# Frequency and Distinctive Spectrum of *KRAS* Mutations in Never Smokers with Lung Adenocarcinoma

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**Abstract**

**Purpose:** *KRAS* mutations are found in ~25% of lung adenocarcinomas in Western countries and, as a group, have been strongly associated with cigarette smoking. These mutations are predictive of poor prognosis in resected disease as well as resistance to treatment with erlotinib or gefitinib.

**Experimental Design:** We determined the frequency and type of *KRAS* codon 12 and 13 mutations and characterized their association with cigarette smoking history in patients with lung adenocarcinomas.

**Results:** *KRAS* mutational analysis was done on 482 lung adenocarcinomas, 81 (17%) of which were obtained from patients who had never smoked cigarettes. *KRAS* mutations were found in 15% (12 of 81; 95% confidence intervals, 8-24%) of tumors from never smokers. Similarly, 22% (69 of 316; 95% confidence intervals, 17-27%) of tumors from former smokers, and 25% (21 of 85; 95% confidence intervals, 16-35%) of tumors from current smokers had *KRAS* mutations. The frequency of *KRAS* mutation was not associated with age, gender, or smoking history. The number of pack years of cigarette smoking did not predict an increased likelihood of *KRAS* mutations. Never smokers were significantly more likely than former or current smokers to have a transition mutation (G→A) rather than the transversion mutations known to be smoking-related (G→T or G→C; $P < 0.0001$).

**Conclusions:** Based on our data, *KRAS* mutations are not rare among never smokers with lung adenocarcinoma and such patients have a distinct *KRAS* mutation profile. The etiologic and biological heterogeneity of *KRAS* mutant lung adenocarcinomas is worthy of further study.

Since the identification of somatic epidermal growth factor receptor (*EGFR*) mutations, there has been heightened interest in the molecular basis of lung cancer in patients who never smoked cigarettes (1–3). Somatic mutations in *EGFR* have been identified in ~15% of all patients with lung adenocarcinoma, with the proportion increasing to 50% in patients who never smoked cigarettes. There is an inverse relationship between cigarette smoking history and frequency of *EGFR* mutations, with the frequency of *EGFR* mutations decreasing significantly among patients who smoked more than 15 pack years (4). Such refined understanding of the relationship between smoking history and presence of *EGFR* mutations has allowed the design of clinical trials which use smoking history to enrich the number of patients with somatic *EGFR* mutations (5–7).

In contrast to *EGFR* mutations, *KRAS* mutations were initially identified in patients with lung adenocarcinoma who had a history of heavy cigarette smoking and were thought to be uncommon in patients without a history of smoking cigarettes (8). These mutations are found in ~25% of lung adenocarcinomas in western countries but are less common in Asian populations (9, 10). *KRAS* mutations have been associated with poor prognosis in resected non–small cell lung cancer (NSCLC; refs. 11–13), lack of survival benefit from adjuvant chemotherapy (14), and resistance to erlotinib or gefitinib (15). More than 95% of *KRAS* mutations in lung cancer occur in codons 12 and 13. In both *KRAS* and *TP53*, transversions (substituting a pyrimidine for a purine or purine for a pyrimidine) are more common than transitions (substituting purine for purine or pyrimidine for pyrimidine) identifying a molecular signature for the carcinogenic effects of cigarette smoke (16, 17). A detailed analysis of *KRAS* mutations in relation to smoking history has not been done. Using a cohort of patients with lung adenocarcinoma, we sought to determine the frequency and type of *KRAS* mutations in a large series of patients with known smoking histories.

## Materials and Methods

Tumor specimens were obtained from an institutional tumor bank of patients who had undergone NSCLC resections between 2002 and 2007, as well as from patients with metastatic NSCLC who had *KRAS* mutation testing done as part of clinical trials or during routine clinical practice. Because *KRAS* mutations are rare in squamous tumors, only

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**Note:** V.A. Miller and M. Ladanyi contributed equally to this work.

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## Translational Relevance

Mutations in the *KRAS* oncogene are found in ~25% of lung adenocarcinomas in Western countries. Studies have linked *KRAS* mutations with poor prognosis in non–small cell lung cancer as well as resistance to treatment with erlotinib or gefitinib. These mutations have been reported to be strongly associated with cigarette smoking. However, previous studies which explored the association of smoking with *KRAS* mutation did not include large numbers of patients who never smoked cigarettes. We report that *KRAS* mutations are found in 15% of lung adenocarcinomas from patients who never smoked cigarettes compared with 22% in patients with a history of smoking cigarettes, a statistically insignificant difference in this study. Furthermore, the frequency of *KRAS* mutation was not associated with age, gender, or smoking history, making it difficult to predict which tumors have *KRAS* mutations by any clinical characteristics. Based on these data, we believe that molecular testing for *KRAS* mutations is necessary to identify this subgroup of patients with a different response to some treatments.

specimens with a histologic diagnosis of lung adenocarcinoma were included. All tumor specimens used for *KRAS* sequencing had >50% tumor. Specimens were microdissected as needed. This retrospective review was done under a waiver of authorization approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board. Standard direct sequencing was used to identify *KRAS* codon 12 and 13 mutations in tumors (15).

Patient smoking history was obtained by review of a patient-completed smoking questionnaire and the medical record. The prospectively administered questionnaire contained the following questions: Have you smoked more than 100 cigarettes in your life? Are you currently smoking? How many years have you been a regular smoker; and on average, how many cigarettes did you smoke per day? The smoking questionnaire was administered at the time of the first evaluation by a thoracic surgeon or medical oncologist at this institution. Never smokers had smoked <100 cigarettes. Former smokers had previously smoked cigarettes but quit smoking more than 1 year prior to diagnosis of lung cancer. Pack years of smoking was defined as [(average number of cigarettes per day / 20) × years smoking].

## Results

In 482 lung adenocarcinomas, *KRAS* mutations in codons 12 or 13 were found in 21% (102 of 482; 95% confidence intervals, 18-25%). Patients whose tumors harbored *KRAS* mutations were not significantly different from patients with *KRAS* wild-type tumors with regard to gender, age, or prior smoking history (Table 1). *KRAS* mutations were identified in 15% (12 of 81; 95% confidence intervals, 8-24%) of never smokers, 22% (69 of 316; 95% confidence intervals, 17-27%) of former smokers, and 25% (21 of 85; 95% confidence intervals, 16-35%) of current smokers (Fig. 1). No tumor with a *KRAS* mutation had a mutation in *EGFR*. There were no significant differences in frequency of *KRAS* mutations by category of smoking history (Mantel-Haenszel $\chi^2$ $P$ = 0.12). Next, we examined the frequency of *KRAS* mutation by pack years of cigarette smoking (Table 2), and found no trend (Mantel-Haenszel $\chi^2$ $P$ = 0.19). To determine whether there was a cutpoint for pack years of cigarette smoking above which *KRAS* mutations were more frequent, a receiver operating characteristic curve was generated. The area under the receiver operating characteristic curve was 0.56 (data not shown) suggesting no value in using pack years of cigarette smoking to predict *KRAS* mutational status.

To determine whether the type of *KRAS* mutation identified in never smokers correlated with the previously described dominance of transversions in smoking-associated cancers, we compared the type of *KRAS* mutation found in never smokers to those found in former or current smokers (Table 3). Never smokers were significantly more likely (Fisher's exact test $P < 0.0001$) to have a transition mutation. The ratio of transition/transversion for never smokers was 11:1 as compared with 17:73 for former or current smokers.

## Discussion

In these patients with lung adenocarcinoma, we find that *KRAS* mutations are not rare in never smokers. This is a striking finding given the widespread perception that cigarette smoking and *KRAS* mutations are invariably linked (reviewed in ref. 16). The association between cigarette smoking and *KRAS* mutations has been inferred from a number of series that included a relatively small numbers of patients who never smoked cigarettes. For example, Nelson et al. examined tumors from 365 patients with NSCLC, of which only 22 were never smokers (18). Among the patients in that series in which *KRAS* mutational analysis was done, there were only 16 never smokers, none of whom had *KRAS* mutations. However, another series which included some never smokers did identify *KRAS* mutation in 14% (3 of 21) of never smokers (19). A difference between our series and previous series is the method

**Table 1.** *KRAS* codon 12 and 13 mutations and clinical characteristics

| | All | Mutant *KRAS* | Wild-type *KRAS* | *P* |
|---|---|---|---|---|
| Total | 482 | 102 (21%) | 380 (79%) | |
| Men | 197 (41%) | 40 (39%) | 157 (41%) | 0.73* |
| Women | 285 (59%) | 62 (61%) | 223 (59%) | |
| Never smokers | 81 (17%) | 12 (12%) | 69 (18%) | 0.14* |
| Former/current smokers | 401 (83%) | 90 (88%) | 311 (82%) | |
| Age, median (range) | 68 (30-89) | 68 (33-85) | 67 (30-89) | 0.98 † |

*Fisher's exact test.
† Wilcoxon rank sum test.

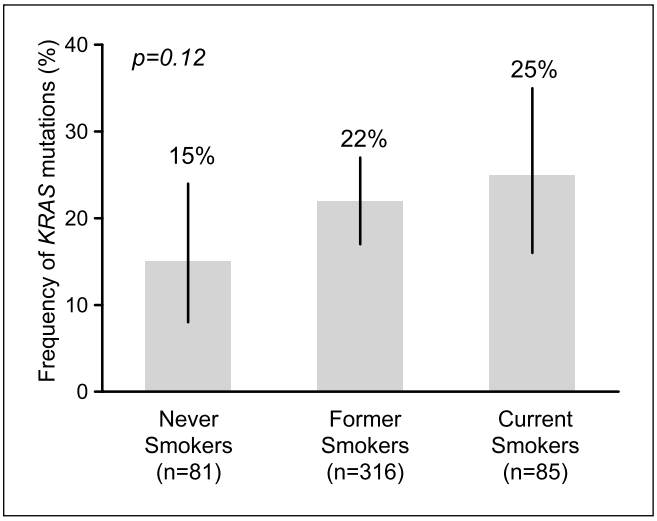


**Fig. 1.** Frequency of *KRAS* mutation by smoking history. Mantel-Haenszel $\chi^2$ test for trend was used to calculate *P* value.

of collection of smoking history. We determined smoking history using prospectively collected smoking questionnaires completed by patients with a diagnosis of lung cancer. These patients completed a detailed questionnaire which included the age of onset of smoking, the average number of cigarettes per day, the number of years in which they smoked cigarettes, and the time that the patient quit smoking cigarettes. The characteristics of patients included in this analysis are similar to the patient population seen at our institution with regard to age, gender, and smoking history.

The *KRAS* mutations observed in these never smokers, in addition to being more frequent than previously reported, are more likely to be transitions, unlike the transversions more common in patients with a history of cigarette smoking. In both *KRAS* and *TP53*, transversions (substituting a pyrimidine for a purine, or purine for a pyrimidine) are more common than transitions (substituting purine for purine, or pyrimidine for pyrimidine; refs. 16, 17). The etiology of G→T transversions in tumors from patients with lung cancer is thought to be related to exposure to polycyclic aromatic hydrocarbons found in cigarette smoke (20). In the case of TP53, investigators have recently noted that TP53 G→T transversions were distinctly uncommon in lung adenocarcinomas with *EGFR* mutations, a mutation more commonly seen in never smokers (21).

**Table 3.** *KRAS* mutation type as a function of smoking history

| *KRAS* | | Former/current | Never | Total |
|---|---|---|---|---|
| **Mutation** | **Nucleotide** | | | |
| G12A | G**G**T→G**C**T | 13 | 0 | 13 |
| G12C | **G**GT→**T**GT | 38 | 0 | 38 |
| G12V | G**G**T→G**T**T | 20 | 1 | 21 |
| G13C | **G**GC→**T**GC | 2 | 0 | 2 |
| G13D | G**G**C→G**A**C | 1 | 0 | 1 |
| G12D | G**G**T→G**A**T | 15 | 10 | 25 |
| G12S | **G**GT→**A**GT | 1 | 1 | 2 |
| Total | | 90 | 12 | |

Because patients without smoking history represent ~15% of patients with lung cancer, it is critical that any analysis seeking to examine the biology of these tumors examine a relatively large number of patients with NSCLC (22, 23). Relatively little is understood about the biology and epidemiology of lung cancer in never smokers. A number of possible causative factors have been suggested including exposure to environmental tobacco smoke or radon, as well as genetic and hormonal abnormalities (reviewed in refs. 24, 25). The distinct profile of *KRAS* mutations observed here in never smokers further suggests that such cancers are only rarely caused by environmental tobacco exposure. Whether this etiologic heterogeneity within *KRAS* mutant lung adenocarcinomas is associated with differences in cooperating genetic lesions and overall biological behavior warrants further investigation. Finally, because tumors from never smokers may have *KRAS* mutations, and such mutations have been associated with resistance to erlotinib and gefitinib (15), molecular analysis of NSCLC specimens for *KRAS* mutations may improve a clinician's ability to predict response and resistance to therapy with erlotinib or gefitinib.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

**Table 2.** Frequency of *KRAS* mutation by smoking history in pack years

| | Mutant | Wild-type | Total | Frequency (%) | 95% Confidence intervals (%) |
|---|---|---|---|---|---|
| Never smokers | 12 | 69 | 81 | 15 | 8-23 |
| <5 py | 3 | 25 | 28 | 11 | 2-28 |
| 6-10 py | 3 | 25 | 28 | 11 | 2-28 |
| 11-15 py | 6 | 13 | 19 | 32 | 13-57 |
| 16-25 py | 10 | 45 | 55 | 18 | 9-31 |
| 26-50 py | 40 | 106 | 146 | 27 | 20-35 |
| 51-75 py | 16 | 51 | 67 | 24 | 14-36 |
| >75 py | 12 | 46 | 58 | 21 | 11-33 |
| Total | 102 | 380 | 482 | 21 | |

Abbreviation: py, pack years.

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