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Research Article

Genetic Mutations Associated with Cigarette Smoking in Pancreatic Cancer

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Abstract

Cigarette smoking doubles the risk of pancreatic cancer, and smoking accounts for 20% to 25% of pancreatic cancers. The recent sequencing of the pancreatic cancer genome provides an unprecedented opportunity to identify mutational patterns associated with smoking. We previously sequenced >750 million bp DNA from 23,219 transcripts in 24 adenocarcinomas of the pancreas (discovery screen). In this previous study, the 39 genes that were mutated more than once in the discovery screen were sequenced in an additional 90 adenocarcinomas of the pancreas (validation screen). Here, we compared the somatic mutations in the cancers obtained from individuals who ever smoked cigarettes (n = 64) to the somatic mutations in the cancers obtained from individuals who never smoked cigarettes (n = 50). When adjusted for age and gender, analyses of the discovery screen revealed significantly more nonsynonymous mutations in the carcinomas obtained from ever smokers (mean, 53.1 mutations per tumor; SD, 27.9) than in the carcinomas obtained from never smokers (mean, 38.5; SD, 11.1; P = 0.04). The difference between smokers and nonsmokers was not driven by mutations in known driver genes in pancreatic cancer (*KRAS, TP53, CDKN2A/p16*, and *SMAD4*), but instead was predominantly observed in genes mutated at lower frequency. No differences were observed in mutations in carcinomas from the head versus tail of the gland. Pancreatic carcinomas from cigarette smokers harbor more mutations than do carcinomas from never smokers. The types and patterns of these mutations provide insight into the mechanisms by which cigarette smoking causes pancreatic cancer. [Cancer Res 2009;69(8):3681–8]

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States (1). It has been estimated that, in the year 2008, ~37,680 Americans were diagnosed with pancreatic cancer and that 34,290 died from this disease (1). Several factors have been identified that increase the risk of pancreatic cancer, including advancing age, diets high in meats and fats, diets low in vegetables and folate, diabetes mellitus, obesity, chronic pancreatitis, partial gastrectomy, radiation, a family history of pancreatic cancer, and cigarette smoking (2–6). Of all of these known risk factors, cigarette smoking remains the leading preventable cause of pancreatic cancer (6, 7). Approximately 20% of cancers of the pancreas are caused by cigarette smoking, and a recent meta-analysis of 82 studies published between 1950 and 2007 on smoking and pancreatic cancer found that current smokers have a 1.74-fold (95% confidence interval, 1.61-1.87) increased risk of developing pancreatic cancer (6, 7). Smoking has also been associated with early-onset pancreatic cancer and smoking cessation has been shown to reduce pancreatic cancer risk (8–12).

Genetic analyses of other cancers caused by cigarette smoking have revealed increased numbers of mutations in cancer-associated genes as well as specific types of mutations in cancers resected from smokers (13–19). This link between cigarette smoking and specific genetic changes in a cancer is strongest for lung carcinomas (14, 19). Smoking is associated with an ~11-fold increased relative risk of lung cancer, and activating point mutations in the *KRAS* gene are more common in adenocarcinomas of the lung resected from smokers than they are in adenocarcinomas from nonsmokers (16, 19–21) Most of these mutations are G:C to T:A transversions, a mutation type associated with carcinogens such as polycyclic aromatic hydrocarbons in tobacco smoke (16, 22). Remarkably, these same mutations can be seen in lung cancers obtained from ex-smokers, suggesting that these *KRAS* gene mutations occurred years before the cancers were resected (15). Similarly, several studies have shown that *TP53* gene mutations are more common in lung cancers from smokers than they are in lung cancers from never smokers; again, the G:C to T:A transversions predominate with a specificity toward CpG sites (14, 17, 23–25). Thus, there is a strong "fingerprint" of tobacco carcinogens in the DNA of lung cancer (24, 26).

The recent analysis of the pancreatic cancer genome, encompassing the sequencing of 20,661 protein coding genes in a series of 24 pancreatic cancers, provides a unique opportunity to correlate the somatic genetic changes in pancreatic cancer with smoking status (27). In this previous study, >750 million bp DNA were sequenced in two phases (27). First, in the discovery screen, the sequences of the protein-coding exons from 20,661 genes were sequenced in 24 advanced adenocarcinomas of the pancreas. Of the 1,562 somatic mutations discovered using this approach, 62.4% were missense, 25.5% were synonymous, 5.0% were small insertions or deletions, 3.8% were nonsense, and 3.3% were in splice sites or

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Distribution of clinical and smoking characteristics among smokers and nonsmokers, separately for the discovery screen and validation screen

	Discovery screen		Validation screen	
	Nonsmokers (*n* = 13)	Smokers (*n* = 11)	Nonsmokers (*n* = 37)	Smokers (*n* = 53)
Age, mean (SD)	64.7 (12.1)	65.5 (9.7)	64.9 (11.3)	65.2 (9.6)
Gender, *n* (%)				
Male	6 (46.2)	4 (36.4)	22 (59.5)	21 (39.6)
Female	7 (53.8)	7 (63.6)	15 (40.5)	32 (60.4)
Race, *n* (%)				
White	12 (92.3)	9 (81.8)	32 (86.5)	47 (88.7)
Other race	1 (7.7)	2 (18.2)	5 (13.5)	6 (11.3)
Surgery, *n* (%)				
Autopsy	4 (30.8)	3 (27.3)	1 (2.7)	0 (0.0)
Whipple	9 (69.2)	6 (54.5)	33 (89.2)	43 (81.1)
Distal pancreatectomy	0 (0.0)	2 (18.2)	3 (8.1)	10 (18.9)
Location of tumor, *n* (%)				
Head	12 (92.3)	7 (63.6)	34 (94.4)	43 (84.3)
Tail	1 (7.7)	4 (36.4)	2 (5.6)	8 (15.7)
Grade*, *n* (%)				
Poor	8 (61.5)	7 (70)	11 (29.7)	22 (42.3)
Moderate/well	5 (38.5)	3 (30)	26 (70.3)	30 (57.7)
Tumor size (cm), *n* (%)				
<3	3 (23.1)	2 (18.2)	13 (35.1)	19 (35.8)
3-5	6 (46.1)	6 (54.5)	20 (54.1)	25 (47.2)
>5	2 (15.4)	2 (18.2)	3 (8.1)	8 (15.1)
Unknown (autopsy cases)	2 (15.4)	1 (9.1)	1 (2.7)	1 (1.9)
Margin, *n* (%)				
Negative	6 (46.2)	5 (45.5)	23 (62.2)	37 (69.8)
Positive	4 (30.8)	3 (27.3)	13 (35.1)	16 (30.2)
Unknown (autopsy cases)	3 (23.1)	3 (27.3)	1 (2.7)	0 (0.0)
Diabetic, *n* (%)				
No	9 (69.2)	7 (63.6)	7 (87.5)	4 (50)
Yes	4 (30.8)	4 (36.4)	1 (12.5)	4 (50)
No. positive lymph nodes, mean (SD)	3 (1.7)	6 (3.6)	3 (4.6)	3 (3)
No. lymph nodes, mean (SD)	22 (12.9)	18 (5.3)	15 (8.2)	15 (8.2)
Smoking status, *n* (%)				
Current		5 (45.5)		21 (39.6)
Former		6 (54.5)		32 (60.4)
Years quit, *n* (%)				
≤10		2 (33.3)		10 (31.2)
>10		4 (66.7)		22 (68.8)
Pack-years, mean (SD)		43 (23.3)		38 (27.9)

NOTE: * Original grade not available on two autopsied patients.

within untranslated regions (27). In addition, 198 homozygous deletions and 144 high copy number amplifications were identified in the cancers included in the discovery screen using high-density oligonucleotide arrays (27). In the second phase of this study, the validation screen, 39 genes that were mutated more than once in the discovery screen were sequenced in an additional panel of 90 well-characterized adenocarcinomas of the pancreas (27).

Here, we correlate these data with patient smoking history as well as with a variety of other clinical factors such as patient age, sex, stage, and location of the cancer within the pancreas.

Materials and Methods

This study was approved by the Johns Hopkins Institutional Review Board.

Patients. All available records were retrospectively reviewed on the 114 patients (24 in the discovery screen and 90 from the validation screen). This included a review of the patient's hospital charts, the electronic patient medical records, and the Johns Hopkins Pancreatic Cancer Research Database (28). Ninety-eight of the 114 (86%) patients included in this study were deceased at the time of the study.

Nonsmokers were defined as patients who reported that they had never smoked in their lives. Smokers were defined as patients who reported that they had smoked in their lives. Ex-smokers were defined as smokers who had quit >1 year before surgery for their pancreatic cancer. Information was not available on secondhand smoking exposure.

Statistical analyses. The total numbers of mutations, deletions, and amplifications were compared between clinical parameters using a Poisson regression model that adjusted for smoking status and included an overdispersion term to account for patient-to-patient variation. A similar approach was used to compare the number of mutations between

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Table 2. Mean (SD) genetic alterations for the discovery screen by clinical parameters

	n	Mutations		Deletions		Amplifications		Mutations, deletions, and amplifications	
		Mean (SD)	*P*	Mean (SD)	*P*	Mean (SD)	*P*	Mean (SD)	*P*
Nonsmoker	13	56.2 (13.9)	0.08	7.8 (4.7)	0.58	5.5 (5.4)	0.73	69.5 (16.4)	0.08
Smoker	11	75.5 (41.7)		8.8 (4.3)		6.5 (9.1)		90.9 (44.4)	
Moderate/well grade	8	58.1 (15.6)	0.59	6.5 (2.7)	0.23	2.6 (4.6)	0.06	67.2 (19)	0.28
Poor grade	15	68.9 (37.4)		8.7 (4.8)		7.4 (7.9)		84.9 (39)	
Ages <70 y	16	60.8 (19.1)	0.16	8.9 (4.6)	0.33	6.6 (7.3)	0.59	76.2 (24.3)	0.37
Ages ≥70 y	8	73.8 (47.1)		7 (4.2)		4.9 (7.3)		85.6 (48.2)	
Black	3	61.3 (15.9)	0.69	7 (2)	0.54	0.3 (0.6)	0.01	68.7 (17.2)	0.39
White	21	65.6 (32.7)		8.4 (4.7)		6.8 (7.3)		80.9 (35.1)	
Female	10	54.3 (13.5)	0.35	6.4 (3.6)	0.08	3 (4.4)	0.05	63.7 (14.5)	0.10
Male	14	69.3 (37)		9.6 (4.7)		8.1 (8.1)		87 (38.7)	
Nondiabetic	16	68.9 (36)	0.34	8.9 (5.1)	0.32	7.8 (7.9)	0.04	85.6 (37.4)	0.13
Diabetic	8	57.4 (15.9)		7 (2.7)		2.4 (3.9)		66.8 (19.8)	
Tail	5	72.6 (24)	0.88	7.8 (3.1)	0.68	9 (10.9)	0.39	89.4 (34.1)	0.76
Head	19	63.1 (32.7)		8.4 (4.8)		5.2 (6)		76.7 (33.6)	
Age (y)		0.96 (0.55)	0.10	−0.12 (0.08)	0.16	−0.06 (0.14)	0.68	0.77 (0.61)	0.22

NOTE: *P* values for smoking-adjusted differences in the rate of mutations between patient groups. Differences between males and females exclude mutations on chromosome X. Values for age in years are regression coefficients (SE) for the average increase in the number of alterations with a yearly increase in age, adjusted for smoking status.

smokers and nonsmokers, adjusted for age and gender. Analyses were adjusted for gender because genes specific to the Y chromosome were not sequenced; therefore, more alleles were sequenced in the cancers obtained from women than in the cancers obtained from men (27). The difference in frequency of specific mutation types (base-pair changes and insertions/deletions) and the context in which the mutations occurred were compared between smokers and nonsmokers using mixed-effect logistic regression models that adjusted for age and gender. The difference in frequency of mutations and deletions of the known driver genes (*KRAS, TP53, SMAD4*, and *CDKN2A/p16*) between smokers and nonsmokers was evaluated with Fisher's exact test. The number of statistical comparisons was not defined before the analyses; therefore, the *P* values presented are not adjusted for the number of comparisons and are included for descriptive purposes only.

Results

Patient demographics. A summary of the patient demographics for the discovery screen and validation screen is provided in Table 1, and the smoking histories of each of the patients included in the original sequencing study are provided in Supplementary Tables S1 and S2 (27). Briefly, the mean age for both smokers and nonsmokers was 65 years. The discovery screen included 10 males and 14 females, and the validation screen included 43 males and 47 females (27). Sixty-four of the 114 patients included were smokers, and 50 were nonsmokers. Of the 64 smokers, 38 had reported that they had quit smoking, and 26 of the 38 ex-smokers had quit >10 years before their diagnosis. The smokers in the discovery screen smoked a mean of 43 pack-years, and the smokers in the validation screen smoked a mean of 38 pack-years. There were no *P* values ≤ 0.05 for any of the clinical parameters examined between smokers and nonsmokers (Table 1).

Mutations in the discovery screen. We first examined the mutations identified by sequencing in the discovery screen and calculated the total number of mutations per sample for each of the clinical parameters evaluated (Table 2).

Table 3. Types of mutations by smoking status for the discovery screen

	Nonsmokers (*n* = 13)	Smokers (*n* = 11)	*P*
	Mean (SD)	Mean (SD)	
Mutations	56.2 (13.9)	75.5 (41.7)	0.06
Mutations not in *KRAS, TP53, SMAD4*, or *CDKN2A/p16*	53.9 (14.1)	73.5 (41.7)	0.05
Mutations not in any driver gene	53.2 (13.8)	73.3 (41.8)	0.05
Synonymous mutations	14.8 (5.4)	18.7 (11.3)	0.26
Nonsynonymous mutations	38.5 (11.1)	53.1 (27.9)	0.04
Transition mutations	35.3 (9)	43.7 (16.9)	0.04
Transversion mutations	20.9 (10.3)	31.8 (25.9)	0.16

NOTE: *P* values for differences between smokers and nonsmokers, adjusted for age and gender.

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Table 4. Frequency of specific sequence mutations and context for the discovery screen by smoking status

	No. mutations in nonsmokers	No. nonsmokers with a specific mutation	No. mutations in smokers	No. smokers with a specific mutation	Odds ratio (95% confidence interval)	*P*
Sequence mutations						
A-to-C	26	10	22	10	0.72 (0.33-1.54)	0.40
A-to-G	32	12	50	11	1.38 (0.86-2.21)	0.18
A-to-T	20	9	29	11	1.25 (0.69-2.27)	0.46
C-to-A	36	13	70	10	1.6 (1.04-2.46)	0.03
C-to-G	34	11	34	10	0.76 (0.45-1.26)	0.28
C-to-T	214	13	197	11	0.78 (0.57-1.07)	0.13
G-to-A	187	13	200	11	1.02 (0.79-1.31)	0.89
G-to-C	39	9	35	10	0.8 (0.36-1.76)	0.57
G-to-T	57	13	83	11	1.27 (0.88-1.85)	0.20
T-to-A	8	6	20	8	2.32 (0.99-5.45)	0.05
T-to-C	26	11	34	8	1.15 (0.64-2.06)	0.63
T-to-G	15	8	16	7	0.85 (0.41-1.79)	0.68
Insertion or deletion	37	13	41	11	1.01 (0.63-1.63)	0.96
Context						
A	78	13	101	11	1.07 (0.78-1.48)	0.66
C	90	13	104	11	0.91 (0.67-1.25)	0.57
C*pG	152	13	140	11	0.82 (0.6-1.12)	0.21
CpG*	128	13	143	11	1.16 (0.8-1.69)	0.43
G	92	13	119	11	1.11 (0.82-1.5)	0.49
G*pA	63	12	56	11	0.79 (0.41-1.53)	0.49
T	49	12	70	11	1.25 (0.81-1.92)	0.31
TpC*	42	12	57	10	1.16 (0.76-1.77)	0.49
All sequence mutations	731	13	831	11	1.26 (0.74-2.15)	0.40

NOTE: Odds ratios for having each specific type of mutation for smokers versus nonsmokers, adjusted for age and gender.
*Altered base.

There was a trend for more mutations in smokers than in nonsmokers. The number of mutations ranged from 40 to 187 per tumor for smokers and from 34 to 72 per tumor for nonsmokers. As has been reported previously with lung cancer, the variance of the number of point mutations for smokers was higher than for nonsmokers (variance ratio estimate, 9.0; 95% confidence interval, 2.7-32.7; $P < 0.001$; ref. 19). The 11 smokers had a mean of 75.5 intragenic mutations per carcinoma (SD, 41.7) and the nonsmokers had a mean of 56.2 mutations (SD, 13.9; $P = 0.06$ when adjusted for age and gender; Table 3). Thus, ~25% of the intragenic mutations in the pancreatic cancers obtained from smokers appear to be smoking related.

When homozygous deletions and amplifications were also included together with the mutations identified by sequencing, the carcinomas from the 11 smokers had a mean of 90.9 (SD, 44.4) genetic alterations per tumor and the carcinomas from the nonsmokers had a mean of 69.5 (SD, 16.4; $P = 0.08$). There were no significant differences observed in the number of amplifications or in the number of deletions in smokers and nonsmokers.

Although the numbers were small, no significant differences were observed between the ex-smokers and the current smokers with respect to mutation number or type.

No significant differences were observed in the number of mutations for the other clinical variables examined for patients included in the discovery screen (Table 2).

Categories of mutations in the discovery screen. Next, we examined the broad categories of alterations observed in the discovery screen (Table 3). As noted above, the number of homozygous deletions and amplifications did not differ between nonsmokers and smokers. Our further analyses therefore focused on the mutations identified by sequencing.

When the *KRAS* and *TP53* genes, the two previously reported targets of tobacco-related carcinogens, were excluded from the analyses, a larger number of mutations were still identified in the cancers obtained from smokers (mean, 73.9; SD, 41.9) than in the cancers obtained from nonsmokers (mean, 54.4; SD, 14.1; $P = 0.06$ when adjusted for age and gender). A similar pattern was observed when all four "gene mountains," the *KRAS, TP53, SMAD4*, and *CDKN2A* genes, were excluded from the analyses, with a mean of 73.5 (SD, 41.7) mutations in the smokers and 53.9 (SD, 14.1) mutations in the nonsmokers ($P = 0.05$ when adjusted for age and gender; Table 3; ref. 27). Finally, we compiled a list of 65 driver genes (Supplementary Table S3). These 65 driver genes included genes identified in our previous genome-wide sequencing analyses, genes reported as driver genes in the literature, and genes with >10 alterations in the Cosmic database[8] (27, 29–31). When these driver genes were excluded from the analyses, the difference persisted, with a mean of 73.3 (SD, 41.8) mutations in smokers and 53.2 (SD, 13.8) mutations in the nonsmokers ($P = 0.05$ when adjusted for age and gender). These results suggest that the differences observed in

[8] http://www.sanger.ac.uk/genetics/CGP/cosmic/. Accessed December 20, 2008.

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the number of mutations detected by sequencing between smokers and nonsmokers are not driven by these major driver genes.

Significantly more nonsynonymous mutations were observed in the cancers from smokers (mean, 53.1; SD, 27.9) than in the cancers from nonsmokers (mean, 38.5; SD, 11.1; $P = 0.04$ when adjusted for age and gender). More synonymous mutations were also observed in the cancers from smokers (mean, 18.7; SD, 11.3; Table 3) compared with nonsmokers (mean, 14.8; SD, 5.4; $P = 0.26$), but this difference was not statistically significant.

Transitions were more common in the cancers from smokers (mean, 43.7; SD, 16.9) than in the cancers from nonsmokers (mean, 35.3; SD, 9; $P = 0.04$ when adjusted for age and gender). There were also more transversions in the cancers from smokers (mean, 31.8; SD, 25.9) than nonsmokers (mean, 20.9; SD, 10.3), but this latter difference was not statistically significant (Table 3).

Types of mutations in the discovery screen. We next examined the specific types of mutations identified in the discovery screen (Table 4). Here, the mutations were placed into 1 of 13 groups: the 12 possible base-pair changes (based on the reading strand) and insertions or deletions. Of the 13 possible mutation types, C:G to A:T (odds ratio, 1.6; 95% confidence interval, 1.04-2.46; $P = 0.03$ adjusted age and gender) and T:A to A:T (odds ratio, 2.32; 95% confidence interval, 0.99-5.45; $P = 0.05$ adjusted age and gender) mutations were both more common in the cancers from smokers than in the cancers from nonsmokers.

There were no significant differences observed in the context in which the mutations occurred (Table 4). Similar analyses for the validation screen are presented in Supplementary Table S4.

***TP53* gene mutations and smoking.** Point mutations in the *TP53* gene were identified in 82% of the cancers (Table 5). Eighteen of the 24 cancers in the discovery screen harbored a *TP53* gene mutation, as did 76 of the 90 cancers in the validation screen. The prevalence of *TP53* gene mutations in cancers from smokers did not differ significantly from the prevalence of *TP53* gene mutations in the cancers from nonsmokers. Fifty of the 64 (78%) cancers from smokers harbored a *TP53* gene mutation compared with 44 of the 50 (88%) cancers from nonsmokers ($P = 0.22$). In addition, the types and context of the *TP53* gene mutations in smokers and in nonsmokers also were similar (Table 6).

***KRAS* gene mutations and smoking.** *KRAS* gene mutations were observed in 113 of the 114 (99%) pancreatic cancers sequenced. With almost universal *KRAS* gene mutations, the number of *KRAS* gene mutations in the cancers from smokers did not differ significantly from the number in cancers from nonsmokers. As was true for the *TP53* gene, the types and context of the *KRAS* gene mutations in smokers and in nonsmokers were similar (Table 6).

Other gene mutations and smoking. There were a total of 1,562 sequence mutations involving 1,315 unique genes in the tumor samples. In addition to the genes presented in Table 5, *TTN* was mutated in 8 carcinomas: 4 smokers and 4 nonsmokers. Of the remaining 1,310 genes, 1,166 were mutated in only one tumor sample. The remaining 144 genes were mutated in 2, 3, or 4 tumor samples and were not analyzed for differences by smoking group.

Discussion

Several studies have linked cigarette smoking with specific genetic alterations in cancer-associated genes in lung cancer (14, 16, 17, 19, 20, 23, 24). For example, Westra and colleagues reported significantly more *KRAS* gene mutations in lung adenocarcinomas obtained from current smokers (30%) and former smokers (32%) than in lung adenocarcinomas obtained from nonsmokers (7%; $P = 0.015$; ref. 15). Similarly, Le Calvez and colleagues found *TP53* gene mutations in the lung cancers of 47.5% of nonsmokers, 55.6% of former smokers, and 77.4% of current smokers (14). More recently, Ding and colleagues sequenced 623 genes in 188 adenocarcinomas of the lung and found significantly more mutations in the cancers from smokers than in the cancers from nonsmokers ($P = 0.02$; ref. 19). All of the cancers obtained from nonsmokers harbored ≤5 mutations, whereas the cancers obtained from smokers had as many as 49 mutations (19). Comparable results have been reported for other cancer types associated with cigarette

Table 5. Frequency distribution of the number of patients with no sequencing mutations or at least one sequencing mutation in *KRAS, SMAD4, CDKN2A/p16*, or *TP53*

	Discovery screen			Validation screen			Combined samples		
	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*
	n (%)	*n* (%)		*n* (%)	*n* (%)		*n* (%)	*n* (%)	
KRAS									
Wild-type	0 (0)	0 (0)	—	0 (0)	1 (2)	—	0 (0)	1 (2)	1.00
Mutated	13 (100)	11 (100)		37 (100)	52 (98)		50 (100)	63 (98)	
SMAD4									
Wild-type	8 (62)	8 (73)	0.68	28 (76)	39 (74)	1.00	36 (72)	47 (73)	1.00
Mutated	5 (38)	3 (27)		9 (24)	14 (26)		14 (28)	17 (27)	
CDKN2A/p16									
Wild-type	12 (92)	10 (91)	1.00	30 (81)	38 (72)	0.33	42 (84)	48 (75)	0.26
Mutated	1 (8)	1 (9)		7 (19)	15 (28)		8 (16)	16 (25)	
TP53									
Wild-type	2 (15)	4 (36)	0.36	4 (11)	10 (19)	0.38	6 (12)	14 (22)	0.22
Mutated	11 (85)	7 (64)		33 (89)	43 (81)		44 (88)	50 (78)	

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Table 6. Frequency distribution of the number of sequencing mutations in *KRAS, SMAD4, TP53*, and *CDKN2A/p16* and all other genes by mutation type and context, comparing nonsmokers and smokers, adjusted for age and gender

	KRAS			*SMAD4*			*TP53*			*CDKN2A/p16*		
	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*
Sequence mutations												
A-to-C	1	2	0.99	1	0	>0.99	0	1	>0.99	0	1	>0.99
A-to-G	0	1	>0.99	0	2	>0.99	4	3	0.89	0	0	—
A-to-T	0	0	—	0	0	—	2	1	0.99	0	1	>0.99
C-to-A	0	0	—	0	0	—	1	2	0.99	0	1	>0.99
C-to-G	0	0	—	1	0	>0.99	1	2	0.99	1	2	0.85
C-to-T	0	0	—	6	3	0.17	9	10	>0.99	4	6	0.32
G-to-A	26	34	0.9	0	3	>0.99	9	12	0.58	0	2	>0.99
G-to-C	6	10	0.99	0	1	>0.99	1	0	>0.99	0	1	>0.99
G-to-T	17	17	0.37	2	1	0.91	5	4	0.85	1	1	0.99
Insertion or deletion	0	0	—	4	7	0.39	11	8	0.36	2	4	0.77
T-to-A	0	0	—	0	0	>0.99	1	2	0.99	0	0	—
T-to-C	0	0	—	1	0	>0.99	2	3	0.99	0	0	—
T-to-G	0	0	—	0	0	—	1	2	0.99	0	1	>0.99
Context												
A	1	3	0.99	1	2	0.99	6	5	0.86	0	2	>0.99
C	0	0	—	1	0	>0.99	2	2	>0.99	2	5	>0.99
C*pG	0	0	—	4	1	0.14	8	11	0.99	3	4	0.34
CpG*	0	0	—	0	0	—	5	8	0.97	0	0	—
G	49	61	0.99	1	4	0.98	4	7	0.99	0	3	>0.99
G*pA	0	0	—	1	1	0.99	6	1	0.67	1	1	0.99
T	0	0	—	1	0	>0.99	4	7	0.99	0	1	>0.99
TpC*	0	0	—	2	2	>0.99	1	1	>0.99	0	0	
Total mutations	50	64	>0.99	15	17	0.12	47	50	>0.99	8	20	0.02

NOTE: Some patients had more than one mutation on an individual gene, and total represents the number of mutations across all patients within smoking category.
*Altered base.

smoking such as head and neck cancer and bladder cancer (18, 32, 33).

Smoking also has been associated with pancreatic cancer through epidemiologic studies and smoking has been linked to specific genetic mutations in pancreatic cancers (6, 13, 34). Pancreatic cancers from cigarette smokers have been reported to have more *KRAS* and more *TP53* gene mutations than pancreatic cancers from nonsmokers (13, 34). For example, Jiao and colleagues found that smoking was associated with G:C to A:T mutations in the *KRAS* gene in pancreatic cancer (35). It should be noted, however, that not all studies have found a link between smoking and specific genetic changes in pancreatic cancer (36, 37). For example, Crous-Bou and colleagues reported on 107 pancreatic cancers and found no relationship between *KRAS* gene mutations and smoking (37).

The sequencing of the pancreatic cancer genome provided a unique opportunity to correlate cigarette smoking and other clinical parameters with specific genetic mutations (27). We found that although the number of smoking-related mutations did not appear to be as high as it was for lung cancer, pancreatic cancers obtained from smokers harbored more mutations than cancers obtained from nonsmokers (19). As has been reported previously with lung cancer, the variance of the number of point mutations in the pancreatic cancers obtained from smokers was higher than the variance of the number of point mutations in the pancreatic cancers obtained from nonsmokers (19). We estimate that one in four of the mutations in the pancreatic cancers obtained from smokers may be smoking related.

In contrast to several previous reports, however, we did not observe an association between smoking and *KRAS* gene mutations (13). This likely reflects the selection criteria used to include cases in the sequencing project (27, 34, 38). Cancers with variant morphologies, such as medullary carcinoma, were excluded from the project in an effort to increase the uniformity of the cancers sequenced. Medullary carcinomas, as we have reported before, are often microsatellite-unstable, they lack *KRAS* gene mutations, and some are caused by germline mutations in a DNA mismatch repair gene (39). Thus, the selection criteria for the pancreatic cancer genome project tended to exclude the *KRAS* wild-type cases driven by a pathway unrelated to smoking. Simply put, with 99% of the cancers harboring a *KRAS* gene mutation, it would have been virtually impossible to detect an effect of smoking on the *KRAS* gene.

The differences between the number of mutations in smokers and nonsmokers were not found in the other genes known to be "driver" genes in pancreatic cancer, such as *TP53, CDKN2A*, and *SMAD4* (27, 40–44). This observation can be explained by the fact that these mutations are likely required for pancreatic cancer to occur and are highly selected for during the tumorigenic process. Passenger mutations, but not driver mutations, provide a molecular clock that can be used to infer mutation rates (45). Smokers may develop pancreatic cancer more frequently and at a

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younger age of onset, but the driver genes that are mutated appear to be the same in the two groups (8). Although this distinction between the passenger mutations and driver mutations has been overlooked in prior literature, it may explain the often unconvincing associations between smoking and driver genetic mutations(37).

We also examined the types of mutations and the context in which these mutations occurred. We did not identify a signature tobacco-related mutation in the smokers. A possible explanation for this heterogeneity is that it reflects the multiple DNA-damaging compounds (>100) found in cigarette smoke and that perhaps the mutagenicity of cigarette smoke is not limited to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo[*a*]pyrene, two well-studied tobacco-derived carcinogens (46). The data would also be consistent with the hypothesis that the carcinogens in tobacco damage the DNA in the pancreas in a nonspecific way, but this latter hypothesis is not consistent with extensive data from the study of lung cancer and with the finding of specific tobacco-derived carcinogens in the pancreatic juice of smokers. Other possible explanations include that non-tobacco-related mutagenic risk factors for pancreatic cancer may share mutagenic properties with the tobacco mutagens active in pancreatic tissues and that the end-organ metabolic products of diverse tobacco carcinogens differ in the lung and the pancreas (19, 47).

We examined the number of mutations in the cancers relative to several other clinical parameters, such as location within the pancreas (head versus tail), sex of the patient, age of the patient, tumor grade, margin status, and stage. No statistically significant differences were found.

Limitations of this study should be acknowledged. Because 86% of the patients were deceased at the time of this study, all of the clinical parameters were collected retrospectively by review of the patient's hospital charts, the electronic patient medical records, and the Johns Hopkins Pancreatic Cancer Research Database (28). Although several studies have suggested that self-reporting may underestimate cigarette smoking, the magnitude of this under-reporting is likely small enough to have only a modest effect on our results (48–50).

In conclusion, we found that cigarette smoking is associated with greater numbers of mutations in pancreatic cancer but that these mutations do not produce a characteristic profile.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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