

# Mutational Activation of *K-ras* in Nonneoplastic Exocrine Pancreatic Lesions in Relation to Cigarette Smoking Status

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The authors dedicate this article to the late Adrian Mayer, M.D.

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**BACKGROUND.** Cigarette smoking is among the few unequivocal risk factors for the development of pancreatic ductal adenocarcinoma (PDAC). Activating mutations in codon 12 of the *K-ras* protooncogene is a frequent and early molecular event in the pathogenesis of PDAC and a variety of nonmalignant ductal pancreatic lesions. The molecular epidemiologic relation between heavy cigarette smoking and mutational activation of *K-ras* in PDAC has been examined to a limited extent. The authors have examined the mutational status of *K-ras* in nonneoplastic pancreata in relation to cigarette smoking status.

**METHODS.** Archival formalin fixed paraffin embedded specimens of nonneoplastic pancreata (n = 39) were obtained from the American Cancer Society and evaluated histopathologically. Specimens from age- and gender-matched individuals were stratified into three groups: 1) those who never smoked cigarettes (n = 16), 2) those who smoked 1–2 packs/day for more than 20 years (n = 10 cases), and 3) those who smoked more than 2 packs/day for 20 or more years (n = 13). Cases were preselected from 77 specimens based on the quality, suitability, and cellularity of the archival tissues for analyses. Furthermore, none of the patients died of primary PDAC or had evidence of pancreatic metastases from an extrapancreatic primary tumor. Tissue sections were microdissected and deparaffinized, and genomic DNA was purified by standard proteinase K-phenol-chloroform extraction techniques. Genomic DNA was analyzed for mutations in codon 12 of the *K-ras* protooncogene by two mutant-allele-enriched polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays and by multiplex PCR-based ligase chain reaction (LCR) analyses.

**RESULTS.** Analyses of multiple microdissected pancreata specimens from 39 cases revealed wild-type *K-ras* codon 12 sequences in both nonsmoking individuals and those who smoked 1–2 packs/day for 20 or more years. *K-ras* codon 12 mutations were confirmed by PCR-RFLP and PCR-LCR assays in 5 of 13 pancreata cases (39%) obtained from individuals who smoked more than 2 packs of cigarettes/day for 20 years or more ( $P < 0.005$ ). The *K-ras* mutation spectra revealed two G→T transversions, one G→C transversion and two G→A transitions. There was no clear relation between the incidence or spectra of mutations and pancreatic histopathology, as overtly normal pancreata as well as pancreata with squamous metaplasia, periductal fibrosis, and ductal atypia revealed reproducible *K-ras* alterations. Similarly, among those 34 cases in which a wild-type *K-ras* sequence was revealed by both approaches, a similar histopathologic profile was evident.

**CONCLUSIONS.** Mutational activation of codon 12 of the *K-ras* protooncogene was confirmed reproducibly by mutant allele-enriched PCR-RFLP and multiplex PCR-LCR analyses in 39% (5 of 13) of archival nonneoplastic pancreata from age- and gender-matched individuals who smoked more than 2 packs of cigarettes/day for 20 or more years. The presence of a mutated or wild-type or *K-ras* was independent of the histopathologic profile of the 39 cases examined. The data provide further suggestive molecular epidemiologic evidence of an association between a major and unequivocal risk factor for PDAC (heavy cigarette smoking) and mutations in a molecular target (*K-ras*), the activation of which is an important and early event

both in the pathogenesis of PDAC and in the development of a variety of nonneoplastic ductal pancreatic lesions. *Cancer* 1999;85:326–32.

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**KEYWORDS:** pancreatic adenocarcinoma, *K-ras*, cigarette smoking, restriction fragment length polymorphism, ligase chain reaction.

**P**ancreatic ductal carcinoma (PDAC) is a virtually fatal malignancy. In the United States, PDAC ranks as the fourth leading cause of cancer death in men and fifth in women.<sup>1</sup> Although the etiology of PDAC is unknown, epidemiologic evidence suggests a weak, albeit unequivocal, association between cigarette smoking and PDAC. The incidence of PDAC is approximately two and one-half times greater in smokers than in nonsmokers.<sup>2–4</sup> More recent case control studies have concluded that, despite the weakness of the dose response data, the correlation between cigarette smoking duration and risk of PDAC is likely to be casual.<sup>2</sup> Histologic substantiation for the epidemiologic link between cigarette smoking and PDAC has been demonstrated in several studies.<sup>5–7</sup> The frequency of focal acinar cell dysplasias and ductal epithelial abnormalities, including squamous cell metaplastic and hyperplastic changes in nonneoplastic pancreata, was elevated among heavy cigarette smokers compared with nonsmokers.<sup>5,6</sup> A significant dose dependent relation between the extent and duration of cigarette smoking and both the percentages of atypical nuclei in the ductal and acinar portions of the exocrine pancreas and the degree of fibrotic and hyaline thickening of arterioles and arteries within the pancreas has been demonstrated.<sup>7</sup> Finally, the histologic abnormalities observed in human pancreata from smokers resemble lesions induced in rodent models of pancreatic carcinoma upon treatment with tobacco-related nitrosamines.<sup>8</sup>

An extensive body of experimental data indicates that mutational activation of the *K-ras* oncogene occurs in greater than 90% of PDAC. The *K-ras* mutations are localized almost exclusively to codon 12.<sup>9–14</sup> Activating mutations in *K-ras* have been identified in the pancreatic juice,<sup>15,16</sup> fine-needle aspirate biopsies,<sup>17</sup> and stools<sup>18</sup> of patients with PDAC. Moreover, recent evidence suggests that *K-ras* mutations may be an early event in PDAC, because mutations have been identified in pancreatic juice 18–40 months prior to clinical evidence of carcinoma.<sup>19</sup> In addition to ductal adenocarcinomas, a high frequency of *K-ras* mutations likewise have been identified in ductal<sup>8</sup> and mucinous<sup>20</sup> hyperplasias of the pancreas as well as intraductal papillary tumors,<sup>21</sup> although others have reported the absence of *K-ras* mutations in intraductal papillary lesions and ductal papillary hyperplasias de-

spite the prevalence of mutations in preinvasive ductal lesions.<sup>22</sup>

Mutations in *K-ras* likewise have been identified in the pancreatic juice,<sup>16,19</sup> stools,<sup>18</sup> and hyperplastic ductal lesions<sup>23</sup> of patients with chronic pancreatitis. Evidence for an association between *K-ras* mutations and microsatellite instability in both chronic pancreatitis and PDAC patients has been presented.<sup>24</sup> Most recently, a unique spectra and lower frequency (24%) of *K-ras* mutations were observed in hyperplastic ductal epithelium in the absence of carcinoma or chronic pancreatitis.<sup>25</sup> Collectively, these studies are evidence of the importance of *K-ras* mutations as an early and frequent event in a variety of noninvasive ductal pancreatic lesions and in the pathogenesis of PDAC. The relation between cigarette smoking and the frequency and spectra of *K-ras* mutations in PDAC has not been examined extensively, and the limited studies have often yielded conflicting findings.<sup>13,26,27</sup>

The current study was directed at examining the frequency and spectra of *K-ras* mutations in nonneoplastic archival pancreatic lesions in relation to patient cigarette smoking status. The sensitivity and selectivity of these analyses of microdissected pancreatic lesions were enhanced by the use of both a primer-mediated, mutant allele specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach and a PCR-based ligase chain reaction (PCR-LCR) assay. We report an association between cigarette smoking status and the presence of codon 12 of *K-ras* mutations in nonneoplastic pancreatic lesions from individuals with a history of heavy cigarette smoking. This work provides further suggestive molecular epidemiologic evidence of a link between cigarette smoking and elevated risk for PDAC. However, the present uncertainties regarding the histogenesis of PDAC and the definitive sequelae of premalignant lesions leading to PDAC necessitate cautious interpretation of these data.

## PATIENTS AND METHODS

### Patients

Archival formalin fixed, paraffin embedded autopsy specimens of nonneoplastic pancreata were obtained from the American Cancer Society-Auerbach collection (Atlanta, GA). The collection, stratification, and histologic evaluation of these specimens has been de-

scribed previously.<sup>7</sup> The patients were divided into five categories by the original investigators<sup>7</sup> based largely on their cigarette smoking histories. In this study, three groups of patients were examined: 1) those who never smoked cigarettes (n = 16 cases); 2) those who smoked 1–2 packs/day (n = 10 cases); and 3) those who smoked more than 2 packs/day (n = 13 cases). It is estimated that each of the smokers in this study had consumed cigarettes regularly for 20 years or more. American Cancer Society data reveal that over 95% of male smokers born in the 1920s began their habit prior to the age of 25 years.<sup>28</sup> Specimens were selected from a larger subset of 77 pancreata based on the quality and suitability of the archival tissues for analyses. Of the 39 tissue samples examined, all were from males with a mean age of 58.1 years (range, 29–80 years). None of the patients died of primary PDAC or had pancreatic metastases from an extrapancreatic primary malignancy. Moreover, none of the patients examined had a clearly documented familial predisposition to any particular type of cancer. Histologic evaluation of pancreatic tissues was performed by a board certified pathologist (M.W.).

#### DNA Isolation from Formalin Fixed, Paraffin Embedded Tissues

Multiple 5- $\mu$ m tissue sections of pancreata were microdissected using sterile scalpel blades, deparaffinized with two successive xylene extractions, rinsed twice with 100% ethanol, and air dried. Samples were digested overnight at 50°C with 50 mM Tris-HCL, 20 mM ethylenediamine tetraacetic acid (EDTA), 10 mM NaCl, pH 9.0, 1% sodium dodecyl sulfate (SDS), and 500  $\mu$ g/mL proteinase K, followed by successive phenol-chloroform extractions as detailed previously.<sup>14,29</sup> Digestion buffer alone was utilized as a negative control for the DNA extraction procedure. Samples were resuspended in 50–75  $\mu$ L of double-distilled, sterile water for subsequent molecular analyses.

#### Mutant Specific PCR-RFLP Analyses of K-ras Codon 12 Mutations

Genomic DNA isolated from formalin fixed tissue sections and tumor-derived cell lines was analyzed for K-ras codon 12 mutations using a nested-mutant, allele-enriched PCR approach as detailed by Scarpa et al.<sup>14</sup> and Hruban et al.<sup>13</sup> For tissue sections, amplified K-ras sequences encompassing codon 12 were subject to nested PCR using mutated primer sequences,<sup>14</sup> generating a novel *HphI* restriction enzyme site. Second-set PCR conditions consisted of 94°C for 5 minutes followed by 35 cycles at 94°C for 15 seconds, 50°C for 15 seconds, and 72°C for 30 seconds. Following digestion with *HphI*, samples were electrophoresed on

a 12% denaturing polyacrylamide gel and stained with ethidium bromide. Controls included buffer blanks for PCR and the ductal pancreatic carcinoma cell lines, ASPC-1 (mutated K-ras codon 12), BxPC-3 (wild type K-ras codon 12), and PANC-89 (mutated K-ras codon 61). Similarly, as an alternative method, the mutant-allele enriched RFLP method detailed by Hruban et al.<sup>13</sup> was employed, but using the primer sequences A and C for the initial set of PCRs and primers A and B for the second series of amplifications (primer A: 5'-ACT GAA TAT AAA CTT CTG GTA GTT GGA CCT-3'; primer B: 5'-TCA AAG AAT GGT CCT GGA CC-3'; primer C: 5'-CAT GAA ATG GTC AGA GAA ACC-3'). Characterization of wild type versus mutant K-ras allele banding patterns upon electrophoresis was done as detailed previously.<sup>13,14</sup> All PCR-RFLP reactions were carried out a minimum of three times.

#### PCR-Based LCR of K-ras Mutations

The LCR used DNA ligation of adjacent mutant and/or wild type K-ras primers to identify the presence or absence of single base mutations, and the assay was carried out as detailed previously.<sup>30,31</sup> Briefly, the region encompassing codon 12 of K-ras was amplified from the extracted DNA of microdissected pancreata increase the number of target sequences for the LCR assay. Primers RS 53 and RS 54<sup>30</sup> were used at 35 cycles of 94°C for 1 minute, at 55°C for 1 minute, and at 72°C for 30 seconds to generate a 115-base pair (bp) product. This PCR product was added to a multiplex LCR mix consisting of a mixture of six diagnostic mutant K-ras primers and two  $\alpha^{32}$ P-labeled invariable primers under the conditions detailed<sup>30,31</sup> in order to determine the first base only of codon 12 of the K-ras gene. Each LCR assay mixture consisted of 20 mM Tris-HCL, pH 7.6; 25 mM potassium acetate; 10 mM magnesium acetate; 10 mM dithiothreitol; 1 mM nicotinamide adenine(NAD<sup>+</sup>); 0.1% Triton X-100; 0.4  $\mu$ g salmon sperm DNA; 15 units of Taq DNA ligase; 40 fmol of each diagnostic primer; 40 fmol of each  $\alpha^{32}$ P-end-labeled invariant primer; and approximately 1 fmol of extracted DNA. Reaction mixtures were incubated at 94°C for 2.5 minutes, followed by 94°C for 1 minute and 65°C for 4 minutes for 30 cycles. Ligation products ranging from 47 bp to 59 bp representing altered K-ras codon 12 sequences were electrophoresed on a denaturing 10% polyacrylamide gel. Each LCR result was confirmed a minimum of two times using two independent PCR reactions to ensure that neither Taq DNA polymerase nor Taq DNA ligase errors had been introduced at the nucleotide base of interest. Control lines<sup>30,31</sup> for the assay and screening of pancreata DNA samples included HeLa cells (wild type control, GGT-Gly), Calu-1 cells for mutant T (TGT<sup>Cys</sup>), A549 cells for

**TABLE 1**  
**Summary of Histopathologic and *K-ras* Abnormalities in Pancreatic Specimens from Cigarette Smokers<sup>a</sup>**

Cases	Smoking status <sup>b</sup>	Age/sex	<i>K-ras</i> , codon 12 status <sup>c</sup>	Histopathologic evaluation of pancreatic tissue
1	2+	54/M	G GT → C GT	Normal
2	2+	55/M	G GT → T GT	Normal
3	2+	55/M	G GT → GAT	Focal periductal fibrosis; squamous metaplasia with ductal atypia
4	2+	76/M	G GT → G TT	Normal
5	2+	42/M	G GT → G AT	Marked periductal fibrosis

M: male.

<sup>a</sup> Data listed are for those five cases of the 39 cases total analyzed that revealed a reproducible *K-ras* alteration by two different methods.

<sup>b</sup> A status designation of 2+ refers to individuals who smoked two packs of cigarettes per day for 20 years or more.

<sup>c</sup> Methods are detailed in the text. Cases 1 and 2 were confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)<sup>13,14</sup> and by multiplex PCR-based ligase chain reaction analyses.<sup>31,32</sup> Cases 3, 4, and 5 were confirmed by two distinct PCR-RFLP assays,<sup>13,14</sup> as illustrated in Figure 1.

mutant A (AGT<sup>Ser</sup>), and H157 cells for mutant C (CGT<sup>Arg</sup>).

### Statistical Analyses

The relation between wild type or mutated *K-ras* status and cigarette smoking duration in these patient populations was analyzed by chi-square analysis on a contingency table. The power of the performed test with alpha = 0.05 was 0.8. Analyses were done by using Sigma Stat software (Jandel Corporation, San Rafael, CA).

### RESULTS

Molecular analyses of multiple microdissected pancreatic specimens from 39 men revealed wild type codon 12 *K-ras* sequences in nonsmoking individuals (n = 16) and in those who smoked 1–2 packs per day for 20 or more years (n = 13). In contrast, 5 of 13 pancreatic tissue specimens (39%) from men who consumed more than 2 packs of cigarettes per day for 20 years or more revealed *K-ras* mutations in codon 12 (Table 1). These findings were confirmed employing both mutant allele-enriched PCR/RFLP assays<sup>13,14</sup> and a PCR-based LCR assay<sup>30,31</sup> were and found to be reproducible in several distinct microdissected tissue preparations obtained from each archival specimen. The sensitivity of mutation detection in these assays was approximately 1% in the current study and elsewhere.<sup>13,30,31</sup> There was a statistically-significant difference observed between heavy cigarette smokers and nonsmokers in their frequency of *K-ras* mutations

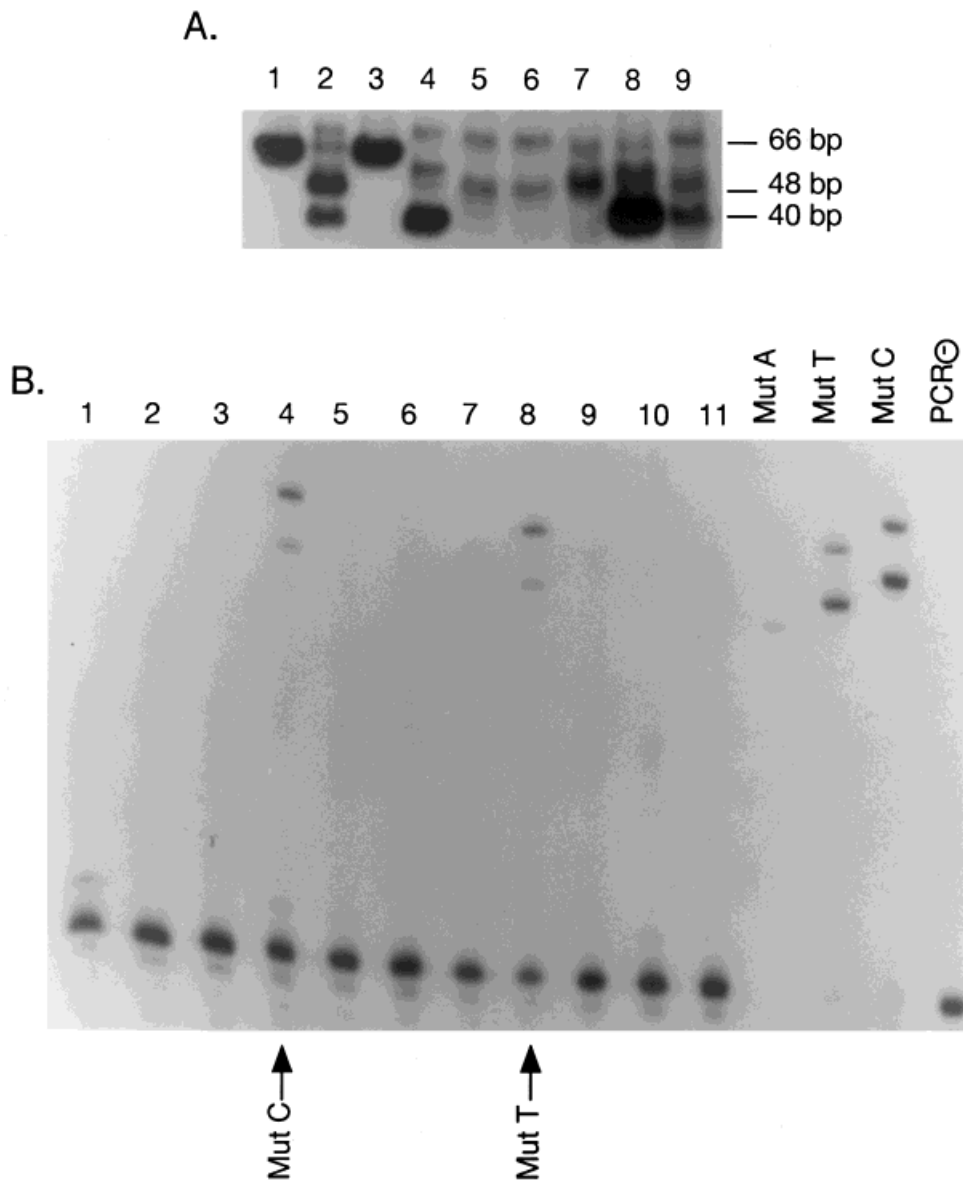
( $P < 0.005$ ) in nonneoplastic PDAC lesions. Results of the RFLP and LCR assays for these five pancreatic specimens from heavy cigarette smokers are illustrated in Figure 1. Moreover, the validity of these two assays was confirmed by analyses of a panel of pancreatic and nonpancreatic, tumor derived cell lines in which the *K-ras* mutation status was known (Fig. 1).<sup>32</sup> The *K-ras* codon 12 mutation spectra for the five cases revealed two G→T transversions, one G→C transversion and two G→A transversions in either the first or second nucleotide of codon 12. Hence, no unequivocally distinctive *K-ras* mutation spectra was apparent relative to the heavy cigarette consumption patterns of the individuals.

Table 1 shows that there was no clear correlation between the presence of mutated *K-ras* and the age of the individuals studied or the histologic characteristics of the pancreatic specimens analyzed. Specifically, three of the five pancreatic specimens exhibiting a *K-ras* mutation were histologically normal for an individual of that age, and two cases exhibited varying degrees of periductal fibrosis and squamous metaplasia with ductal atypia (Table 1). Furthermore, none of the five individuals died of PDAC or had evidence of metastatic disease from an extrapancreatic primary tumor. Conversely, among the remaining 34 pancreatic specimens exhibiting a wild type *K-ras* by two different analytical approaches, a range of histologic abnormalities were evident. These alternations included varying degrees of fibrosis, ductal hyperplasia, goblet cell metaplasia, focal dysplasia, and varying levels of inflammatory infiltrate and inflammatory atypia (data not shown).

### DISCUSSION

A substantial body of experimental data exists demonstrating the prevalence and high frequency of activating mutations in the *K-ras* oncogene early in the pathogenesis of PDAC.<sup>9–14,22</sup> Nonetheless, these mutations are not specific for PDAC, because such mutations have been observed in chronic pancreatitis,<sup>16,18,19</sup> ductal and mucinous hyperplasias,<sup>18,20</sup> and intraductal papillary lesions and ductal papillary hyperplasias<sup>21</sup> to varying degrees in patients with and without subsequent development of PDAC. Moreover, evidence has been presented for geographic differences both in the site and the spectra of *K-ras* mutations in PDAC<sup>14,26</sup> and for an association between different *K-ras* mutation spectra in pancreatic lesions of differing malignant potential.<sup>25</sup>

Despite the prevalence of *K-ras* mutations in PDAC and the body of evidence for an association between cigarette smoking and increased risk for PDAC,<sup>2–4</sup> relatively few studies have examined the



**FIGURE 1.** Mutant-allele specific polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP; A) and PCR-based ligase chain reaction (PCR-LCR; B) analyses of *K-ras*, codon 12 mutations from microdissected paraffin sections of pancreata from heavy cigarette smokers and nonsmokers. (A) Representative mutant-allele specific PCR-RFLP analyses of template DNA from microdissected and extracted pancreata were conducted as detailed in Methods and by Scarpa et al.,<sup>14</sup> electrophoresed on a 12% polyacrylamide gel, and stained with ethidium bromide. Lanes 1 and 2: Genomic DNA from cell line AsPC-1 possessing the *K-ras* codon 12 mutation (positive control). Lanes 3 and 4: Genomic DNA from cell line Panc-89 possessing the *K-ras* codon 61 mutation (negative control). Lanes 5–9: Genomic DNA from microdissected pancreata from heavy cigarette smokers described in Table 1 (cases 1–5). Lanes 1 and 3 represent undigested second-set PCR products from controls; lanes 2, 4, and 5–9 are second-set PCR products digested with *HpaI*. Uncut PCR products exhibit a 65-base-pair (bp) fragment; wild-type *K-ras* alleles from *HpaI*-digested samples yield 40-bp fragments; codon 12 mutated *K-ras* alleles from *HpaI*-digested samples yield 49-bp fragments. Numbers on the right depict size markers in base pairs. (B) Representative PCR-LCR analyses of first-base codon 12 *K-ras* mutations of template DNA from microdissected pancreata in relation to cigarette smoking. Multiplex PCR-LCR of codon 12 of *K-ras* on microdissected and extracted template DNA from pancreata and controls was performed as detailed in Methods and by Scott et al.<sup>31</sup> by electrophoresis on 10% denaturing polyacrylamide gels. Lanes 1, 2, 3, and 5: Pancreata DNA from representative nonsmokers. Lanes 4, 6, 7, and 8: Pancreata DNA from the group that smoked more than 2 packs/day. Lanes 9–11: Pancreata DNA from the group that smoked 1–2 packs/day. Controls: Mut A, DNA from A549 cell line exhibiting GGT→AGT; Mut T, DNA from Calu 1 cell line exhibiting GGT→TGT; Mut C, DNA from H157 cell line exhibiting GGT→CGT; PCR-, negative PCR control with normal DNA. Lanes 4 and 8 depict cases 1 and 2 (Table 1) for a G→C and G→T base change, respectively, in the first base of codon 12.

relation between cigarette smoking status and the frequency and K-*ras* mutation spectra in PDAC. An initial report<sup>26</sup> of 38 PDAC cases analyzed K-*ras* mutations by dot blot hybridization assays and found no association of K-*ras* mutation frequency and pattern with cigarette smoking history. A subsequent comprehensive study analyzed 82 archival PDAC specimens by mutant allele specific RFLP analyses and allele specific oligonucleotide hybridization. This study revealed a substantially higher frequency of K-*ras* mutations in PDAC from cigarette smokers (88%) compared with nonsmokers (68%), with  $P < 0.05$  for this association. The K-*ras* mutation spectra, however, were comparable in both groups.<sup>13</sup> The finding of an association between K-*ras* mutation frequency and smoking was substantiated in a subsequent study of 51 PDAC patients who were grouped as nonsmokers or smokers at some point in their lives.<sup>27</sup> This study employing mutant-allele specific RFLP analyses revealed a higher incidence of K-*ras* mutations in PDAC from smokers (67%) versus nonsmokers (57%), a finding that was not statistically significant. A more significant correlation ( $P < 0.05$ ) was observed for moderate-heavy alcohol consumption and prevalence of K-*ras* mutations in the cases of PDAC examined.<sup>27</sup>

In all three published accounts<sup>13,26,27</sup> discussed above, the relation between cigarette smoking history and K-*ras* mutation status was examined specifically in PDAC lesions. In view of the evidence for K-*ras* mutational activation as an early event in pancreatic tumorigenesis and the occurrence of mutations in non-PDAC lesions, we undertook the current studies to examine the K-*ras* mutation status of nonneoplastic pancreatic lesions in relation to cigarette smoking history. The pancreata were obtained from sex- and age-adjusted individuals,<sup>7</sup> none of whom subsequently died from PDAC or had pancreatic metastases or died from a nonpancreatic malignancy in which K-*ras* may have been implicated. By using two confirming approaches with comparable mutation detection sensitivities (approximately 1%), we observed a clear association between the presence of K-*ras* codon 12 mutations and history of heavy cigarette consumption (more than 2 packs/day for 20 or more years) in 39% of the cases examined. These findings were statistically significant among the groups analyzed ( $P < 0.005$ ). Moreover, there was no correlation between the incidence or type of mutations in this group and pancreatic histopathology, because both overtly normal pancreata (cases 1, 2, and 4) and those with squamous metaplasia, periductal fibrosis, and ductal atypias (cases 3 and 5) revealed reproducible K-*ras* abnormalities. The types of K-*ras* mutations observed (G→A, G→T and G→C) were similar to those reported elsewhere.<sup>9-14</sup> To our knowledge, this is the first report of

a clear molecular epidemiologic association between heavy cigarette consumption and K-*ras* mutational activation in a substantial percentage of noncancerous pancreatic lesions. These data provide support for an association between a known risk factor for PDAC (heavy cigarette smoking) and mutations in a molecular target (K-*ras*) that is believed to be an early and important event both in ductal pancreatic tumorigenesis and in a variety of non-PDAC lesions, including chronic pancreatitis, ductal and mucinous hyperplasia, and intraductal papillary lesions. An important caveat in interpreting data of this nature is the fact that the histogenesis of PDAC and its origin from acinar or ductal elements remains controversial based on available *in vitro*<sup>35,36</sup> and *in vivo*<sup>37</sup> transgenic mouse studies. This issue is complicated further by the documented phenotypic plasticity of differentiated pancreatic ductal epithelia *in vitro* and *in vivo* (for review, see Hall and Lemoine<sup>38</sup>). Finally, the definitive sequelae of premalignant stages or precise relationship between particular nonneoplastic lesions and unequivocal PDAC remain unresolved.<sup>21,22,33</sup> In this context, it is well established that chronic pancreatitis is a significant risk factor for human PDAC.<sup>39,40</sup> It could be argued that chronic pancreatitis may constitute a potential precursor lesion in the pathogenesis of PDAC.

This study is illustrative of how molecular analyses of archival lesions may be useful for assessing the risk of mutational damage from exposure to known etiologic agents associated with particular cancers. These findings of mutational activation of K-*ras* in nonneoplastic lesions, independent of histological abnormalities and in association with cigarette smoking, requires substantiation on a larger series of cases. Nonetheless, these data support studies of human PDAC cases and in specific N-nitrosamine-induced animal models of PDAC,<sup>33,34</sup> suggesting that carcinogenic agents present in cigarette smoke (e.g., aromatic amines and N-nitrosamines) can induce specific activating mutations in the K-*ras* protooncogene in nonneoplastic target tissues, including the exocrine pancreas.

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