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Original Article

Sequence variants in antioxidant defense and DNA repair genes, dietary antioxidants, and pancreatic cancer risk

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Abstract: To investigate whether polymorphisms in genes related to oxidative stress act alone or in combination with antioxidants to modulate pancreatic cancer risk. Cases (n=189), ages ≥ 20 years, were ascertained in 1994-1998 from all hospitals in the Twin Cities and the Mayo Clinic. Controls (n=486) were randomly selected from the general population and frequency matched to cases by age and sex. After adjustment for confounders, individuals who were homozygous or heterozygous for the variant allele of *SOD2* polymorphism (Ala16Val, rs4880) experienced a 43% lower risk than those who were homozygous for the wild-type allele [OR (95% CI): 0.57 (0.37, 0.89)]. Conversely, an increased risk was observed for the variant allele of *hOGG1* polymorphism (Ser326Cys, rs1052133) compared with the wild-type allele [OR (95% CI) for Ser/Cys or Cys/Cys vs. Ser/Ser: 1.57 (1.04, 2.39)]. The protective effect of the variant allele of *SOD2* was more pronounced among subjects with a low dietary intake (<median) of lutein/zeaxanthin, lycopene, α-carotene, and α-tocopherol [OR (95% CI): 0.46 (0.27, 0.81), 0.42 (0.23, 0.75), 0.47 (0.26, 0.85), and 0.48 (0.27, 0.87), respectively]. Individual variations in the capacity to defend against oxidative stress and to repair oxidative DNA damage influence pancreatic cancer risk, and some of these genetic effects are modified by dietary antioxidants.

Keywords: Antioxidants, DNA repair, oxidative stress, pancreatic cancer, polymorphisms

Introduction

Pancreatic cancer is one of the leading causes of cancer death in the United States and other Western countries [1-3]. However, little is known about its etiology, with cigarette smoking as the only well established risk factor. Diabetes had been linked to pancreatic cancer in epidemiologic studies but reverse causality could not be entirely ruled out in some of those studies [4]. Because no effective screening tools are available for this malignancy, most patients are diagnosed at an advanced stage and have a dismal prognosis. To prevent pancreatic cancer, it is critical to identify environmental and genetic factors that influence the occurrence of this dreadful disease.

Several lines of evidence suggest that oxidative stress plays a role in pancreatic cancer etiology. Superoxide dismutase (SOD) and catalase (CAT) are primary intracellular antioxidant enzymes in mammalian cells. Mitochondrial manganese SOD (Mn-SOD or SOD2) catalyzes the dismutation of superoxides into hydrogen peroxide and water. Hydrogen peroxide is further converted into oxygen and water, thereby preventing cells from attacks by highly reactive oxygen species [5]. Expression of *SOD2* and *CAT* has been shown to be lower in pancreatic tumor than in normal pancreas [6]. Furthermore, enforced expression of *SOD2* into a rapidly-growing pancreatic cancer cell line increased SOD2 activity and decreased growth rate [7]. Cigarette smoke contains a number of oxidants and a long-term exposure to cigarette smoking enhances oxidative stress [8]. Chronic pancreatitis has been associated with an elevated risk of pancreatic

cancer [9], whereas high dietary intakes of some antioxidants (e.g. vitamins C and E, lycopene) were reported to reduce risk [2,10,11].

Oxidative stress induces oxidative DNA lesions, including 8-hydroxy-2-deoxyguanine (8-OH-dG). A major form of such DNA damage, 8-OH-dG can cause transversions of GC to TA in oncogenes and tumor suppressor genes and eventually leads to carcinogenesis [12-14]. Human oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing group 1 (XRCC1) are key proteins in the base-excision repair pathway that is responsible for repairing oxidative DNA damage. After a damaged base is excised and removed by hOGG1, XRCC1 functions as a scaffold to bring together a complex of DNA repair enzymes (polymerase-α, DNA ligase III, etc.) in the subsequent restoration of the site [12-14].

Therefore, it is possible that sequence variants in genes involved in antioxidant defense and repair of oxidative DNA damage act alone or in combination with dietary antioxidants to influence pancreatic cancer risk. To date, only a few epidemiologic studies [5,15] have investigated this hypothesis, with inconsistent results. We sought to address this question in a population-based case-control study in Minnesota.

Materials and methods

Study population

The case-control study of pancreatic cancer conducted from April 1994 to September 1998 in Minnesota has been described in detail elsewhere [16,17]. Briefly, cases were patients diagnosed with pathologically-confirmed cancer of the exocrine pancreas (*International Classification of Disease for Oncology, third edition, code C25*). Cases were ascertained from all hospitals in the seven-county metropolitan area of the Twin Cities (Minneapolis and St. Paul) and the Mayo Clinic. The cases recruited from the latter were confined to subjects residing in the Upper Midwest of the US. Because pancreatic cancer is rapidly fatal in a high proportion of cases, an ultra-rapid case-ascertainment system was adopted to maximize response rate of cases. As a result, the mean and median numbers of days between diagnosis and first contact for the study were only 34 and 13 days for the cases enrolled to the study, respectively. To be eligible for the study, subjects had to be 20 years of age or older, English-speaking, and mentally competent. Of 460 eligible cases identified, 202 failed to participate in the study because of occurrence of death before contact or interview (n = 85), refusal of cases (n = 79), refusal of physicians (n = 31), and inability to contact cases (n = 7). After these exclusions, 258 cases participated in the study with a response rate of 56%.

Controls were recruited from the geographic areas where cases lived. Specifically, controls were randomly selected from residents of the seven-county metropolitan area of the Twin Cities and the Upper Midwest of the US. Potential controls were identified from the drivers' license and State identity card databases for subjects aged 20-64 years and from US Health Care Financing Administration (now the Centers for Medicare and Medicaid Services) records for those aged 65 years or older. Controls were frequency matched to cases by age (within 5 years) and sex. Inclusion criteria for controls were the same as those for cases, disallowing diagnosis of pancreatic cancer. Of 1,141 eligible controls identified, 676 participated in the study, which yielded a response rate of 59%. Of the 934 subjects (258 cases and 676 controls) who completed at least some portion of the study, genotyping data were missing for 259 subjects (69 cases and 190 controls) because they neither donated a blood sample nor had sufficient amounts of remaining DNA samples for genotyping the polymorphisms evaluated in this study. Therefore, a total of 189 cases and 486 controls were available for the present analysis. The institutional review boards of the University of Minnesota and the Mayo Clinic approved the protocol of this study. Each participant provided written, informed consent prior to interview.

Data collection

All participants were interviewed in person with a risk factor questionnaire and a food frequency questionnaire (FFQ). The risk factor questionnaire elicited information on demographics, socioeconomics, physical activity, medical history, family history, and cigarette smoking (status, duration, and amount).

The diet of cases and controls was assessed by a slightly modified version of the Willett FFQ (18), a well-validated dietary assessment instru-

ment widely used in nutritional epidemiologic studies. It contained 153 items of food or food groups commonly eaten in the U.S. diet as well as questions on alcohol consumption (serving/week). In the dietary survey, subjects were asked to recall the average frequency of consumption of each food item included in the questionnaire during the year before pancreatic cancer diagnosis for cases or the past year for controls. Dietary intakes of energy and nutrients were calculated by multiplying the amount in a pre-defined portion size of each food item by the reported frequency of consumption and summing over all food items. The amounts of energy and nutrients for the portion size of each food item were estimated using a nutrient database that was developed for the Minnesota Colon Cancer Prevention Research Unit studies. The antioxidants used in our data analysis included intake of these nutrients from both diet and supplements in single or multivitamin *formulations*.

Genotyping

DNA was extracted from peripheral blood lymphocytes using a commercial kit (Qiagen Inc., Valencia, CA). The polymorphisms examined in this study were *CAT* (rs1001179), *SOD2* (rs4880), *hOGG1* (rs1052133), and *XRCC1* (rs25487). These polymorphisms were selected because they are involved in either defense against oxidative stress or repair of oxidative DNA damage, have functional impact (e.g., altered enzyme activity, protein structure or DNA repair capacity), and are common (>5%) among Caucasians (vast majority of the participants). Genotyping of these variants was performed with TaqMan SNP Genotyping Assays (Carlsbad, CA) at the laboratory (Dr. Kadlubar) of the Division of Medical Genetics, University of Arkansas for Medical Sciences. Quality control measures were taken to minimize genotyping error. Specifically, genotyping personnel were blinded to the case-control status of DNA specimens, and a 10% of the tested samples were randomly replicated and were found to be 100% concordant.

Statistical analysis

Prior to data analysis, a test for the deviation from the Hardy-Weinberg equilibrium was performed among controls to detect potential genotyping errors. Characteristics of cases and controls were compared with t-test for continuous variables and with chi-square test for categorical variables. Pancreatic cancer risk in relation to selected polymorphisms was evaluated by unconditional logistic regression analysis. In the multivariable models, subjects who were homozygous for wild-type allele were treated as the reference group to estimate odds ratios (OR) and 95% confidence interval (95% CI) for those who were heterozygous or homozygous for variant allele. Given the relatively small number of cases (n=189), individuals who carried one or two copies of variant allele were combined to maximize statistical power. The confounders adjusted for in the logistic regression were age, sex, race, education (three levels), cigarette smoking (pack-year), alcohol intake (serving/week), physical activity (hour/week), and energy intake (kcal/day).

To investigate whether the main effects of genes of interest on pancreatic cancer risk were modified by dietary intake of carotenoids and other antioxidants (vitamin C and α-tocopherol), an interaction term between each of selected polymorphisms and each of dietary antioxidants were put into the multivariable models described above. The likelihood ratio test was used to examine the significance of interaction terms. Because it is biologically plausible that antioxidants interact with genetic variation in antioxidant defense and repair of oxidative DNA damage, the associations between selected polymorphisms and pancreatic cancer risk were stratified by low and high levels of dietary antioxidants (defined as $<$ and $\geq$ median values of controls, respectively) regardless of whether the interaction terms constructed reached statistical significance level. Statistical analysis was undertaken with the SAS software (version 9.1; SAS Institute, Inc., Cary, NC). A two-sided p-value of < 0.05 was considered statistically significant.

Results

Characteristics of cases and controls are presented in **Table 1**. Study subjects were predominantly of European origin (93.5% for cases and 97.9% for controls). Risk of pancreatic cancer in relation to four selected genetic variants was shown in **Table 2**. After adjustment for confounders, individuals who were homozygous or heterozygous for the variant allele of the *SOD2* polymorphism (rs4880) appeared to have a

Table 1. Characteristics of cases and controls in a population-based case-control study of pancreatic cancer in Minnesota, 1994-1998*

Characteristic	Cases (n=189)	Controls (n=486)	*P* Value
Age (years)†	65.9 (11.2)	65.6 (12.4)	0.76
Sex			
Male	112 (60.5%)	277 (57.0%)	
Female	73 (39.5%)	209 (43.0%)	0.41
Race			
Caucasian	173 (93.5%)	476 (97.9%)	
African-American	8 (4.3%)	4 (0.8%)	
Other	4 (2.2%)	6 (1.3%)	0.006
Education			
Some high school or less	34 (18.1%)	56 (11.5%)	
High school graduate	68 (36.2%)	121 (24.9%)	
Some college or more	86 (45.7%)	309 (63.6%)	0.0001
Cigarette smoking			
Never smoker	75 (42.1%)	223 (45.9%)	
Former smoker	75 (42.1%)	209 (43.0%)	
Current smoker	28 (15.8%)	54 (11.1%)	0.26
Pack-year†‡	33.4 (22.1)	33.2 (29.1)	0.95
Alcohol intake (serving/week)†	3.4 (6.9)	4.7 (8.5)	0.065
Physical activity (hr/week)†	42.9 (26.6)	50.1 (25.5)	0.003

* Data for some variables were missing for cases and/or controls.
† Values given are mean (standard deviation).
‡ Means calculated for former and current smokers combined.

Table 2. Risk of pancreatic cancer in relation to SNPs in genes involved in oxidative stress in a population-based case-control study of pancreatic cancer in Minnesota, 1994-1998*

	Cases (n=189)		Controls (n=486)		
Genotype	Number	%	Number	%	OR (95% CI)†
CAT (-262C>T) (rs1001179)					
CC	103	54.5	271	55.8	1.00‡
CT or TT	86	45.5	215	44.2	0.98 (0.65, 1.47)
SOD2 (Ala16Val) (rs4880)					
Ala/Ala	60	31.8	121	24.9	1.00
Ala/Val or Val/Val	129	68.2	365	75.1	0.57 (0.37, 0.89)
hOGG1 (Ser326Cys) (rs1052133)					
Ser/Ser	100	53.2	299	61.9	1.00
Ser/Cys or Cys/Cys	88	46.8	184	38.1	1.57 (1.04, 2.39)
XRCC1 (Arg399Gln) (rs25487)					
Arg/Arg	79	41.8	208	42.8	1.00
Arg/Gln or Gln/Gln	110	58.2	278	57.2	1.11 (0.73, 1.70)

Ala, Alanine; Val, Valine; Ser, Serine; Cys, Cytosine; Arg, Arginine; Gln, Glutamine.
* Data on hOGG1 were missing for one case and three controls due to failed genotyping.
† Adjusted for age, sex, race, education (three levels), cigarette smoking (pack-year), alcohol intake (serving/week), physical activity (hour/week), and energy intake (kcal/day).
‡ Reference.

43% lower risk than those who were homozygous for the wild-type allele [OR (95% CI): 0.57 (0.37, 0.89)]. Conversely, a statistically significantly increased risk was observed for the variant allele (326Cys) of *hOGG1* polymorphism (rs1052133) compared with the wild-type allele (326Ser) [OR (95% CI) for Ser/Cys or Cys/Cys vs. Ser/Ser: 1.57 (1.04, 2.39)].

Table 3. Risk of pancreatic cancer in relation to the SOD2 polymorphism (rs 4880) stratified by dietary intake of antioxidants in a population-based case-control study of pancreatic cancer in Minnesota, 1994-1998*

	Ala/Ala		Ala/Val or Val/Val	
Antioxidant†	Cases/Controls	OR‡	Cases/Controls	OR (95% CI)§
Lutein/zeaxanthin				
Low	36/62	1.00	57/167	0.46 (0.27, 0.81)
High	16/53	1.00	41/177	0.94 (0.44, 2.11)
β-cryptoxanthin				
Low	26/60	1.00	53/168	0.57 (0.31, 1.05)
High	26/55	1.00	45/176	0.54 (0.29, 1.04)
Lycopene				
Low	32/55	1.00	52/174	0.42 (0.23, 0.75)
High	20/60	1.00	46/170	0.84 (0.43, 1.73)
α-carotene				
Low	31/58	1.00	52/171	0.47 (0.26, 0.85)
High	21/57	1.00	46/173	0.75 (0.38, 1.51)
β-carotene				
Low	30/60	1.00	61/169	0.56 (0.32, 0.99)
High	22/55	1.00	37/175	0.57 (0.28, 1.18)
Vitamin C				
Low	25/58	1.00	56/171	0.67 (0.36, 1.27)
High	27/57	1.00	42/173	0.49 (0.25, 0.93)
α-tocopherol				
Low	30/60	1.00	46/169	0.48 (0.27, 0.87)
High	22/55	1.00	52/175	0.73 (0.38, 1.46)

Ala, Alanine; Val, Valine. * Data on antioxidants were missing for 39 cases and 27 controls. † Median values of controls were used as cut-off points to define low (<median) and high (≥median) groups, and were 2.2 mg, 0.05 mg, 3.8 mg, 0.72 mg, 4.9 mg, 210 mg and 8.5 mg for lutein/zeaxanthin, β-cryptoxanthin, ycopene, α-carotene, β-carotene, vitamin C, and α-tocopherol, respectively. ‡ Reference. § Adjusted for age, sex, race, education (three levels), cigarette smoking (pack-year), alcohol intake (serving/week), physical activity (hour/week), and energy intake (kcal/day).

The gene-nutrient interactions on pancreatic cancer risk were analyzed for the *SOD2* polymorphism and the *hOGG1* polymorphism. A clear pattern of the interactions between some dietary antioxidants and the *SOD2* missense mutation was identified. Specifically, a reduced risk of pancreatic cancer associated with the valine allele of *SOD2* was more pronounced among subjects with a low dietary intake (<median) of lutein/zeaxanthin, lycopene, α-carotene, and α-tocopherol [OR (95% CI): 0.46 (0.27, 0.81), 0.42 (0.23, 0.75), 0.47 (0.26, 0.85), and 0.48 (0.27, 0.87), respectively] (**Table 3**). No apparent effect modification of dietary antioxidants was detected on the association between the *hOGG1* polymorphism and the risk of pancreatic cancer (**Table 4**). The selected genetic variants of *CAT* and *XRCC1* neither influenced the risk of pancreatic cancer alone (**Table 2**) nor interacted with dietary antioxidants to modulate risk (data not shown).

Discussion

This study is one of the first investigations of whether genetic variability in antioxidant defense and repair of oxidative DNA damage interacts with dietary intake of carotenoids and other antioxidants to modulate the risk of pancreatic cancer. We found that polymorphisms in *SOD2* and *hOGG1* were associated with an altered risk of this malignancy. Furthermore, the protective effect of the mutant allele of *SOD2* (Ala16Val) was modified by dietary intake of antioxidants.

To date, only two studies have evaluated the association between polymorphisms in antioxidant genes and the risk of pancreatic cancer. In a case-control study conducted in Massachusetts General Hospital [15], the homozygous variant genotype of *SOD2* (rs4880) was marginally associated with an elevated risk of pancreatic cancer [OR (95% CI): 1.96 (1.0, 3.8) for Val/

Table 4. Risk of pancreatic cancer in relation to the hOGG1 polymorphism (rs1052133) stratified by dietary intake of antioxidants in a population-based case-control study of pancreatic cancer in Minnesota, 1994-1998*

Antioxidant†	Ser/Ser Cases/Controls	OR‡	Ser/Cys or Cys/Cys Cases/Controls	OR (95% CI)§
Lutein/zeaxanthin				
Low	49/140	1.00	43/88	1.48 (0.85, 2.55)
High	27/139	1.00	30/89	1.63 (0.83, 3.21)
β-cryptoxanthin				
Low	42/141	1.00	37/87	1.78 (0.99, 3.25)
High	34/138	1.00	36/90	1.55 (1.02, 2.36)
Lycopene				
Low	45/144	1.00	38/83	1.73 (0.98, 3.06)
High	31/135	1.00	35/94	1.39 (0.74, 2.62)
α-carotene				
Low	42/136	1.00	41/90	1.52 (0.87, 2.67)
High	34/143	1.00	32/87	1.64 (0.86, 3.14)
β-carotene				
Low	42/136	1.00	48/90	1.74 (1.01, 3.01)
High	34/143	1.00	25/87	1.24 (0.63, 2.43)
Vitamin C				
Low	44/140	1.00	36/88	1.29 (0.72, 2.31)
High	32/139	1.00	37/89	1.76 (0.95, 3.28)
α-tocopherol				
Low	41/145	1.00	34/82	1.41 (0.79, 2.51)
High	35/134	1.00	39/95	1.67 (0.90, 3.14)

Ser, Serine; Cys, Cytosine. *Data were missing on antioxidants for 39 cases and 27 controls and on genotyping for one case and three controls. †Median values of controls were used as cut-off points to define low (<median) and high (≥median) groups, and were 2.2 mg, 0.05 mg, 3.8 mg, 0.72 mg, 4.9 mg, 210 mg and 8.5 mg for lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene, vitamin C, and α-tocopherol, respectively. ‡ Reference. § Adjusted for age, sex, race, education (three levels), cigarette smoking (pack-year), alcohol intake (serving/week), physical activity (hour/week), and energy intake (kcal/day).

Val vs. Ala/Ala]. Of note, this risk estimate was only based on 64 cases and 166 controls. In a study of 575 cases and 648 controls recruited at the MD Anderson Cancer Center [5], a modest reduced risk was observed for the variant allele (16Val), although this effect was not statistically significant. Our study showed that individuals who had one or two copies of the 16Val allele experienced a 43% lower risk than those who only carried the wild-type allele (16Ala). Scarce data are available for the functional impact of this nonsynonymous polymorphism; one study suggested that it alters the secondary structure and transport of the protein [18]. More studies are warranted to investigate the functionality of this common sequence variant and its influence on pancreatic cancer risk.

It has been reported that *hOGG1* polymorphisms (rs1052133) had functional significance [19]. Specifically, the DNA repair capacity of 326Ser allele was 7-fold higher than that of 326Cys allele [19]. Surprisingly, only one epidemiologic study has evaluated the effect of this genetic variant on pancreatic cancer risk [12]. In that case-control study carried out at the Mayo Clinic, no significant association was detected for all subjects, although an increased risk was suggested for never smokers [OR (95% CI): 1.18 (0.80, 1.74 for Ser/Cys or Cys/Cys vs. Ser/Ser] [12]. We found a significantly increased risk associated with the variant allele (326Cys) [OR (95% CI): 1.57 (1.04, 2.39) for Ser/Cys or Cys/Cys vs. Ser/Ser]. Our results were consistent with the data available for the functionality of this polymorphism.

We demonstrated a clear pattern of the interaction between the *SOD* Ala16Val polymorphism and dietary intake of some carotenoids and vitamin E on the occurrence of pancreatic cancer. Specifically, reduction in risk associated with the variant allele (16Val) was greater among individuals with a low intake (<median) of lutein/zeaxanthin, lycopene, α-carotene, and α-tocopherol (one form of vitamin E family with highest bioavailability). Abundantly present in fruits and vegetables, lutein/zeaxanthin

(isomers with identical chemical formulas), lycopene, and α-carotene are carotenoids that possess antioxidant properties [20]. A long-term low consumption of foods rich in carotenoids may promote oxidative stress and hereby induces oxidative DNA damage. Our results suggested that the potential beneficial effect of this genetic polymorphism was more remarkable when oxidative stress was likely present due to habitual suboptimal or inadequate intake of antioxidants. The effect modification observed between this genetic variant and vitamin E in the present study was generally confirmed in the MD Anderson Cancer Center study [5].

Besides the molecular-functional studies mentioned above [18,19], gene expression and epidemiologic studies lend additional support for the main effects of the genetic polymorphisms of interest and their interactions with dietary intake of antioxidants on pancreatic cancer risk. In mammals, there are three primary intracellular antioxidant enzymes to prevent or repair oxidative DNA damage: SOD, catalase, and glutathione peroxidase. Pancreatitis has been associated with an increased risk of pancreatic cancer [9]. An immunohistochemical study [6] revealed a gradual decrease in the expression of manganese SOD, copper/zinc SOD, and catalase in pancreatic cells when comparing normal pancreas to chronic pancreatitis to pancreatic cancer. Furthermore, another study from the same research group [7] showed that increased expression of manganese SOD in the rapidly growing cell line MIA PaCa-2 by adenovirus transfection suppressed the growth rate of these pancreatic cancer cells.

A few epidemiologic studies that have examined the association between antioxidants and pancreatic cancer risk offer another line of credibility for the interactions of antioxidants with the candidate genes considered on the occurrence of this disease. In a Canadian case-control study [11], a significantly reduced risk was associated with dietary intake of lycopene among men and of β-carotene among never smokers. An inverse and statistically significant association was also observed for high intake of vitamin C and vitamin E from dietary and supplemental sources in a case-control study in the San Francisco Bay Area [10]. The protective effect of vitamin E was also reported in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study of male Finnish smokers [2]. In the latter study, higher serum concentrations of α-tocopherol were significantly associated with a lower risk of pancreatic cancer. All of these epidemiologic data, although not substantial, indicate that it is biologically plausible that external antioxidants interact with genetic variants in internal antioxidant enzymes to influence pancreatic carcinogenesis by modulating oxidative stress and repairing its resultant oxidative DNA damage.

No significant associations of *CAT* (-262 C/T, rs1001179) and *XRCC1* (Arg399Gln, rs25487) with the risk of pancreatic cancer were observed in the present study. Previous studies evaluating the effect of these polymorphisms on pancreatic cancer are scanty and inconsistent. It has been revealed that catalase activity (in a red blood cell model) was significantly higher for the CC genotype than for the CT or TT genotype [21]. The C allele has been associated with a lower risk of breast cancer than the T allele in the Long Island Breast Cancer Study [21]. The only one study that has examined the association between the *CAT* (-262 C/T) variant and pancreatic cancer risk yielded null results [5], which is consistent with the results of our study. The variant allele of *XRCC1* (Arg399Gln) polymorphism was reported to increase levels of aflatoxin B1-DNA adducts in placental tissue, polyphenol DNA adducts in mononuclear cells, sister chromatin exchange in lymphocytes, and somatic glycophorin A mutation in erythrocytes [22,23]. Therefore, it is possible that this functional variant confers an elevated risk of pancreatic cancer. A modest insignificant increase in risk was found in the present study and the San Francisco Bay Area study [13] for all subjects and in the Mayo Clinic study among heavy smokers (>40 pack-years) [12].

Several limitations need to be considered in interpreting the results of the present study. Selection bias might arise from relatively low response rates for cases and controls (<60%), although a rate of this size is not uncommon in studies of pancreatic cancer [11,24]. Assessment of diet by food frequency questionnaire is subject to recall bias (i.e. under- or over-reporting of some food items) [25]. This misreporting error was less likely to be substantial because our hypothesis was unknown to the study participants. The food frequency questionnaire is designed to evaluate usual dietary intake. However, changes in dietary habits among

some patients after the diagnosis and treatment of pancreatic cancer might have affected the recall of their usual diet. It is unlikely that such a dietary change was considerable because cases were invited to participate in the study within an average of about one month after diagnosis. Genotyping error should not be a concern because all polymorphisms examined were in *Hardy-Weinberg* equilibrium. Body mass index was not adjusted as a confounder due to lack of data, but energy intake and physical activity (a major determinant of energy expenditure [26]) were controlled for in the regression models. In addition, it is possible that some of our findings are observed by chance alone because of small sample size.

In this study, we found that polymorphisms in *SOD2 and hOGG1* were associated with an altered risk of pancreatic cancer and that the potential protective effect of *SOD2* (Ala16Val) polymorphism was modified by dietary intake of some carotenoids and vitamin E. Our observation suggests that oxidative stress may play a role in the etiology of pancreatic cancer. If the findings of this study are confirmed in other epidemiologic studies, it may be possible to reduce pancreatic cancer risk by increasing intake of antioxidants especially among subjects who carry risk alleles of genes involved in antioxidant defense and repair of oxidative DNA damage.

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References

[1] American Cancer Society. Cancer Facts & Figures. Atlanta, GA: American Cancer Society, 2009.

[2] Stolzenberg-Solomon RZ, Sheffler-Collins S, Weinstein S, Garabrant DH, Mannisto S, Taylor P, Virtamo J, Albanes D. Vitamin E intake, alpha-tocopherol status, and pancreatic cancer in a cohort of male smokers. Am J Clin Nutr 2009; 89: 584-591.

[3] Parkin DM. International variation. Oncogene 2004; 23: 329-6340.

[4] Gullo L, Pezzilli R, Morselli-Labate AM. Diabetes and the risk of pancreatic cancer. Italian pancreatic cancer study group. N Engl J Med 1994; 331: 81-84.

[5] Tang H, Dong X, Day RS, Hassan MM, Li D. Antioxidant genes, diabetes and dietary antioxidants in association with risk of pancreatic cancer. Carcinogenesis 2010; 31: 607-613.

[6] Cullen JJ, Mitros FA, Oberley LW. Expression of antioxidant enzymes in diseases of the human pancreas: Another link between chronic pancreatitis and pancreatic cancer. Pancreas 2003; 6: 23-27.

[7] Cullen JJ, Weydert C, Hinkhouse MM, Ritchie J, Domann FE, Spitz D, Oberley LW. The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. Cancer Res 2003; 63: 1297-1303.

[8] Cross CE, van der Vliet A, Eiserich JP. Cigarette smokers and oxidant stress: A continuing mystery. Am J Clin Nutr 1998; 67: 184-185.

[9] Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andren-Sandberg A, Domellof L. Pancreatitis and the risk of pancreatic cancer. International pancreatitis study group. N Engl J Med 1993; 328: 1433-1437.

[10] Gong Z, Holly EA, Wang F, Chan JM, Bracci PM. Intake of fatty acids and antioxidants and pancreatic cancer in a large population-based case-control study in the san francisco bay area. Int J Cancer 2010; 127: 1893-904.

[11] Nkondjock A, Ghadirian P, Johnson KC, Krewski D. Dietary intake of lycopene is associated with reduced pancreatic cancer risk. J Nutr 2005; 135: 592-597.

[12] McWilliams RR, Bamlet WR, Cunningham JM, Goode EL, de Andrade M, Boardman LA, Petersen GM. Polymorphisms in DNA repair genes, smoking, and pancreatic adenocarcinoma risk. Cancer Res 2008; 68: 4928-4935.

[13] Duell EJ, Holly EA, Bracci PM, Wiencke JK, Kelsey KT. A population-based study of the arg399gln polymorphism in x-ray repair cross-complementing group 1 (xrcc1) and risk of pancreatic adenocarcinoma. Cancer Res 2002; 62: 4630-4636.

[14] Zhang J, Dhakal IB, Greene G, Lang NP, Kadlubar FF. Polymorphisms in hogg1 and xrcc1 and risk of prostate cancer: Effects modified by plasma antioxidants. Urology 2010; 75: 779-785.

[15] Wheatley-Price P, Asomaning K, Reid A, Zhai R, Su L, Zhou W, Zhu A, Ryan DP, Christiani DC, Liu G. Myeloperoxidase and superoxide dismutase polymorphisms are associated with an increased risk of developing pancreatic adenocarcinoma. Cancer 2008; 112: 1037-

1042.
[16] Anderson KE, Sinha R, Kulldorff M, Gross M, Lang NP, Barber C, Harnack L, DiMagno E, Bliss R, Kadlubar FF. Meat intake and cooking techniques: Associations with pancreatic cancer. Mutat Res 2002; 506-507: 225-231.
[17] Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, Delongchamp R, Kadlubar F. Distribution and concordance of n-acetyltransferase genotype and phenotype in an american population. Cancer Epidemiol Biomarkers Prev 1999; 8: 683-692.
[18] Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The ala16val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics 2003; 13: 145-157.
[19] Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H, Yokota J. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 1998; 16: 3219-3225.
[20] Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 2001; 385: 20-27.
[21] Ahn J, Gammon MD, Santella RM, Gaudet MM, Britton JA, Teitelbaum SL, Terry MB, Nowell S, Davis W, Garza C, Neugut AI, Ambrosone CB. Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. Am J Epidemiol 2005; 162: 943-952.
[22] Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA: Xrcc1 polymorphisms. Effects on aflatoxin b1-DNA adducts and glycophorin a variant frequency. Cancer Res 1999; 59: 2557-2561.
[23] Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, Mark EJ, Wain JC, Christiani DC, Kelsey KT. Polymorphisms in the DNA repair genes xrcc1 and ercc2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 2000; 21: 965-971.
[24] Howe GR, Ghadirian P, Bueno de Mesquita HB, Zatonski WA, Baghurst PA, Miller AB, Simard A, Baillargeon J, de Waard F, Przewozniak K, McMichael AJ, Jain M, Hsieh CC, Maisonneuve P, Boyle P, Walker AM. A collaborative case-control study of nutrient intake and pancreatic cancer within the search programme. Int J Cancer 1992; 51: 365-372.
[25] Kroke A, Klipstein-Grobusch K, Voss S, Moseneder J, Thielecke F, Noack R, Boeing H. Validation of a self-administered food-frequency questionnaire administered in the european prospective investigation into cancer and nutrition (epic) study: Comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. Am J Clin Nutr 1999; 70: 439-447.
[26] Westerterp KR. Physical activity as determinant of daily energy expenditure. Physiol Behav 2008; 93: 1039-1043.