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The Association Between Selected Risk Factors for Pancreatic Cancer and the Expression of *p53* and K-*ras* Codon 12 Mutations

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Abstract
Background Pancreatic cancer is a major contributor to cancer mortality. Studies suggest that a few risk factors, including cigarette smoking, body mass index, having a relative with pancreatic cancer, and diabetes may be related to pancreatic cancer risk.
Aim of the Study We conducted a case–control study in southeastern Michigan to examine the relation between the abovementioned risk factors and mutations of the K-*ras* oncogene and *p53* tumor suppressor gene.
Methods Two hundred forty-five patients with newly diagnosed pancreatic cancer and 420 general population controls were enrolled in the study. For this analysis, all case subjects were restricted to the pancreatic cancer patients that had tissue blocks available for study (n=51). In-person interviews were conducted to ascertain information on demographic and lifestyle factors. Adjusted logistic regression analyses were conducted to compare various subject characteristics of pancreatic cancer patients with K-*ras* and *p53* mutations and their subtypes to the characteristics of the general population controls.
Results Smoking (adjusted odds ratio [aOR]=2.0; 95% confidence interval [95%CI]=0.9–4.3) and diabetes diagnosed 5 or more years before interview (aOR=3.4; 95%CI=1.3–8.8)

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were associated with pancreatic cancer patients positive for K-*ras* codon 12 mutations, but not with pancreatic cancer patients negative for K-*ras* codon 12 mutations. On the other hand, none of the examined risk factors were meaningfully related to patients with *p53* mutations.
Conclusions This study suggests that some recognized risk factors for pancreatic cancer may also be associated with K-*ras* codon 12 mutations. However, further large-scale studies are warranted.

Keywords pancreatic cancer · risk factors · K-*ras* codon 12 mutations · *p53* mutations · epidemiology

Introduction

An estimated 34,000 new cases of pancreatic ductal adenocarcinoma (pancreatic cancer) and 32,000 pancreatic cancer deaths occurred in the US in 2006 [1]. Pancreatic cancer is the second most common cause of cancer death among gastrointestinal cancers, and the fourth among all cancers with a 5-year survival rate under 5% [1]. Few long-term successful treatment options are available for those who are stricken with pancreatic cancer.

A variety of occupational, environmental, and lifestyle factors have been studied, but risk factors generally remain elusive [2]. Recent evidence suggests that a family history of pancreatic cancer [3], cigarette smoking [2], diabetes [2], and obesity [4] may be important in the etiology of pancreatic cancer. However, the focus of many past analytical studies has mainly been descriptive. It has been observed that both the K-*ras* oncogene and the tumor suppressor gene *p53* are often highly mutated in pancreatic cancer [5], but the prevalence of these mutations varies across studies [5, 6]. If these markers of genetic damage are related to environmental or life-style exposures, it can be hypothesized that this variation may be because of differences in exposures to potential carcinogens.

To understand more fully the relationship between these factors, we completed a large case–control study of pancreatic cancer in southeast Michigan. We collected histological material from pancreatic cancer patients during their diagnostic workup and ascertained information on a variety of potential exposures related to pancreatic cancer risk, including smoking habits, body mass index (BMI), family history of pancreatic cancer, obesity, and history of diabetes. In earlier reports of this study, we evaluated the associations between pancreatic cancer and a family history of pancreatic cancer and BMI [3, 4]. In this report, we examined whether potential exposures related to pancreatic cancer risk were associated with mutations of the K-*ras* oncogene and the *p53* tumor suppressor gene.

Subjects and Methods

Study Population

Subjects were recruited between October 1, 1996 and March 31, 1999. Eligible patients were residents of 1 of 18 counties in southeastern Michigan (Hillsdale, Lenawee, Monroe, Jackson, Washtenaw, Wayne, Ingham, Livingston, Oakland, Macomb, Shiawassee, Genesee, Lapeer, St. Clair, Bay, Gratiot, Midland, or Saginaw) at the time of diagnosis, aged 30 to 79 years, English-speaking, with newly diagnosed pancreatic cancer (ICD10 C25.0–C25.3 and C25.7). The study pathologist (J.K. Greenson) reviewed pathology reports, histology slides, and selected medical records to confirm the pancreatic cancer diagnosis. Because survival is short, incident pancreatic cancer patients were ascertained through a rapid case-finding system built in eight large hospital pathology departments, various oncology and surgical physician groups, and hospital-based tumor registries in southeastern Michigan. Sometimes, we were able to interview patients before their diagnosis. No proxy interviews were conducted. The median time for eligible cases for identification by the rapid case-finding system after diagnosis was 19 days (range=52 days before diagnosis to 180 days after diagnosis) and the median time for interview after identification was 48 days (range=0 to 441 days). To validate our rapid case-finding system, we searched the population-based metropolitan Detroit Surveillance, Epidemiology, and End Results Program (SEER), which also ascertains information on incident pancreatic cancer patients, and found only three additional pancreatic cancer patients indicating that our rapid case-finding system captured nearly all of the SEER registry patients.

Random digit dialing based on established methods [7] was used to recruit general population controls throughout the ascertainment period. Controls were frequency matched by age group (30–44, 45–59, 60–69, 70–79 years), sex, ethnicity (African-American, Caucasian, and other), and county of residence (counties were grouped into nine different areas on the basis of population size and proximity to each other).

The Institutional Review Boards of the University of Michigan and the participating centers approved the study protocol. All study subjects provided written informed consent before enrollment into the study.

Data Collection

All study subjects underwent a face-to-face interview. The interviewers were unaware of the study hypotheses and were trained to interview subjects in a standardized manner. Using a structured, pretested questionnaire, interviewers

ascertained information on height, weight at various points in time, smoking, and specific medical conditions including diabetes, family history of cancer, and demographics. All data were entered using the double-entry method and computer algorithms were built to detect outliers, logical errors, and omissions.

Genetic Analyses

Pancreatic cancer patients with tissue blocks available from pretreatment tissue (n=51) were included for the analysis of somatic mutations for the K-*ras* and *p53* genes. Polymerase chain reaction-mediated amplification of DNA from formalin-fixed, paraffin-embedded tissue was used to detect K-*ras* mutations at codon 12, as previously described [8]. DNA was analyzed by dot blot hybridization assays with oligonucleotide probes appropriate for testing specific mutations of wild-type glycine. The antibody (clone) and dilutions used for detection of *p53* mutations was DO-7 (Vector Laboratories), 1:100. Any definite staining was considered positive.

Statistical Analysis

Simple descriptive characteristics were calculated for the general population controls, pancreatic cancer patients, and pancreatic cancer patients with histological material. For pancreatic cancer patients, the mean month survival after pancreatic cancer diagnosis was determined. Date of death information was found by searching the publicly available Social Security Master file and the files at the Detroit SEER registry.

To estimate the main effects for the factors under study, case–control analyses were conducted to compare various subject characteristics (smoking, BMI, family history of pancreatic cancer, and diabetes) of pancreatic cancer patients with K-*ras* and *p53* mutations and their subtypes to the characteristics of the general population controls. Pancreatic cancer patients are characterized by rapid weight loss [9], so BMI at 20 years before interview was used in all analyses. All case subjects were restricted to the pancreatic cancer patients that had histological material (n=51). Because of the small number of pancreatic cancer patients involved in the analyses for specific genetic mutations, continuous variables (e.g., pack years of cigarette smoking, BMI=body weight divided by height raised to the second power [kg/m^2]) were dichotomized based on the mean value among the general population controls. As the subjects were frequency matched, unconditional logistic regression models were built to estimate the association between the studied risk factors and pancreas cancer while controlling for potential confounders.

Results

A total of 358 pancreatic cancer patients were eligible for the study. Fifty-three died before contact could be made and 37 were not contacted because their physician would not give permission. The remaining 268 were invited to join the study. Of these, 245 (91%) agreed to participate in the study. Fifty-one had tissue blocks available from pretreatment tissue. Of the 597 general population controls eligible for the study, 19 could not be reached by phone, 1 died before being contacted, and 27 were not contacted because there was an overselection of controls under 45 years of age early in the study period. The remaining 550 people were invited to participate and 420 (76%) agreed.

The characteristics of the pancreatic cancer patients overall, pancreatic cancer patients with tissue blocks available for analyses of K-*ras* codon 12 and *p53* mutations, and general population controls are shown in Table 1. Reflecting the matching criteria, the pancreatic cancer patients and controls were similar in age (p=0.06), gender (p=0.36), and race (p=0.54). Those with histological material were slightly younger (mean=60.6 years for pancreatic cancer patients with histological material vs 61.5 years for all pancreatic cancer patients) and more likely female (61% for pancreatic cancer patients with histological material vs 49% overall) compared to the pancreatic cancer patients overall. As expected, tumor location was less likely to be specified for pancreatic cancer patients overall (65%) than for those with histological material (28%). The majority of the tumors for pancreatic cancer patients that had histological material were located in the head of the pancreas (59%) compared to 29% for all pancreatic cancer patients. The mean number of months survived was not significantly different (p=0.398) for pancreatic cancer patients with histological material (mean survival=13.5 months; range=1.4 to 41.4 months) compared to those without histological material (mean survival=12.0 months; range=1.1 to 60.7 months).

The distributions of K-*ras* codon 12 mutations and *p53* mutations are displayed in Table 2. Of the cases, 59% (n=30) with histological material had a K-*ras* codon 12 mutation. The majority of these mutations were either a glycine to asparagine mutation (n=12; 40%) or a glycine to valine mutation (n=12; 40%), although 5 (17%) glycine to arginine mutations and 1 (3%) glycine to cysteine were also seen. Of the cases with histological material, 37% (n=19) had a *p53* mutation. Most of these mutations were of exon 4 (n=9; 47%) and exon 7 (n=6; 32%). Less frequent were mutations of exon 5 (n=3; 16%) and exon 6 (n=1; 5%).

Table 3 shows the relation between various subject characteristics and pancreatic cancer patients negative for K-*ras* codon 12 mutations, pancreatic cancer patients positive for K-*ras* codon 12 mutations, and pancreatic

Table 1 Characteristics of the General Population Controls (n=420), Pancreatic Cancer Cases (n=245), and Pancreatic Cancer Cases with Tissue for Analysis (n=51)

Characteristic	General population controls		Pancreatic cancer cases		Pancreatic cancer cases with tissue	
	N	%	*n*	%	*n*	%
Age						
30–52	98	23	47	19	8	16
53–60	104	25	45	18	12	24
61–68	109	26	60	24	18	35
69+	109	26	93	38	13	25
Race						
White	351	84	207	84	40	78
Black/other	69	16	38	16	11	22
Gender						
Male	196	47	124	51	20	39
Female	224	53	121	49	31	61
County						
Wayne	154	37	93	38	21	41
Maccomb and Oakland	157	37	87	36	17	33
Other	109	26	65	27	13	25
Tumor location						
Head			71	29	30	59
Body			5	2	2	4
Tail			11	4	5	9
NOS			158	65	14	28
Mean month survival, range			12.2	1.1–60.7	13.5	1.5–51.4

cancer patients with specific subtypes of K-*ras* codon 12 mutations. Pancreatic cancer patients negative for K-*ras* codon 12 mutations were not more likely to have smoked cigarettes than the general population controls (adjusted odds ratio [aOR]=1.1; 95% confidence interval [95%CI]=0.4–2.7). A borderline statistically significant risk for the association between cigarette smoking and K-*ras* codon 12 mutations overall was observed (aOR=2.0; 95%CI=0.9–4.3) with an elevated, nonstatistically significant risk found for a glycine to asparagine mutation (aOR=2.2; 95%CI=0.7–7.3). Whereas high BMI was significantly associated with pancreatic cancer patients negative for K-*ras* codon 12 mutations (aOR=2.8; 95%CI=1.1–7.2), no risk was found for those with K-*ras* codon 12 mutations overall (aOR=1.1; 95%CI=0.5–2.3). No statistically significant relationship was found for those having a relative with pancreatic cancer and positive or negative for K-*ras* codon 12 mutations (aOR for K-*ras* negative=3.0; 95%CI=0.6–14.4 and aOR for K-*ras* positive=1.5; 95%CI=0.3–7.2). On the other hand, a history of diabetes was elevated for pancreatic cancer patients negative for K-*ras* codon 12 mutations (aOR=5.6; 95%CI=2.1–15.1) and for pancreatic cancer patients positive for K-*ras* codon 12 mutations (aOR=3.1; 95%CI=1.3–7.3). When we only considered diabetes diagnosed 5 or more years before pancreatic cancer diagnosis, the relation with pancreatic cancer patients negative for K-*ras* codon 12 mutations decreased and became statistically nonsignificant (aOR=1.3; 95%CI=0.3–6.2), whereas the relation with pancreatic cancer patients positive for K-*ras* codon 12 mutations remained virtually the same (aOR=3.4; 95%CI=1.3–8.8).

Table 2 Summary of K-*ras* Codon 12 Mutations and *p53* Mutations in Pancreatic Cancer Specimens (n=51)

	Number	Percent
K-*ras*, codon 12 mutation		
Total	30	59
Gly→Asp	12	40
Gly→Val	12	40
Gly→Arg	5	17
Gly→Cys	1	3
p53 mutations		
Total	19	37
Exon 4	9	47
Exon 5	3	16
Exon 6	1	5
Exon 7	6	32

Pancreatic cancer patients positive for *p53* mutations were not more likely to smoke (aOR=1.1; 95%CI=0.4–2.9) nor have a BMI of 24.9 or higher (aOR=1.2; 95%CI=0.5–3.1) than the general population controls (Table 4). No statistically significant relationships were found for pancreatic cancer patients negative or positive for *p53* mutations

Table 3 ORs and 95%CIs for Pancreatic Cancer Cases without K-*ras* Mutations and with K-*ras* Mutations Compared to the General Population Controls for Various Characteristics

Characteristic	Controls (n=420) n (%)	K-*ras* negative		K-*ras* positive					
		Wild-type (n=21) n (%)	OR[a] (95%CI)	Any mutation (n=30) n (%)	OR[a] (95%CI)	Gly→Val (n=12) n (%)	OR[a] (95%CI)	Gly→Asp (n=12) n (%)	OR[a] (95%CI)
Cigarette smoking 22 or more pack years	162 (39)	8 (38)	1.1 (0.4–2.7)	16 (53)	2.0 (0.9–4.3)	5 (42)	1.3 (0.4–4.4)	7 (58)	2.2 (0.7–7.3)
BMI[b] 24.9 or higher	185 (44)	14 (67)	2.8 (1.1–7.2)	14 (47)	1.1 (0.5–2.3)	5 (42)	0.9 (0.3–3.0)	6 (50)	1.1 (0.3–3.5)
Relative with pancreatic cancer	16 (4)	2 (10)	3.0 (0.6–14.4)	2 (7)	1.5 (0.3–7.2)	0 (12)	0.0 (0.0–10.0)	1 (8)	2.8 (0.3–25.1)
Diabetes	48 (11)	8 (38)	5.6 (2.1–15.1)	9 (30)	3.1 (1.3–7.3)	3 (25)	2.2 (0.6–9.1)	5 (42)	5.1 (1.5–17.3)
Diabetes 5 or more years before interview	32 (8)	2 (10)	1.3 (0.3–6.2)	7 (23)	3.4 (1.3–8.8)	2 (17)	2.0 (0.4–10.0)	4 (33)	6.0 (1.7–21.9)

[a] Adjusted for age, gender, race, and county group.
[b] BMI 20 years before interview, measured in kg/m^2.

and having a family history of pancreatic cancer. aORs were slightly higher for the association between diabetes and pancreatic cancer patients negative for *p53* mutations (aOR=4.7; 95%CI=2.1–10.7) than the association between diabetes and pancreatic cancer patients positive for *p53* (aOR=2.8; 95%CI=1.0–7.9). Similar associations were found when only those diagnosed with diabetes 5 or more years before the interview were considered.

Discussion

Smoking and diabetes diagnosed 5 or more years before interview were associated with pancreatic cancer patients positive for K-*ras* codon 12 mutations, but not with pancreatic cancer patients negative for K-*ras* codon 12 mutations. There were no meaningful relations with K-*ras* codon 12 mutations and having a relative with pancreatic cancer. None of the examined risk factors were meaningfully related to patients with *p53* mutations.

It has been observed that the K-*ras* oncogene and the tumor suppressor gene *p53* are often highly mutated in pancreatic cancer [5, 6]. Whereas K-*ras* mutations are present in a few other human tumors, such as the colon and lung, the highest incidence of K-*ras* mutations are found in pancreatic cancer. The *ras* family of genes includes three very similar *ras* genes (Ha-*ras*, Ki-*ras*, and N-*ras*), all of which produce a protein product of molecular mass 21 kDa (p21ras). This product is an essential element in the pathway by which growth signals are transmitted from receptors on the cell surface to the nucleus, resulting in the stimulation of gene expression [10]. The RAS family proteins mediate a number of pleiotropic effects including cell proliferation, survival, and migration. A mutation in RAS results in a gain of function, because the RAS protein remains trapped in the activated state [11]. On the other

Table 4 ORs and 95%CIs for Pancreatic Cancer Cases Negative for *p53* Mutations and Positive for *p53* Mutations Compared to the General Population Controls for Various Characteristics

Characteristic	Controls (n=420) n (%)	*p53* negative		*p53* positive	
		Wild-type (n=32) n (%)	OR[a] (95%CI)	Any mutation (n=19) n (%)	OR[a] (95%CI)
Cigarette smoking 22 or more pack years	162 (39)	17 (53)	1.9 (0.9–4.0)	7 (37)	1.1 (0.4–2.9)
BMI[b] 24.9 or higher	185 (44)	18 (56)	1.8 (0.8–3.7)	10 (53)	1.2 (0.5–3.1)
Relative with pancreatic cancer	16 (4)	3 (9)	2.8 (0.7–10.3)	1 (5)	1.2 (0.1–10.1)
Diabetes	48 (11)	11 (34)	4.7 (2.1–10.7)	6 (32)	2.8 (1.0–7.9)
Diabetes 5 or more years before interview	32 (8)	6 (19)	3.2 (1.2–8.6)	3 (16)	1.6 (0.4–6.2)

[a] Adjusted for age, gender, race, and county group.
[b] BMI 20 years before interview, measured in kg/m^2.

hand, the *p53* tumor suppressor gene is found to be altered in almost all human tumors, reflecting its critical role as a tumor suppressor [12]. *p53* maintains genomic stability and its loss of functionality, through extracellular stress, is associated with aneuploidy and, ultimately, cell cycle arrest or apoptosis. In our study, K-*ras* codon 12 mutations were found in 59% of the examined tumors and *p53* mutations were detected in 37% of the tumors. Other studies have observed a wide variation in the prevalence of these mutations, reporting K-*ras* codon 12 mutations in 20% to 100% of pancreatic cancer tumors [11, 13] and *p53* mutations in 40% to 75% of pancreas tumors [13–15]. The reasons for such variations across studies are unclear but may be because of biases in patient selection, type of sample analyzed, mutation-detection techniques, or susceptibility to specific carcinogens.

Smoking is an established risk factor for pancreatic cancer [2]. As other smoking-related cancers, such as bladder and lung cancer, are associated with *ras* mutations [16], it is reasonable to examine the relation between smoking and K-*ras* mutations in pancreatic cancer patients. One early case–case study found that pancreatic cancer patients with a K-*ras* mutation were more likely to be nonsmokers rather than smokers (OR=0.25; p=0.289), although this association was not statistically significant [17]. Similar to our study, two studies have reported a positive association with smoking [18, 19], but only one of these was borderline statistically significant [18]. A more recent study [20] found that a comparable percentage of K-*ras* positive patients and K-*ras* negative patients were smokers (61% vs 60%). Given that smoking is weak risk factor for pancreatic cancer, these conflicting studies are not surprising and any increased risk for smoking is probably modest at best.

Few studies have evaluated other risk factors for pancreatic cancer and K-*ras* codon 12 mutations. Slebos et al. [20] observed that pancreatic cancer patients negative for K-*ras* codon 12 mutations reported diabetes at the time of interview more often compared to those pancreatic cancer patients positive for K-*ras* codon 12 mutations. We also found a higher prevalence of self-report of diabetes at the time of interview among pancreatic cancer patients negative for K-*ras* codon 12 mutations. However, one concern regarding the association between diabetes and pancreatic cancer is the possibility that diabetes may be a consequence of pancreatic cancer rather than a cause as a number of studies have reported higher risks with increasing years before diagnosis of pancreatic cancer [21]. When we considered only diabetes diagnosed 5 or more years before interview, it is interesting to note that in the case–control analyses, only the relation between pancreatic cancer patients positive for K-*ras* codon 12 mutations remained statistically significantly elevated. There was no statistically significant relation between pancreatic cancer patients negative for K-*ras* codon 12 mutations and diabetes diagnosed 5 or more years before interview. Although the case–case analyses lacked precision, elevated risks for pancreatic cancer patients positive for K-*ras* codon 12 mutations remained. It has been suggested that diabetics may receive extra medical care because of their disease, making an earlier diagnosis of pancreatic cancer more likely. However, this is unlikely to explain our findings that the relation between diabetes and pancreatic cancer is modified by the presence of K-*ras* codon 12 mutations. Although our data suggest a strong association with glycine to asparagine K-*ras* codon 12 mutations, other larger studies are needed so that more precise risk estimates may be calculated.

This is the first study to examine the relation between BMI and pancreatic cancer patients with K-*ras* codon 12 mutations. Pancreatic cancer patients negative for K-*ras* codon 12 mutations had higher BMI 20 years before the interview than pancreatic cancer patients positive for K-*ras* codon 12 mutations, indicating that BMI is probably not an important factor for K-*ras* codon 12 mutations. A few studies have suggested a genetic predisposition for pancreatic cancer within families [2], but the exact etiology is unknown. Similar to our study, one case series of pancreatic cancer found that K-*ras* codon 12 mutations were not statistically different for patients groups with and without a family history of cancer [22].

We found little evidence that any of the examined factors were associated with *p53* mutations. Whereas the *p53* tumor suppressor gene is mutated in a large proportion of pancreatic cancer, it often occurs late in the progression model [11]. It could be hypothesized that the factors we examined may be involved in early stage events.

We were concerned that those patients with histological material may be long-term survivors and that the associations we described may be associated with survival rather than etiology. However, we found that the mean survival time for patients with histological material was similar to the mean survival time for all pancreatic cancer patients. In addition, patients with histological material were similar to all pancreatic cancer patients on age, sex, and race, giving us confidence that the pancreatic cancer patients with histological material were probably not a biased group. This study had other strengths. It is one of the few studies to rely on direct interviews of pancreatic cancer patients. Because of poor survival, most studies of pancreatic cancer have relied on surrogate interviews in which accurate exposure information is difficult to obtain. In addition, the exposure information was ascertained through in-person interviews, which provide more reliable information than self-administered questionnaires or telephone interviews. We also assessed the completeness of our case-finding

system for Wayne, Oakland, and Macomb counties by also searching the regional SEER registry to identify eligible cases at participating hospitals who had been missed during the study period. We found only three additional cases, indicating that selection bias was probably not important. The satisfactory participation rate among cases and controls further lessened the likelihood of a significant selection bias introduced into the study design. Our study was further strengthened by its strict case definition. Among the controls, nonparticipants were similar to participants on age ($p=0.15$), sex ($p=0.047$), and county ($p=0.19$). However, the proportion of blacks who were nonparticipants (21%) was higher than the proportion of blacks who were participants (15%). If nonparticipation among blacks was also related to the factors in our study, then a bias could be present. Pathology slides and reports were reviewed by the study pathologist to ensure that diagnostic inclusion criteria were met and only patients with histologically confirmed adenocarcinoma of the exocrine pancreas were included. This increased the reliability of our findings because the results are for a single category of pancreatic cancer. Interview training and a structured interview minimized recall bias.

In summary, this study suggests that some recognized risk factors for pancreatic cancer, such as smoking and diabetes, may also be associated with K-*ras* codon 12 mutations. Future research must include larger numbers of pancreatic cancer patients with histological material so that more precise estimates of risk may be determined. Further understanding of the relationship between these genetic mutations and environmental and lifestyle factors in the etiology of pancreatic cancer may be important for identifying the population at greatest risk for pancreatic cancer for the primary purpose of preventing this devastating disease.

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