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DNA Mismatch Repair Network Gene Polymorphism as a Susceptibility Factor for Pancreatic Cancer

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Abstract

DNA repair plays a critical role in human cancers. We hypothesized that DNA mismatch repair gene variants are associated with risk of pancreatic cancer. We retrospectively genotyped 102 single-nucleotide polymorphisms (SNPs) of 13 mismatch repair related genes in 706 patients with pancreatic cancer and 706 cancer-free controls using the mass spectroscopy–based MassArray method. Association of genotype with pancreatic cancer risk was tested by multivariate logistic regression models. A significance level of $P \leq 0.0015$ was set at the false discovery rate (FDR) <1% using the Beta-Uniform Mixture method. We found 28 SNPs related to altered pancreatic cancer risk ($P<0.05$). Adjusting for multiple comparisons, *MGMT* I143V AG/GG, *PMS2* IVS1-1121C>T TC/TT, and *PMS2L3* Ex1+118C>T CT/TT genotypes showed significant main effects on pancreatic cancer risk at FDR <1% with OR (95% CI) of 0.60 (0.46-0.80), 1.44 (1.14-1.81) and 5.54 (2.10-14.61), respectively ($P \leq 0.0015$). To demonstrate genotype-phenotype association, we measured O^6-ethylguanosine (O^6-EtGua) adduct levels *in vitro* by immunoslot blot assay in lymphocytes treated with N-ethyl-N-nitrosourea (ENU) in 297 case/control subjects. *MGMT* I143V GG, *MGMT* K178R GG, *MSH6* G39E AG/AA, *PMS2L3* IVS3+9A>G GA and *TP73* IVS1-7449G>C CG/CC genotypes correlated with a higher level of ENU-induced DNA adducts. Haplotypes of *MGMT, MSH6, PMS2, PMS2L3,* and *TP73* were significantly associated with pancreatic cancer risk ($P \leq 0.0015$). Our findings suggest that mismatch repair gene variants may affect susceptibility to pancreatic cancer.

Keywords

mismatch repair; single-nucleotide polymorphism; pancreatic cancer; susceptibility; haplotype

Introduction

Pancreatic cancer ranks as the fourth-leading cause of cancer mortality in the United States. [1] It is a notoriously aggressive and difficult-to-treat malignancy expressing global genomic instability (e.g., mutation, translocation, and deletion) and aneuploidy.[1,2] Identification of predisposing genetic factors and environmental and lifestyle risk factors is critical for the primary prevention of this fatal disease. Five to ten percent of pancreatic cancer patients have inherited germline disorders, including mutations of genes responsible for Peutz-

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Jeghers syndrome (*STK11*), hereditary pancreatitis (*PRSS1*, *SPINK1*), Lynch syndrome (*MLH1*, *MSH2*), familial atypical multiple-mole melanoma (*p16*), cystic fibrosis (*CTFR*), hereditary breast-ovarian cancer (*BRCA1*, *BRCA2*), familial adenomatous polyposis (*APC*), or family X site-specific pancreatic cancer (*PALLD*).[2-4] Some of these genes (e.g., *MLH1*, *MSH2*, *BRCA1*, and *BRCA2*) function in DNA repair and cellular response to DNA damage. Most pancreatic cancers harbor genetic alterations in *KRAS2*, *p16*, *TP53*, or *SMAD4*.[2-4] Moreover, DNA damage control and apoptosis pathways have been identified as part of the core signaling pathways in human pancreatic cancers.[5] Several case-control studies have examined the associations between risk of pancreatic cancer and genes involved in base excision repair, nucleotide excision repair, and homologous recombination DNA repair. [6-10] Although these genes were observed to have little significant main effect, several of them showed significant interactions with cigarette smoking, a well-established risk factor for pancreatic cancer.[11]

The genes of the mismatch repair (MMR) pathway have yet to be examined in these studies of DNA repair genes. As a multifaceted DNA-repair system, MMR improves the fidelity of DNA replication, maintains genome stability, and affects DNA damage signaling, apoptosis, and cell type–specific processes.[12,13] MMR deficiency confers a mutated phenotype susceptible to cancer.[12] Human MMR is complicated machinery encompassing several functional complexes.[12,13]

To test the hypothesis that genetic variants in MMR modify the risk of pancreatic cancer, we evaluated 13 genes encoding key nodes for DNA mismatch recognition and removal (MutS homolog 2 [*MSH2*], *MSH3*, *MSH6*, MutL homolog 1 [*MLH1*], *MLH3*, postmeiotic segregation increased 1 [*PMS1*], *PMS2*, *PMS2*-like 3 [*PMS2L3*], exonuclease I [*EXO1*], and three prime repair exonuclease 1 [*TREX1*]), or MMR interaction component O^6-methylguanine-DNA methyltransferase [*MGMT*], or MMR-induced apoptosis transducer (tumor protein 73 [*TP73*]), or MMR regulator RecQ protein-like/DNA helicase Q1-like (*RECQL*).[14] MutSα homolog MSH2/MSH6 recognizes base-base mismatches and small insertion/deletion loops (IDLs).[12] MutSβ homolog MSH2/MSH3 recognizes small and large IDLs.[12] MutLα homolog MLH1/PMS2 forms a ternary complex with mismatched DNA and MutSα, increases discrimination between heteroduplexes and homoduplexes, and acts in meiotic recombination.[12] Loss of MLH1 or PMS2 brings about mutated and microsatellite instability phenotypes.[12] MMR *(MLH1*, *MSH2*, *MSH6*, and *PMS2)* germline mutations cause Lynch syndrome/hereditary nonpolyposis colorectal cancer.[12] *PMS2L3* encodes a polypeptide homologous to PMS2.[15] As a member of the PMS2 family, PMS2L3 contains motifs conserved in MutL elements.[15] MutLβ homolog MLH1/PMS1 and MutLγ homolog MLH1/MLH3 act in meiotic recombination and as backup for MutLα in repairing base-base mismatches/small IDLs.[12] *EXO1* encoding 5′-3′ exonuclease is critical in MMR and recombination by interacting with MSH2 and removing biosynthetic errors.[16] The *TREX1* gene encodes 3′-5′ DNA exonuclease to proofread for DNA polymerase and degrade the 3′ ends of nicked DNA.[17,18] MGMT, involved in DNA direct reversal repair, defends against alkylating agents by interacting with MutSα, MutLα, and EXO1.[19,20] *RECQL*, a RecQ DNA helicase family member, has been found to interact with MMR proteins in regulating genetic recombination.[21] *TP73* encodes p73, a p53 family member, which acts in cellular growth, development, and responses to DNA damage. [22,23] MMR-mediated apoptosis can be induced by p73.[24] We examined 102 single-nucleotide polymorphisms (SNPs) of the 13 genes in 706 pancreatic cancer patients and 706 controls.

Materials and Methods

Study population and data collection

The study design and data collection have been described in detail previously.[25] This hospital-based case-control study included 706 patients with pathologically confirmed primary pancreatic ductal adenocarcinoma and 706 controls recruited at The University of Texas MD Anderson Cancer Center between 2000 and 2007.[7,26] The recruitment response rates of cases and controls were 80.6% and 76.9%, respectively.[7] All study subjects were U.S. residents and communicated in English. By personal interview, we collected information on demographics (age, sex, and self-reported race), cigarette smoking, alcohol consumption, medical history (e.g., diabetes), family history of cancer, and other risk factors. Control subjects were recruited from healthy spouses, friends, and non-blood relatives of patients with various types of cancers other than smoking-related or gastrointestinal cancers. Controls were frequency matched to cases by sex, race, and age at enrollment (±5 years). We collected a blood sample from each participant at enrollment. Cumulative smoking was calculated in "pack-years" [pack-years = (packs per day) × (years smoked)]. Alcohol consumption was calculated in terms of milliliters of ethanol consumed daily, with 12.0 oz of beer, 4.0 oz of wine, and 1.5 oz of hard liquor each considered to be equivalent to ~12.0 grams of ethanol. Body mass index (BMI, kg/m^2) was calculated on the basis of self-reported weight and height at age 34 to 39 years in 363 cases and 425 controls (this information was collected only after January 2004). Family history of cancer in first-degree relatives was noted. Each study participant signed a written informed consent for the interview and blood sample donation. The study was approved by the MD Anderson Cancer Center institutional review board and conducted according to all current ethical guidelines.[7,27]

DNA extraction, SNP selection, and genotyping

DNA was extracted from peripheral-blood lymphocytes of 1385 study participants and from paraffin sections of normal tissues of 27 patients using Qiagen DNA isolation kits (Valencia, CA).[7,27] From the HapMap Project database (www.hapmap.org), we selected 45 tagging SNPs using the SNPbrowser (Applied Biosystems, Carlsbad, CA; www.allsnps.com/snpbrowser) with a cutoff of r^2=0.8 and a minor allele frequency (MAF) ≥10% in Caucasians. For more comprehensive coverage of the polymorphisms, we chose 57 additional SNPs from the coding region (nonsynonymous or synonymous), untranslated region (UTR), promoter region, or splicing sites with a MAF ≥1% in Caucasians. The genes, nucleotide substitutions, functions (e.g., encoding amino acid changes), reference SNP identification numbers, and reported allele frequencies of the 102 SNPs were summarized in Supplemental Table 1. The mRNA transcripts, protein sequences, structures, homology models, and predicted functions for the SNPs were evaluated by F-SNP software (Queen's University, Kingston, Ontario, Canada).[28] Genotyping was performed with the mass spectroscopy–based MassArray system (Sequenom, Inc, San Diego, CA). Twenty percent of the DNA samples were genotyped in duplicate, with 99.6% concordance. We excluded the inconsistent genotyping data from the final analysis.

In vitro ENU-Induced DNA adduct assay

As a phenotypic marker for the repair of DNA alkylation adducts, the N-ethyl-N-nitrosourea (ENU)-induced O^6-ethylguanine (O^6-EtGua) level was measured in peripheral blood lymphocytes that were treated with 0.64 mM ENU *in vitro* as previously described.[29] Semi-quantification of ENU-induced O^6-EtGua was conducted by immunoslot blot.[29] 5μg DNA was slot-blotted onto Hybond-N+ Nylon membranes using Convertible Filtration Manifold System. After single-stranded DNA was immobilized onto the nitrocellular membranes, the membranes were blocked and hybridized with the (O^6-EtGua)-specific

mouse monoclonal antibody EM-2-3.[29] The membrane was hybridized with the secondary antibody goat anti-mouse horseradish peroxidase-labeled IgG. Enzymatic activity was visualized by chemiluminescence reaction with an ECL™ Western blotting detection kit. A standard for DNA measurement was generated by *in vitro* reaction of calf thymus DNA with ^{3}H-labeled N-methyl-N-nitrosourea, and the DNA adduct levels were determined by scintillation counting. Standard curves were established from the series of DNA concentrations by using the immunoslot blot assay. The intensity of the band in each slot was compared with the standard curve to semi-quantify the adduct levels. Each sample was analyzed in duplicates and the arithmetic mean was computed from the parallel samples to represent the actual value of each sample.

Statistical analysis

We examined the genotype distribution for the Hardy-Weinberg equilibrium (HWE) by the goodness-of-fit χ^2 test and calculated genotype/allele frequency of the SNPs by direct gene counting. We calculated haplotype diversity and linkage disequilibrium index (Lewontin's |*D*'| and r^2) by using SNPAlyze (DYNACOM Co., Ltd., Mobara, Japan) and Haplostats (Mayo Clinic, Rochester, MN). The heterozygous and homozygous genotypes were combined if the homozygous variant had a very low frequency (number of homozygote < 4) or if the homozygous and heterozygous genotypes exerted a similar effect on the risk for pancreatic cancer.

Odds ratio (OR) and 95% confidence interval (CI) were estimated by logistic regression analysis with adjustment for age, race, sex, smoking history (never, ≤20 pack-years, >20 pack-years), alcohol consumption (never, ≤60 grams of ethanol/day, >60 grams of ethanol/day), diabetes (yes or no), family history of cancer in first-degree relatives (yes or no), and BMI (≤25 kg/m^2 or >25 kg/m^2) in some analyses when appropriate. Non-drinkers and light drinkers were defined as consuming 0 gram and up to 60 grams of ethanol/day, respectively, and heavy drinkers as consuming >60 grams of ethanol/day. Statistical analysis was carried out by SPSS (SPSS Inc, Chicago, IL) and Stata (StataCorp LP, College Station, TX). We calculated the false discovery rate (FDR) using the Beta-Uniform Mixture method.[30] We found a *P*-value of 0.0015 corresponded to an FDR of 1%. We defined $P \leq 0.0015$ in the genotype analysis as statistically significant.

The value of O^6-EtGua measurement was square-root transformed to ensure the normal distribution for parametric testing and for stabilizing the group variance.[29] An analysis of covariance (ANCOVA) was used to compare the difference in mean adduct levels between genotypes adjusting for age, sex, race, smoking and alcohol consumption. The heterozygous and homozygous genotype was combined when both showed the same effect on the level of adducts. In the multiple linear regression, the percentage of the variance of dependent variable (adduct level) explained by the polymorphism and other variable was evaluated by subtracting the r^2 value for the full model from the r^2 for a model that excludes the test variable. The full models contained covariates age, race, sex, alcohol, smoking and the polymorphisms terms. Separate models were run for the nonsmokers and the smokers. Multiplicity adjusted *P* value was calculated.[29]

Results

Characteristics of study subjects

The demographics and risk factors in the study population were summarized in Table 1. Cases and controls were well matched by sex, age, and race. As reported previously, smoking, diabetes, obesity, and family history of cancer had been associated with increased risk of pancreatic cancer.[25]

Genotype distribution and allele frequencies

The observed MAFs of the 102 SNPs in the study population were comparable to those reported in the general population (Supplemental Table 1). Linkage disequilibrium data were presented in Supplemental Table 2. Most of the 102 SNPs followed HWE; exceptions were *MGMT* IVS4-44836G>A, *MLH1* I219V, *MSH2* IVS11-62G>A, *PMS2* P470S, N775S, −153C>G, *PMS2L3* IVS3+9A>G, and *RECQL* IVS8+190A>G in both cases and controls ($P<0.05$).

Main effect of genotype

Twenty-eight SNPs of MGMT, MLH1, MLH3, MSH2, MSH3, PMS2, PMS2L3, RECQL, TP73, and *TREX1* correlated with altered risk of pancreatic cancer after adjusting for other risk factors ($P\leq0.05$, Table 2). *MGMT* I143V AG/GG, *PMS2* IVS1-1121C>T TC/TT, and *PMS2L3* Ex1+118C>T CT/TT remained statistically significant predictors for altered pancreatic cancer risk after adjusting for multiple comparisons ($P\leq0.0015$). The corresponding ORs (95% CI) were 0.60 (0.46-0.80), 1.44 (1.14-1.81), and 5.54 (2.10-14.61), respectively (Table 2).

Genotype association with O^6-EtGua levels by smoking status

To demonstrate whether the genotypes are associated with the DNA repair capacity, we compared the O^6-EtGua levels between genotypes for those SNPs that showed altered risk of pancreatic cancer as listed in Table 2. The square-root transformed value (mean ± SE) of O^6-EtGua (fmol/μg DNA) was 9.3 ± 0.4 in 123 patients with pancreatic cancer and 8.8 ± 0.2 in 122 non-cancer controls ($p=0.19$). For power consideration, we analyzed the pooled data from both cases and controls, and the results were comparable when the analysis was conducted in controls only (Table 3). We found that *MGMT* I143V, *MGMT* K178R, *MSH6* G32E, *PMS2L3* IVS3+9A>G and *TP73* IVS1-7449G>C genotypes correlated with O^6-EtGua level ($p<0.05$). Multiple linear regression analyses showed a differential genotype effect on DNA adduct level by smoking status. *MGMT* and *MSH6* genotypes correlated with DNA adduct levels mainly in smokers; while *PMS2L3* and *TP73* genotypes affected the DNA adduct levels mainly in nonsmokers. For example, *MGMT* 143V GG and *MGMT* K178R GG genotypes had higher adduct levels in smokers but lower adduct levels in non-smokers than their counterparts (Fig. 1).

Association of haplotype diversity with pancreatic cancer risk

MGMT, *MSH6*, *PMS2*, *PMS2L3*, and *TP73* haplotypes correlated with pancreatic cancer risk after adjusting for multiple comparisons ($P\leq0.0015$, Table 4). The associations manifested the effects of *MGMT* AATA of IVS4-44836G>A, IVS4-75473G>A; IVS4-7901C>T, IVS5+23129G>A; *MSH6* G39E AG/AA (protective), IVS4-101G>C CC; *PMS2* IVS1-1121C>T TC/TT, IVS7+442G>T GT/TT; *PMS2L3* IVS3+9A>G GA/GG, IVS2-1578A>G AG, Ex1+118C>T CT/TT; and *TP73* IVS1-7449G>C CG/CC (protective) and A610A GA/AA genotypes on pancreatic cancer risk. Other haplotypes with $P>0.0015$ but <0.05 in logistic regression models were shown in Supplemental Table 3.

Discussion

With this study, we demonstrated a significant association between MMR network gene variants and risk of pancreatic cancer. *MGMT*, *PMS2*, *PMS2L3* and *TP73* genotypes and haplotypes showed effects on susceptibility to pancreatic cancer. As a multifunctional system, MMR maintains genome stability by removing mismatched or distorted DNA structures and stimulates the apoptosis cascade when cells are overwhelmed with genotoxic or cytotoxic damage.[12,31] MMR dysfunction causes genomic instability and abnormal

DNA damage response.[31] Through failure to recognize or repair the mismatched base-pairs/IDLs or failure to activate apoptosis signaling, MMR dysfunction may promote tumor development by accumulating replication errors. [12,31] Individuals with Lynch syndrome have increased risk of pancreatic cancer.[32] The observed association between MMR genetic variation and risk of pancreatic cancer supports the notion that MMR may contribute to pancreatic carcinogenesis.

A recent field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility revealed sparse association signals with strong epidemiological credibility.[33] Among the DNA repair genes studied in various types of human cancers, *MGMT* I143V showed credibility in prostate cancer.[33] We observed that *MGMT* I143V and K178R (in linkage) variant alleles were significantly related to decreased risk of pancreatic cancer. Consistently, the I143V variant has been reported as acting alone[34] or interacting with dietary factors[35] to reduce risk of colorectal cancer or breast cancer.[36] We found that *MGMT* I143V GG and K178R GG genotypes correlated with increased DNA damage level. MGMT is the major enzyme to remove O^6Et-Gua. I143V polymorphism is located close to the alkyl group acceptor pocket at codon 145, which may affect the acceptance of an alkyl group. We infer that the 143V might repair the DNA damage less effectively. We observed the interaction of I143V with smoking. The protective effect of *MGMT* 143V/178R genotype on risk of pancreatic cancer may not simply explained by its function in DNA adduct removal, however, it may reflex the activation of apoptosis signaling triggered by the high level of DNA damage.

The variant allele of *PMS2* IVS1-1121C>T, which was associated with increased pancreatic cancer risk, had also been associated with increased risk of ovarian cancer.[37] IVS1-1121C>T is located close to the R20Q variant, which is defective in activating the p73-dependent apoptotic response to cisplatin.[38] *PMS2L3* Ex1+118C>T (5′UTR) and IVS3+9A>G were related to pancreatic cancer risk. These SNPs were in linkage with the splice site variant IVS1-8C>T, which was predicted to cause frame-shift mutation.[28] *PMS2L3* IVS3+9A>G GA genotype correlated with increased DNA damage level. GA genotype carriers may be less active in recognizing O^6Et-Gua-mimic mismatches but more effective to activate apoptosis to clear the damaged cells.

We found that haplotypes of *MGMT*, *MSH6*, *PMS2*, *PMS2L3*, and *TP73* correlated with pancreatic cancer risk. mutSα and mutSβ initiate repair of base-base mismatches and IDLs. *MSH6* haplotype demonstrated the protective effect of the G39E variant against pancreatic cancer. G39E had been related consistently to decreased breast cancer risk.[39] G39E AG/AA genotype showed increased DNA damage level, which may be less effective to recognize O^6Et-Gua-mimic mismatches to initiate the MMR machinery, but act more efficiently to activate apoptosis pathway to maintain the genome stability. p73 is a determinant of apoptosis and a therapeutic target in cancer treatment.[40] Interaction of p73 isoform TA with truncated ΔN regulates differentiation, proliferation, and apoptosis.[41] We found that *TP73* genetic variation may modify cancer susceptibility, which supports a role for p73 in pancreatic carcinogenesis. *TP73* IVS1-7449G>C CG/CC genotype showed increased DNA damage level, which may be more effective in inducing apoptosis with the DNA damage accumulation and genome instability.

Previously we reported that *RECQL* Ex15+159A>C predicted clinical outcome in pancreatic cancer.[42] Currently we found that several other *RECQL* SNPs might be associated with altered pancreatic cancer risk. Genomic instability is detectable in chronic pancreatitis PanIN lesions.[43] RecQ helicases are caretakers of the genome to maintain genomic stability by acting at interface between DNA replication and DNA repair.[44] The role of

RECQL as a regulator for MMR and cell cycle checkpoints in pancreatic carcinogenesis[45] needs further exploration.

The strengths of our study include hypothesis-driven gene selection and comprehensive gene/SNP coverage. The limitations include a small sample size, the potential for false-positive findings due to multiple comparisons, lack of a replication set, and lack of SNP function investigation. To mitigate this, we applied a stringent FDR (1%)-controlled *P*-value. Because the MAFs of the functional SNPs were relatively low, a larger sample size is needed to demonstrate their associations with risk of cancer. Although we used FDR-p-cut-off to correct for multiple comparisons, our findings need to be confirmed in different populations. The associated SNPs in our study may not be functional but in linkage with other functional variants, since in candidate-gene studies most of the variants assayed are not causal but tagging causal ones. Another limitation is that we did not analyze the interaction of genotypes with risky factors (e.g. smoking, alcohol, diabetes). To clarify the gene-environment interaction, we need a larger sample size to get statistical power. Moreover, the study was conducted in a single tertiary referral hospital; our findings may not be applicable to the general population. Furthermore, several genes correlated with patient survival,[27] and thus the association with pancreatic cancer risk might be biased if we missed those patients who died rapidly of the aggressive tumor. However, the three SNPs with $P \leq 0.0015$ were not associated with overall survival, which indicated these genotypes' potential predictive value for pancreatic cancer risk. Finally, eight SNPs deviated from the HWE might indicate the genotyping artifact, selection bias in recruitment, or causal effect on pancreatic cancer pathogenesis. Among those, *PMS2L3* IVS3+9A>G showed protective effect on pancreatic cancer risk. However, none of the 8 SNPs remained significant after adjusting for multiple comparisons. So the major effects of those SNPs that followed HWE on pancreatic cancer risk were unlikely biased.

To conclude, we observed an association between MMR polymorphism and pancreatic cancer risk, providing supporting evidence of a role for MMR dysfunction in pancreatic carcinogenesis. Our findings need to be validated in diverse populations. If confirmed, such information may help to identify individuals at high risk who may benefit from the primary prevention of pancreatic cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANCOVA	analysis of covariance
BMI	Body mass index
CI	confidence interval
ENU	N-ethyl-N-nitrosourea
FDR	false discovery rate
HWE	Hardy-Weinberg equilibrium

IDL	insertion/deletion loop
MAF	minor allele frequency
MMR	mismatch repair
O^6-EtGua	O^6-ethylguanine
OR	odds ratio
SNP	single-nucleotide polymorphism
UTR	untranslated region

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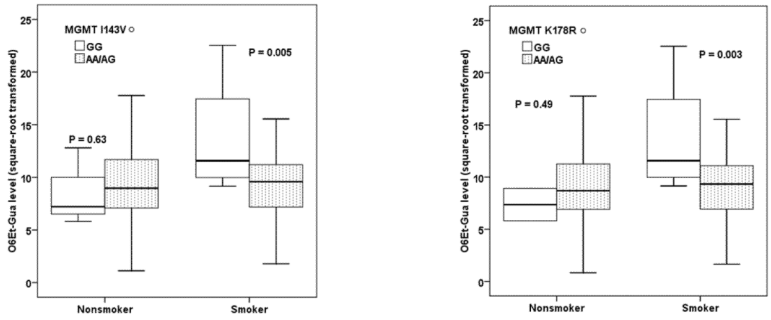

Fig.1. Box-plot of adduct level by genotype and smoking status

The square-root transformed value of original DNA O^6Et-Gua-adduct levels were associated with *MGMT* I143V (left panel) and *MGMT* K178R (right panel) in 297 subjects. *P* value was from ANCOVA F test. The solid line indicated median; the box extents marked the 25th and 75th percentile of the observed value, and the capped bars indicated the 10th and 90th percentile. Symbol indicated the outliers of adduct level.

Table 1
Characteristics of the study population

Variables	Case, n (%) (N=706)	Control, n (%) (N=706)	OR[1] (95% CI)	P
Sex			Matching Factor	
Female	281 (39.8)	269 (38.1)		
Male	425 (60.2)	437 (61.9)		
Race			Matching Factor	
White	624 (88.4)	630 (89.2)		
Nonwhite	82 (11.6)	76 (10.8)		
Age, years			Matching Factor	
< 50	77 (10.9)	99 (14.0)		
50-60	173 (24.5)	197 (27.9)		
60-70	265 (37.5)	251 (35.6)		
≥ 70	191 (27.1)	159 (22.5)		
Diabetes				
No	518 (73.4)	627 (88.8)	1.0	
Yes	188 (26.6)	79 (11.2)	2.77 (2.04-3.76)	<0.001
Smoking				
Never	285 (40.4)	360 (51.0)	1.0	
≤ 20 pack years	175 (24.8)	175 (24.8)	1.32 (0.99-1.76)	0.057
> 20 pack years	246 (34.8)	171 (24.2)	1.68 (1.27-2.22)	<0.001
Alcohol consumption[2]				
Never	319 (46.9)	325 (46.2)	1.0	
≤ 420 g/week	284 (41.8)	324 (46.1)	1.05 (0.81-1.35)	0.72
> 420 g/week	77 (11.3)	54 (7.7)	1.44 (0.94-2.20)	0.09
0-420 g/week vs. >420 g/week			1.48 (1.03-2.13)	0.03
Family history of cancer[3]				
No	262 (37.3)	318 (45.4)	1.0	
Yes	441 (62.7)	382 (54.6)	1.56 (1.24-1.96)	<0.001
Body mass index (kg/m^2)[4]				
< 25	188 (51.8)	254 (59.8)	1.0	
25-30	130 (35.8)	144 (33.9)	1.22 (0.90-1.65)	0.20
≥ 30	45 (12.4)	27 (6.4)	2.25 (1.35-3.76)	0.002

[1] Odds ratios and *P* values were from logistic regression analysis adjusted for sex, race, age (continuous), diabetes, smoking (non-smoker, ≤20 pack years and >20 pack years), alcohol consumption (0-420 g/week vs. >420 g/week), body mass index, and family history of cancer in a first-degree relative.

[2] Missing values from 26 cases and 3 controls.

[3] Missing values from 3 cases and 6 controls.

[4] Information was available for only 363 cases and 425 controls.

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Table 2

Association of genotype with pancreatic cancer risk

Function		Genotype	Case, n/n	Control, n/n	OR[1] (95% CI)	*P*[1]
Recognition	O^6G	*MGMT* I143V (AA vs. AG/GG)	501/112	472/170	0.60 (0.46-0.80)	**<0.001**
		MGMT K178R (AA vs. AG/GG)	533/170	490/215	0.73 (0.57-0.93)	0.01
		MGMT IVS5+23129G>A (AA vs. AG/GG)	226/473	185/517	0.76 (0.60-0.97)	0.027
	MutSα	*MSH2* IVS9-9A>T (AA/TA vs. TT)	525/18	547/5	3.81 (1.36-10.68)	0.01
		MSH6 G39E (GG vs. AG/AA)	430/247	382/284	0.73 (0.58-0.92)	0.008
	MutSβ	*MSH6* IVS4-101G>C (CC vs. CG/GG)	371/215	335/274	0.70 (0.55-0.89)	0.0037
		MSH3 I79V (GG vs. GA/AA)	367/245	344/166	1.33 (1.03-1.72)	0.027
		MSH3 Ex24-318G>A (GG/GA vs. AA)	689/17	694/4	3.39 (1.11-10.3)	0.03
	MutLα (γ)	*MLH1* IVS3-659A>C (AA vs. CA/CC)	231/472	187/516	0.73 (0.57-0.92)	0.009
		MLH1 IVS12-169C>T (CC vs. CT/TT)	224/482	187/519	0.74 (0.59-0.91)	0.006
		MLH1 IVS14-19A>G (AA vs. AG/GG)	200/489	171/534	0.78 (0.61-1.00)	0.05
		MLH3 P844L (CC vs. TC/TT)	216/480	183/515	0.74 (0.60-0.92)	0.007
		PMS2 T485K (CC vs. CA/AA)	620/81	584/118	0.67 (0.49-0.91)	0.01
		PMS2 IVS1-1121C>T (CC vs. TC/TT)	260/354	317/322	1.44 (1.14-1.81)	**0.0015**
		PMS2 IVS7+442G>T (GG vs. GT/TT)	345/236	326/279	0.77 (0.60-0.98)	0.036
		PMS2L3 Ex1+118C>T (CC vs. CT/TT)	653/30	674/5	5.54 (2.10-14.61)	**<0.001**
		PMS2L3 IVS2-1578A>G (GG/AA vs. AG)	365/238	322/301	0.70 (0.55-0.88)	0.0027
		PMS2L3 IVS3+9A>G (AA/GG vs. GA)	271/434	219/486	0.70 (0.56-0.89)	0.0029
Excision	Exonuclease	*TREX1* Ex14+782T>C (TT/CT vs. CC)	677/24	662/44	0.55 (0.32-0.93)	0.026
Apoptosis	TP73	*TP73* A610A (GA/AA vs. GG)	629/69	643/50	1.54 (1.03-2.29)	0.03
		TP73 IVS1-7449G>C (GG vs. CG/CC)	251/438	210/476	0.73 (0.58-0.93)	0.0097
Regulation	RECQL	*RECQL* -5349A>G (GG/GA vs. AA)	627/63	640/41	1.54 (1.01-2.34)	0.046
		RECQL IVS1-92C>T (CC/TC vs. TT)	599/43	592/25	1.74 (1.02-2.95)	0.04
		RECQL IVS4-795C>T (CC/TT vs. CT)	375/255	290/276	0.72 (0.57-0.91)	0.006
		RECQL IVS5-717C>A (CC/CA vs. AA)	651/48	666/30	1.63 (1.00-2.65)	0.05
		RECQL IVS8+190A>G (AA vs. AG/GG)	292/401	338/359	1.27 (1.02-1.58)	0.03
		RECQL IVS11+582T>A (TT/AA vs. TA)	293/230	219/250	0.68 (0.53-0.89)	0.004
		RECQL *1236A>G (AA vs. AG/GG)	212/283	146/261	0.73 (0.55-0.97)	0.029

O^6G, O(6)-alkyl-guanine; OR, Odds ratio.

SNPs with P>0.05 are not listed.

[1] OR and *P* values were from logistic regression analysis adjusted for sex, race, age, diabetes, smoking, alcohol consumption, and family history of cancer.

Table 3

Association of genotype with DNA damage level

	O^6-EtGua[§] (Mean ± SE)							
	Case/control				Control			
Genotype	**N**	**All**	**Nonsmoker**	**Smoker**	**N**	**All**	**Nonsmoker**	**Smoker**
MGMTI143V								
GG	7	11.5 ± 2.1	8.6 ± 2.1	13.7 ± 3.0	6	12.2 ± 2.3	9.3 ± 3.5	13.7 ± 3.0
AG	55	9.4 ± 0.4	9.5 ± 0.6	9.2 ± 0.5	48	9.4 ± 0.4	9.5 ± 0.7	9.4 ± 0.6
AA	179	9.3 ± 0.2	9.5 ± 0.3	9.0 ± 0.3	147	9.2 ± 0.3	9.5 ± 0.4	8.8 ± 0.4
AA/AG		9.3 ± 0.2	9.5 ± 0.3	9.1 ± 0.3		9.2 ± 0.2	9.5 ± 0.3	8.9 ± 0.3
p[*]		0.02	0.63	0.005		0.03	0.94	0.007
MGMTK178R								
GG	6	11.6 ± 2.4	7.4 ± 1.5	13.7 ± 3.0	6	11.6 ± 2.3	7.4 ± 1.5	13.7 ± 3.0
AG	78	9.0 ± 0.4	9.2 ± 0.5	8.8 ± 0.5	65	9.0 ± 0.4	9.2 ± 0.6	8.7 ± 0.6
AA	211	8.8 ± 0.2	8.9 ± 0.3	8.8 ± 0.3	163	8.8 ± 0.3	9.1 ± 0.4	8.5 ± 0.4
AA/AG		8.9 ± 0.2	9.0 ± 0.3	8.8 ± 0.2		8.9 ± 0.2	9.2 ± 0.3	8.5 ± 0.3
p		0.05	0.49	0.003		0.058	0.46	0.004
MSH6 G39E								
AG/AA	116	9.6 ± 0.3	9.5 ± 0.5	9.7 ± 0.5	98	9.8 ± 0.4	9.9 ± 0.5	9.4 ± 0.5
GG	158	8.8 ± 0.2	9.1 ± 0.4	8.6 ± 0.3	117	8.6 ± 0.3	9.0 ± 0.4	8.2 ± 0.4
p		0.009	0.51	0.047		0.01	0.15	0.03
PMS2L3 IVS3+9A>G								
GA	243	9.1 ± 0.2	9.3 ± 0.3	8.9 ± 0.3	201	9.1 ± 0.2	9.4 ± 0.3	8.8 ± 0.4
AA/GG	54	8.1 ± 0.4	7.5 ± 0.5	8.8 ± 0.6	34	8.0 ± 0.5	7.7 ± 0.6	8.3 ± 0.8
p		0.05	0.01	0.86		0.077	0.046	0.60
TP73 IVS1-7449G>C								
CG/CC	193	9.3 ± 0.2	9.5 ± 0.3	9.2 ± 0.3	151	9.3 ± 0.3	9.6 ± 0.4	9.1 ± 0.4
GG	89	8.4 ± 0.3	8.1 ± 0.5	8.7 ± 0.5	72	8.4 ± 0.4	8.4 ± 0.5	8.4 ± 0.5
p		0.03	0.03	0.38		0.058	0.098	0.33

O^6-EtGua, O^6-ethylguanosine; SE, standard error.

§ Square-root transformed value of O^6-EtGua original level (fmol/μg DNA).

* *P* was from ANCOVA F test adjusted for age, race, sex, smoking, and alcohol consumption.

Table 4
Association of haplotype with pancreatic cancer risk

Haplotype	Frequency (Case/Control)	OR[1] (95% CI)	P[1]
MGMT			
GGCG	0.1268/0.1113	1.0	
AATG	0.0912/0.0756	2.95 (2.09-4.18)	<0.001
GG**TA**	0.0766/0.0955	0.54 (0.37-0.78)	0.001
AATA	0.094/0.05	3.07 (1.74-5.41)	<0.001
MSH6			
GTCGA	0.1908/0.2241	1.0	
GTC**C**A	0.1709/0.1216	1.93 (1.35-2.75)	<0.001
ATCGA	0.1116/0.126	0.61 (0.45-0.82)	0.001
PMS2			
TACCGGTA	0.1382/0.2323	1.0	
CAC**TTT**TA	0.0948/0.0776	2.03 (1.41-2.92)	<0.001
TACCGG**CG**	0.07/0.0464	4.11 (2.74-6.16)	<0.001
CGGC**T**GTA	0.061/0.048	3.13 (2.04-4.80)	<0.001
CAC**TT**G**CG**	0.0502/0.0322	6.83 (3.83-12.1)	<0.001
CACC**T**GTA	0.0495/0.0368	2.98 (1.84-4.83)	<0.001
CA**G**C**T**GTA	0.0482/0.0198	3.00 (1.92-4.69)	<0.001
CACC**TTCG**	0.0466/0.0406	2.81 (1.78-4.43)	<0.001
PMS2L3			
GAACC	0.3663/0.3768	1.0	
GA**G**CC	0.1997/0.178	2.85 (2.24-3.63)	<0.001
G**G**ACC	0.1923/0.1749	1.82 (1.35-2.50)	<0.001
G**GG**CC	0.1278/0.1048	3.67 (2.82-4.78)	<0.001
TP73			
GCCCTGCG	0.2632/0.2626	1.0	
GCCC**AC**CG	0.0846/0.1148	0.51 (0.38-0.68)	<0.001
GCCCT**C**CG	0.0473/0.0535	0.32 (0.22-0.47)	<0.001

[1] Odds ratios and *P* values were from logistic regression analysis adjusted for sex, race, age, diabetes, smoking, alcohol consumption, and family history of cancer. Haplotypes with *P*>0.0015 but <0.05 in logistic regression models are listed in Supplemental Table 3.