cancer in combination with the *APE1* Asp^{148}Glu or *MGMT* Leu^{84}Phe polymorphism. These observations suggest that defects in DNA base damage repair pathway conferred by genetic polymorphisms may play a role in the development of pancreatic cancer.

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Both the *MGMT* ^{84}Phe and *MGMT* ^{143}Val variant alleles have previously been associated with either significantly increased [20-22] or decreased risk [23] of various types of human cancer. Shen et al. [24] reported a positive interaction of the *MGMT* ^{84}Phe allele with heavy smoking or fruit and vegetable intake in modulating breast cancer risk. The frequency of the *MGMT* ^{84}Phe variant allele (0.12) in our control population, which was predominantly represented by non-Hispanic whites, was comparable with that reported in a white population [25]. One previous study found a significant positive association between the presence of the ^{84}Phe allele and tobacco carcinogen-induced chromosome aberrations in human lymphocytes [26], but most other studies found no significant functional difference between the variant alleles and the wild type [27-29], which to some extent support the overall null association of the two *MGMT* polymorphisms with pancreatic cancer risk in our study. The frequency of the *MGMT* ^{143}Val allele (0.12) was similar to that reported in two European studies (0.11) [25,30] but higher than that reported in other two studies (0.07) [20,21]. The discrepancy in the genotype frequencies may be related to the variations in study populations and sensitivity of the genotyping methods used in these studies. In spite of the fact that smoking was confirmed as a significant risk factor of pancreatic cancer in our study population, the current study did not observe a significant interaction between *MGMT* polymorphisms and smoking. This may be related to the limited sample size.

APE1 has several distinct physiological functions and is highly conserved from plants to humans [9]. It is the rate-limiting enzyme in the BER pathway. AP sites in DNA are the most common lesions in cells. It has been estimated that the majority of the AP sites are readily cleaved and converted into SSBs [31]. If not repaired, SSBs cause genomic instability leading to increased mutation rates and chromosome rearrangement [9,32]. The Asp^{148}Glu polymorphism is the most common variation among the *APE1* gene polymorphisms, with an allelic frequency of 0.38 [33]. Biochemical assays did not reveal any apparent differences in the endonuclease activity between the *APE1* ^{148}Glu and ^{148}Asp proteins [33]. Some previous studies showed that the *APE1*^{148} variant genotype did not affect the frequency of X-ray- or ultraviolet-induced chromosome aberrations [34], but the Glu allele may have higher sensitivity to ionizing radiation [35]. In the present study, we did not find an association between the *APE1*^{148} polymorphism and the risk of developing pancreatic cancer, indicating that this polymorphism alone may not play a major role in pancreatic carcinogenesis.

The *XRCC1* ^{194}Trp variant allele results in a non-conservative substitution in a hydrophobic region of XRCC1, and the Arg^{399}Gln variation is located within the BRCA1 C-terminal domain known to react with poly(ADP-ribose) polymerase [14]. The frequency of the variant allele of these two polymorphisms in our controls was comparable with that reported in other white populations [36-39]. Results of previous epidemiological studies of the *XRCC1* Arg^{194}Trp and Arg^{399}Gln polymorphisms [36,38-42] and cancer risk have been inconsistent. A significant protective effect of the *XRCC1* ^{194}Trp allele for tobacco-related cancer has been reported [43]. However, findings in the current study were consistent with previous reports showing the *XRCC1* ^{194}Trp as the at-risk allele in cancer development. A previous population-based study of pancreatic cancer with 309 patients and 964 controls found an interaction between the *XRCC1* ^{399}Gln allele and smoking and the interaction was more prominent in women than men [44]. However, we could not duplicate this finding in the current analysis. We observed an increased risk associated with the *XRCC1* ^{399}Gln allele among smokers who had daily cigarette consumption less than a pack only. Since XRCC1 protein acts as a scaffold in the restoration of repaired DNA sites rather than being directly involved in the repair process, its functional significance may depend on other interacting proteins.

Genes involved in the same DNA repair pathway may interact with each other and elicit a combined effect on phenotype. XRCC1 has been shown to coordinate the initial and late stages of DNA AP site repair through protein-protein interaction [45]. A previous study showed a

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joint effect of the *APE1* Asp^{148}Glu and *XRCC1* Arg^{399}Gln polymorphisms on elevated sensitivity to ionizing radiation [34] and increased risk of lung cancer in current smokers [46]. We found that the *XRCC1* ^{194}Trp allele significantly predisposed individuals carrying the *APE1*^{148} homozygous Asp allele to pancreatic cancer and this effect was not present among those carrying at least one copy of the ^{194}Glu allele. The exact mechanism for this observation is unknown. In BER, the balanced level of different proteins involved in various steps of the enzymatic repair is a requisite to modulate their respective activities, and to avoid the accumulation of potentially toxic repair intermediate [47]. It is possible that the polymorphisms of these two genes cause the imbalance of the action of the proteins in processing DNA damages. For example, the SSBs lesion resulted from the repair of the AP site by APE1 coded by *APE1*^{148}Asp cannot be efficiently processed by XRCC1 coded by the ^{194}Trp variant. It is also possible that these two polymorphisms affect the adequate protein-protein interaction in repair of either AP site or intermediate SSBs lesion.

We also observed an interaction between the *XRCC1*^{194} and *MGMT*^{84} polymorphisms. The XRCC1 N-terminal domain (residues 1-183) interacts with DNA containing a single-strand break. The conserved residues 159-250 adjacent to this domain have been proposed to have a role in facilitation of DNA binding [48]. The R194W mutation may confer an altered DNA binding capacity of XRCC1. The altered capacity in repair of alkylating DNA adducts by *MGMT* variant protein, together with defects of the XRCC1 protein in repairing the resulting SSBs, could act in a synergistic manner to contribute to cancer development. Because the functional impact of single protein is low, interaction between two proteins with reduced functional activity may be required to significantly decrease the DNA repair capacity and increase the risk of cancer [49].

The present study has several strengths. For example, we minimized the outcome misclassification by including pathologically confirmed cases only. Also, we reduced information bias by performing the direct interviews in collecting exposure information. This study has some limitations. Voluntary participation of the patients and controls might have introduced bias to the association investigation. However, the genotype frequency is unlikely to be related to participation. Nevertheless, we did find that patients who did not donate blood after consenting were more likely to have metastatic cancer than those who provided blood. We do not know how this will bias the associations studied. Although the gene-gene interaction effect was significant, the role of chance findings cannot be excluded because of the small sample size and the low allele frequency. The current study is underpowered in examining the gene-environment interaction. Thus, the interpretation of our findings needs caution.

In conclusion, the current study found that the *XRCC1*^{194} polymorphism might interact with *APE1*^{148} or *MGMT*^{84} polymorphism in modulating the risk of pancreatic cancer. Although the mechanisms of these observations remain elusive, these results are in agreement with the notion that APE1 and *MGMT* coordinate with XRCC1 in the repair of DNA base damage. There is a need for further larger-scale study including other BER genes to confirm our findings, and to fully examine the biologically plausible gene-environment interaction effect.

Acknowledgements

People who agreed to participate in the research made this study possible. We thank Rabia Khan and Kaustubh Mestry for their contributions to patient recruitment and data collection. We also thank all physicians and clinical staff at the M.D. Anderson Gastrointestinal Center who helped with patient recruitment. We appreciate the constant laboratory support provided by Ping Chang, Yanan Li, Jijiang Zhu, and Yingqiu Du. We thank Don Norwood for editing the manuscript.

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Table 1

Distribution of selected factors in patients and controls

Variable	Patients (%) (*n* = 384)	Controls (%) (*n* = 357)	*p*-Value (for χ^2 test)
Age (years)			
<50	51 (13.3)	60 (16.8)	0.21
50-59	110 (28.6)	86 (24.1)	
60-69	119 (31.0)	125 (35.0)	
≥70	104 (27.1)	86 (24.1)	
Gender			
Female	170 (44.3)	173 (48.5)	0.25
Male	214 (55.7)	184 (51.5)	
Race			
Non-Hispanic	337 (87.8)	316 (88.5)	0.96
White			
Hispanic	20 (5.2)	19 (5.3)	
African American	20 (5.2)	17 (4.8)	
Others	7 (1.8)	5 (1.4)	
Smoking status			
Never	149 (38.8)	166 (46.5)	0.03
Ever	235 (61.2)	191 (53.5)	
Years of smoking			
0	149 (38.8)	166 (46.5)	0.008
<21	91 (23.7)	95 (26.6)	
≥21	144 (37.5)	96 (26.9)	
Cigarette/day			
0	149 (38.8)	166 (46.5)	0.02
<20	72 (18.8)	73 (20.4)	
≥20	163 (42.4)	118 (33.1)	
Pack-year of smoking			
0	149 (38.8)	166 (46.5)	0.001
<20	81 (21.1)	93 (26.0)	
≥20	154 (40.1)	98 (27.5)	

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Table 2

Genotype distribution among patients and controls and in relation to pancreatic cancer risk

	Patients (%) (*n* = 384)	Controls (%) (*n* = 357)	OR (95% CI)[a]	*p*-Value (for χ^2 test)
*APE1*148				
Asp/Asp	108 (29.4)	85 (25.8)	1.00	0.52
Asp/Glu	180 (49.1)	174 (52.7)	0.82 (0.57-1.16)	
Glu/Glu	79 (21.5)	71 (21.5)	0.86 (0.56-1.33)	
Glu allele frequency	0.46	0.48		
MGMT[84]				
Leu/Leu	264 (71.0)	257 (75.6)	1.00	0.08
Leu/Phe	101 (27.1)	82 (24.1)	1.22 (0.87-1.72)	
Phe/Phe	5 (1.9)	1 (0.3)	7.30 (0.88-60.5)	
Phe allele frequency	0.15	0.12		
MGMT[143]				
Ile/Ile	297 (78.6)	276 (78.0)	1.00	0.91
Ile/Val	77 (20.4)	73 (20.6)	0.98 (0.68-1.40)	
Val/Val	4 (1.0)	5 (1.4)	0.70 (0.18-2.64)	
Val allele frequency	0.11	0.12		
XRCC1[194]				
Arg/Arg	130 (85.6)	301 (89.0)	1.00	0.38
Arg/Trp	49 (13.6)	34 (10.1)	1.43 (0.89-2.30)	
Trp/Trp	3 (0.8)	3 (0.9)	0.92 (0.18-4.63)	
Trp allele frequency	0.08	0.06		
XRCC1[399]				
Arg/Arg	130 (34.2)	135 (38.1)	1.00	0.27
Arg/Gln	197 (51.8)	172 (48.6)	1.20 (0.87-1.65)	
Gln/Gln	53 (14.0)	47 (13.3)	1.17 (0.74-1.87)	
Gln allele frequency	0.40	0.38		

[a] ORs are adjusted for age (<50, 50-59, 60-69, and ≥70 years), gender, race, and pack-year of smoking (0, <20, and ≥20).

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Table 3

Combined effect of *XRCC1* Arg^{194}Trp and other polymorphisms in modifying pancreatic cancer risk

Genotypes		Patients/ controls	OR(95% CI)[a]	*p*-Value[b]
XRCC1^{194}	*APE1*^{148}			
Arg/Arg	Asp/Asp	84/77	1.00	
Arg/Arg	Asp/Glu + Glu/Glu	214/204	0.99 (0.67-1.44)	
Arg/Trp + Trp/Trp	Asp/Asp	20/4	4.98 (1.61-15.4)	
Arg/Trp + Trp/Trp	Asp/Glu + Glu/Glu	31/33	0.85 (0.47-1.53)	0.005
XRCC1^{194}	*MGMT*^{84}			
Arg/Arg	Leu/Leu	225/215	1.00	
Arg/Arg	Leu/Phe + Phe/Phe	78/73	1.04 (0.71-1.51)	
Arg/Trp + Trp/Trp	Leu/Leu	30/31	0.93 (0.54-1.61)	
Arg/Trp + Trp/Trp	Leu/Phe + Phe/Phe	20/6	3.04 (1.14-7.87)	0.02
XRCC1^{194}	*MGMT*^{143}			
Arg/Arg	Ile/Ile	239/236	1.00	
Arg/Arg	Ile/Val + Val/Val	67/62	1.05 (0.71-1.56)	
Arg/Trp + Trp/Trp	Ile/Ile	41/28	1.39 (0.82-2.35)	
Arg/Trp + Trp/Trp	Ile/Val + Val/Val	10/9	1.17 (0.46-2.97)	0.54
XRCC1^{194}	*XRCC1*^{399}			
Arg/Arg	Arg/Arg	102/107	1.00	
Arg/Arg	Arg/Gln + Gln/Gln	204/192	1.09 (0.77-1.54)	
Arg/Trp + Trp/Trp	Arg/Arg	20/18	1.16 (0.57-2.38)	
Arg/Trp + Trp/Trp	Arg/Gln + Gln/Gln	31/19	1.67 (0.87-3.20)	0.55

[a] ORs are adjusted for age (<50, 50-59, 60-69, and ≥70 years), gender, race, and pack-year of smoking (0, <20, and ≥20).

[b] *p*-Value for interaction, likelihood ratio test.