Chronic Obstructive Pulmonary Disease Is Not Associated with KRAS Mutations in Non-Small Cell Lung Cancer

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Abstract

Mutations in epithelial growth factor receptor (EGFR), as well as in the EGFR downstream target KRAS are frequently observed in non-small cell lung cancer (NSCLC). Chronic obstructive pulmonary disease (COPD), an independent risk factor for developing NSCLC, is associated with an increased activation of EGFR. In this study we determined presence of EGFR and KRAS hotspot mutations in 325 consecutive NSCLC patients subjected to EGFR and KRAS mutation analysis in the diagnostic setting and for whom the pulmonary function has been determined at time of NSCLC diagnosis. Information about age at diagnosis, sex, smoking status, forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) was collected. Chronic obstructive pulmonary disease(COPD) was defined according to 2013 GOLD criteria. Chi-Square, student t-test and multivariate logistic regression were used to analyze the data. A total of 325 NSCLC patients were included, 193 with COPD and 132 without COPD. COPD was not associated with presence of EGFR and KRAS hotspot mutations in 325 consecutive NSCLC patients subjected to EGFR and KRAS mutation analysis in the diagnostic setting and for whom the pulmonary function has been determined at time of NSCLC diagnosis. Information about age at diagnosis, sex, smoking status, forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) was collected. Chronic obstructive pulmonary disease(COPD) was defined according to 2013 GOLD criteria. Chi-Square, student t-test and multivariate logistic regression were used to analyze the data. A total of 325 NSCLC patients were included, 193 with COPD and 132 without COPD. COPD was not associated with presence of EGFR and KRAS hotspot mutations, while EGFR mutations were significantly higher in non-COPD NSCLC patients.

Both female gender (HR 2.61; 95% CI: 1.56–4.39; p < 0.001) and smoking (HR 4.10; 95% CI: 1.14–14.79; p = 0.03) were associated with KRAS mutational status. In contrast, only smoking (HR 0.11; 95% CI: 0.04–0.32; p<0.001) was inversely associated with EGFR mutational status. Smoking related G>T and G>C transversions were significantly more frequent in females (86.2%) than in males (61.5%) (p = 0.008). The exon 19del mutation was more frequent in non-smokers (90%) compared to current or past smokers (36.8%). In conclusion, KRAS mutations are more common in females and smokers, but are not associated with COPD-status in NSCLC patients. EGFR mutations are more common in non-smoking NSCLC patients.
**Introduction**

Chronic obstructive pulmonary disease (COPD) is associated with lung cancer also after accounting for other respiratory diseases and smoking [1–2]. An increased risk of lung cancer in COPD patients was evident in a meta-analysis [2]. About one third of smokers with COPD died of lung cancer within a follow-up of 14.5 years [3]. On the other hand, 50–70% of the lung cancer patients have COPD according to results of pulmonary function tests at time of diagnosis [4]. In a more recent, large prospective study, the association between COPD and lung cancer was largely explained by smoking [5]. The odds ratio (OR) for patients diagnosed with COPD to develop lung cancer within a period of 6 months was 11.4. However, the OR dropped to 6.8 after correction for smoking [5]. This is consistent with the notion that COPD has been recognized as an independent risk factor for developing lung cancer [6].

KRAS is involved in regulation of cell proliferation [7]. Mutations in KRAS are mostly found in codons 12, 13 and 61 and result in constitutive activation of the protein [8]. KRAS mutations are observed more frequent in smoking patients with adenocarcinoma (5–40%) than in the other subtypes of lung cancer [7, 9]. Mutations in KRAS are associated with poorer prognosis of NSCLC patients [10]. Moreover, a COPD-like airway inflammation can increase lung carcinogenesis in the presence of the p.G12D K-ras activating mutation in a mouse model [11].

EGFR plays a crucial role in wound healing and tissue repair in the lung, especially in the bronchial wall. Overexpression of EGFR was reported in the bronchial mucosa of non-smoking asthmatic individuals compared to normal controls [12]. Moreover, prolonged activation of EGFR leads to metaplasia [13]. Exposure of epithelial cells to cigarette smoke induced aberrant phosphorylation and activation of EGFR and this may subsequently mediate development of lung cancer [14–15]. Mutations in the kinase domain also lead to activation of the EGFR pathway independent of binding to its ligand [16]. These activating EGFR mutations are common in non-small cell lung cancer (NSCLC) with a frequency of about 10–15% in Caucasians [17–18]. EGFR mutations have been associated with non-smoking NSCLC patients [19]. The p. (L858R) in exon 21 (referred to as L858R) and deletions in exon 19 (referred to as exon 19del) of the EGFR gene are the most commonly observed activating mutations [20]. We previously showed a significant association between EGFR mutations and clinical outcome [21]. In vivo studies in mouse models conditionally expressing either the L858R or an exon19del mutant allele of the human EGFR gene have supported the role of these mutations in initiation and development of lung cancer [22].

Smoking is a known risk factor for both COPD and lung cancer [23–24]. KRAS mutations are described as a signature for cigarette smoking [25], while EGFR mutations are more common in non-smokers. We hypothesize that KRAS mutations are positively associated with COPD status in NSCLC patients, while activating EGFR mutations are negatively associated with COPD in NSCLC patients. To study this hypothesis we analyzed NSCLC patients screened for the presence of EGFR and KRAS mutations in a diagnostic setting and investigated whether the presence of EGFR and KRAS mutations in NSCLC patients was related to COPD.

**Materials and Methods**

**Patients**

Consecutive patients with advanced NSCLC, diagnosed between November 2008 and July 2012, and for whom KRAS and EGFR mutation analysis was performed in a clinical setting, were selected for this study. In this cohort we further selected patients for whom lung function data were available. All patients had stage IV NSCLC and had one or more visceral metastases...
at diagnosis. Previously, 165 of the NSCLC patients have been described in a study on EGFR and KRAS mutations in relation to clinical outcome [21]. Patients with NSCLC post lung transplantation were excluded from this study. For all patients, data on gender, smoking status (including pack year if available), age at diagnosis, stage at diagnosis according to the 6th TNM edition, localization of metastases, start date and (different) lines of treatment were collected. Data on lung function was newly collected for all patients included in this study. All procedures and protocols were performed according to the guidelines for good clinical practice and after informed consent was obtained from all patients.

Informed Consent and Ethics

Written informed consent for blood and tumor tissue from all patients was obtained before biobanking. This procedure was approved by the Medical Ethical Committee of the University Medical Center Groningen. This study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. For this study, all patient data were anonymized and de-identified prior to analysis. Besides the mutational analysis, pulmonary function tests were performed as part of routine diagnostic approach and the outcome of these tests was documented in the patient file and communicated with patients. Due to the retrospective nature of this study, under Dutch Law for human medical research (WMO), no specific permission was compulsory from the Institutional Review Board.

Pulmonary function testing

Spirometry was performed with a daily-calibrated pneumotachograph (MasterscreenPneumo, Jaeger, Wurzburg, Germany) according to standardized guidelines [26]. Lung function tests are provided after bronchodilator (salbutamol 100 microgram). Patients were defined as having COPD if the forced expiratory volume in 1 sec (FEV1)/forced vital capacity (FVC) (FEV1/FVC) was <0.70 with fixed bronchial obstruction over time not due to endobronchial tumor obstruction. Staging of COPD was performed according to GOLD criteria [27].

Histology and KRAS/EGFR molecular testing

Tumor samples were obtained either by bronchoscopy, transthoracic lung biopsies and/or from pulmonary resections. Histological subtyping was performed according to 2004 WHO criteria [28]. Mutational analysis was performed as previously described [21].

Statistics

For normally distributed data we show mean and standard deviation (SD) and used a student t-test to determine significant differences. For not normally distributed data median and range are given and Chi-Square test is used to determine significance. Logistic regression was performed to study whether the presence of COPD had any effect on KRAS or EGFR mutational status using sex, age, histology, and smoking as covariates. Statistical analysis was performed using SPSS version 22.0. Nominal P-values less than 0.05 were considered significant. Data are available in S1 Table.

Results

Patient characteristics and KRAS/EGFR mutations

A total of 325 stage IV NSCLC patients were included. Over 80% had adenocarcinoma, 174 (53.5%) were male and 151 (46.5%) female. The mean age at time of diagnosis was 63.6 (±10.5 years). One hundred and five patients (32.3%) had a KRAS mutation. For 1 out of 105 patients
with a KRAS mutation, the type of mutation was inconclusive with a positive high resolution melting (HRM) PCR result, but with a wild type sequence based on the Sanger sequencing result. For one patient with an EGFR mutation, the KRAS mutation status was not available. The remaining 219 patients did not have mutations in the KRAS hotspot region. Twenty-nine patients (8.9%) had an EGFR mutation. In five patients, the EGFR mutational status was not available; four of these patients did have a KRAS mutation. The other 291 patients did not have EGFR mutations in the hotspot regions. The mean age of the males was higher than the mean age of the females (66.3 ±9.8 years vs. 60.5 ±10.5 years; p<0.001). Males showed a significant higher number of smoking pack years than females (mean 37.5 ±20.6 pack years vs. 30.1 ±15.7 pack years; p = 0.015).

Patient characteristics in COPD stratified subgroups

Almost 60% (193/325) of the NSCLC patients had COPD. Two third of the COPD cases were males. The distribution of females was almost equal in COPD and non-COPD groups (Table 1). Mean age in the COPD group was higher with 65.6 years (±9.9 years) compared to the non-COPD group with 60.6 (±10.8 years) (p<0.001). We found a significant relationship between smoking and COPD, 62.6% of current or past smokers had COPD, while only 18.2% of non-smokers had COPD (p<0.001). A logistic regression model for COPD using sex, age and smoking as covariates revealed significant associations with age (Hazard ratio [HR] 1.05; 95% confidence interval [CI]: 1.02–1.07; p<0.001) and smoking (HR 8.28; 95% CI: 2.61–26.24; p<0.001), but not with gender (Table 2).

Table 1. NSCLC patient characteristics according to COPD status.

| Characteristics                        | COPD (%) N = 193 | Non-COPD (%) N = 132 | p-value |
|----------------------------------------|------------------|----------------------|---------|
| Sex                                    |                  |                      |         |
| Female                                 | 77 (51)          | 74 (49)              | 0.004†  |
| Male                                   | 116 (66.7)       | 58 (33.3)            |         |
| Age at diagnosis, Mean (±SD)           | 65.6 (±9.9)      | 60.6 (±10.8)         | <0.001* |
| Histology                              |                  |                      |         |
| Adenocarcinoma                         | 154 (59)         | 107 (41)             | 0.777†  |
| Adeno-squamous                         | 14 (66.7)        | 7 (33.3)             |         |
| NSCLC-NOS                               | 25 (58.1)        | 18 (41.9)            |         |
| Smoking status*                        |                  |                      |         |
| Current or past smoker                 | 184 (62.6)       | 110 (37.4)           | <0.001† |
| Non-smoker                             | 4 (18.2)         | 18 (81.8)            |         |
| FEV1%, Mean (±SD)                      | 72.8 (±18.7)     | 90.2 (±19.4)         | -       |
| FEV1/FVC ratio, Mean (±SD)             | 0.58 (±0.09)     | 0.76 (±0.05)         | -       |
| KRAS mutation*                         |                  |                      |         |
| Yes                                    | 66 (62.9)        | 39 (37.1)            | 0.404†  |
| No                                     | 127 (58)         | 92 (42)              |         |
| EGFR activating mutation*              |                  |                      |         |
| Yes                                    | 9 (31)           | 20 (69)              | 0.001†  |
| No                                     | 180 (61.9%)      | 111 (38.1)           |         |

* T-test;
†Chi-square test;
*Missing data for smoking (n = 9), KRAS (n = 1) and EGFR (n = 5) status.

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COPD Is Not Associated with KRAS Mutations in NSCLC

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KRAS mutations were observed more often in females compared to males (65/151 (43%) versus 40/173, (23%); p < 0.001) and also more often in current or past smokers (34.5%) than in non-smokers (13.6%) (p = 0.045) (Table 3). KRAS mutations were not significantly different between COPD (34.2%) and non-COPD patients (29.8%) (Table 1), so the presence of KRAS mutations were independent of COPD. Stratification according to FEV1/FVC and GOLD stage did not reveal a significant association with presence of KRAS mutation (Fig 1A and 1B). However, FEV1 percentage as a continuous variable was significantly related to the presence of KRAS mutations (Fig 1C), but not in a multivariate analysis. Putting the variables (sex, age, smoking and COPD) in a logistic regression model confirmed the significant association.

### Table 2. Logistic regression analysis of patient characteristics associated with COPD, KRAS and EGFR.

| Characteristics | COPD HR | 95% CI | p-value | KRAS HR | 95% CI | p-value | EGFR HR | 95% CI | p-value |
|----------------|---------|--------|---------|---------|--------|---------|---------|--------|---------|
| Sex            | 0.78    | 0.48–1.29 | 0.33    | 2.61    | 1.56–4.39 | <0.001 | 1.21    | 0.51–2.90 | 0.66    |
| Age            | 1.05    | 1.02–1.07 | <0.001  | 0.99    | 0.97–1.01 | 0.39   | 0.98    | 0.95–1.02 | 0.39    |
| Smoking        | 8.28    | 2.61–26.24 | <0.001  | 4.10    | 1.14–14.79 | 0.03   | 0.11    | 0.04–0.32 | <0.001 |
| COPD           | -       | -       | -       | 1.29    | 0.77–2.18 | 0.34   | 0.44    | 0.18–1.09 | 0.08    |

HR: Hazard ratio; CI: Confidence interval

### Table 3. NSCLC patient characteristics and KRAS/EGFR mutation*.

| Characteristics | KRAS mutation | Pearson Chi-Square | EGFR mutation | Pearson Chi-Square |
|----------------|---------------|--------------------|---------------|--------------------|
|                | No (%)        | Yes (%)            |               | No (%)             | Yes (%)            |
| Sex            |               |                    |               |                    |
| Female         | 86 (57)       | 65 (43)            | <0.001        | 130 (87.8)         | 18 (12.2)          | 0.07 |
| Male           | 133 (76.9)    | 40 (23.1)          |               | 161 (93.6)         | 11 (6.4)           |      |
| Smoking status |               |                    |               |                    |
| Current or past smoker | 192 (65.5) | 101 (34.5) | 0.045 | 270 (93.4) | 19 (6.6) | <0.001 |
| Nonsmoker      | 19 (86.4)     | 3 (13.6)           |               | 12 (54.5)          | 10 (45.5)          |      |

* Missing data for smoking (n = 9), KRAS (n = 1) and EGFR (n = 5) status.

**Fig 1. KRAS mutations and severity of airflow obstruction.** (A) FEV1/FVC, (B) COPD GOLD classification and (C) FEV1 percentage in KRAS mutant and wildtype patients with NSCLC. *p*-value was calculated by student t-test. **p*-value was calculated by Chi-square test.

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between KRAS hotspot mutations with female sex (HR 2.61; 95% CI: 1.56–4.39; p<0.001) and smoking (HR 4.10; 95% CI: 1.14–14.79; p = 0.03) (Table 2).

EGFR mutations showed a trend to a higher frequency in females compared to males, (p = 0.073). Ten out of 22 (45.5%) non-smokers had activating EGFR mutations, while only 19 out of 289 (6.5%) of the current or past smokers had an EGFR mutation (p<0.001). EGFR mutations were observed more often in the non-COPD (20/131, i.e. 15.3%) as compared to the COPD group (9/189, i.e. 4.8%)(p = 0.001) (Table 3). Using sex, age, smoking and COPD as covariates in a logistic regression model for EGFR mutations, confirmed significant inverse association between smoking (HR 0.11; 95% CI: 0.04–0.32; p<0.001) and EGFR mutational status (Table 2).

COPD and type of KRAS/EGFR mutations

The KRAS p.(G12C) was the most common amino acid change in both male and female with a frequency of approximately 41%. The p.(G12V) and p.(G12D) mutations were the second most frequent mutations in females and males, with a frequency of 20% and 25.6%, respectively (Table 4). Forty-three percent of the KRAS mutations in the current or past smoker group were p.(G12C) mutations, while none of the non-smoking patients had this mutation. In addition, G>T and G>C transversions in KRAS occurred in 86.2% of the females and in 61.5% of the males. The G>A transition was more common in males than in females (p = 0.008)(Table 5). COPD status was not associated with any type of KRAS amino acid changes or nucleotide substitutions.

Of all EGFR mutation positive cases the percentage of patients with an exon 19del was not significantly different between females (11/18) and males (5/11). In non-smokers, 9 out of 10 EGFR mutation positive cases had an exon 19del (Table 6), whereas in current or past smokers only 7 out of 19 patients with an EGFR mutation had an exon 19del.

Discussion

In contrast to our hypothesis we showed that COPD is not associated with the presence of KRAS mutations in lung cancer, whereas presence of EGFR mutations was more frequent in

| Characteristics | p.(G12C) (%) | p.(G12V) (%) | p.(G12A) (%) | p.(G12D) (%) | Other (%) | Pearson Chi-Square |
|-----------------|-------------|-------------|-------------|-------------|----------|------------------|
| Sex             |             |             |             |             |          |                  |
| Female          | 27 (41.5)   | 13 (20)     | 7 (10.8)    | 6 (9.2)     | 12 (18.8) | 0.20             |
| Male            | 16 (41)     | 5 (12.8)    | 2 (5.1)     | 10 (25.6)   | 6 (15.4)  |                  |
| Histology       |             |             |             |             |          |                  |
| Adenocarcinoma  | 41 (42.7)   | 16 (16.7)   | 8 (8.3)     | 13 (13.5)   | 18 (18.8) | 0.26             |
| NSCLC NOS       | 2 (25)      | 2 (25)      | 1 (12.5)    | 3 (37.5)    | 0        |                  |
| Smoking status  |             |             |             |             |          |                  |
| Current or past smoker | 43 (43)   | 17 (17)     | 7 (7)       | 15 (15)     | 18 (18)  | 0.24             |
| Non smoker      | 0           | 1 (33.3)    | 1 (33.3)    | 1 (33.3)    | 0        |                  |
| COPD            |             |             |             |             |          |                  |
| Yes             | 27 (40.9)   | 11 (16.7)   | 6 (9.1)     | 11 (16.7)   | 11 (16.7) | 0.99             |
| No              | 16 (42.1)   | 7 (18.4)    | 3 (7.9)     | 5 (13.2)    | 7 (18.4)  |                  |

* Missing data for smoking (n = 1) status and type of KRAS mutation (n = 1); p.(G12C) and p.(G12V) (G>T), p.(G12A) (G>C), p.(G12D) (G>A).

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non-COPD as compared to COPD lung cancer patients, after correcting for sex and smoking. We found significantly higher mean age in the COPD group as compared to the non-COPD group. This finding is consistent with the fact that the COPD prevalence increases with age [29].

KRAS mutations were identified in 32% of the NSCLC patients, which mainly included adenocarcinoma patients. We observed a relationship between presence of KRAS hotspot mutations and smoking status consistent with previous studies [25, 30], but not with

### Table 5. Distribution of different KRAS nucleotide changes in advanced NSCLC patients.*

| Characteristics | Transversions G>T, G>C (%) | Transitions G>A (%) | Other (%) | Pearson Chi-Square* |
|-----------------|-----------------------------|---------------------|-----------|---------------------|
| **Sex**         |                             |                     |           |                     |
| Female          | 56 (86.2)                   | 7 (10.8)            | 2 (3.1)   | 0.008               |
| Male            | 24 (61.5)                   | 14 (35.9)           | 1 (2.6)   |                     |
| **Histology**   |                             |                     |           |                     |
| Adenocarcinoma  | 75 (78.1)                   | 18 (18.8)           | 3 (3.1)   | 0.41                |
| NSCLC NOS       | 5 (62.5)                    | 3 (37.5)            | 0         |                     |
| **Smoking**     |                             |                     |           |                     |
| Current or past smoker | 77 (77)                   | 20 (20)             | 3 (3)     | 0.83                |
| Nonsmoker       | 2 (66.7)                    | 1 (33.3)            | 0         |                     |
| **COPD**        |                             |                     |           |                     |
| Yes             | 50 (75.8)                   | 14 (21.2)           | 2 (3)     | 0.93                |
| No              | 30 (78.9)                   | 7 (18.4)            | 1 (2.6)   |                     |

* Missing data for smoking (n = 1) status and type of KRAS mutation (n = 1).

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### Table 6. Distribution of different EGFR mutations in advanced NSCLC patients.

| Characteristics | EGFR mutations | Pearson Chi-Square |
|-----------------|----------------|-------------------|
|                 | Exon 19del (%) | p.(L858R) (%)     | Other (%) |                     |
| **Sex**         | Exon 19del (%) | p.(L858R) (%)     | Other (%) |                     |
| Female          | 11 (61.1)      | 4 (22.2)          | 3 (16.7)  | 0.69                |
| Male            | 5 (45.5)       | 3 (27.3)          | 3 (27.3)  |                     |
| **Histology**   | Exon 19del (%) | p.(L858R) (%)     | Other (%) |                     |
| Adenocarcinoma  | 16 (57.1)      | 6 (21.4)          | 6 (21.4)  | 0.20                |
| NSCLC NOS       | 0              | 1 (100)           | 0         |                     |
| **Smoking**     | Exon 19del (%) | p.(L858R) (%)     | Other (%) |                     |
| Current or past smoker | 7 (36.8)       | 6 (31.6)          | 6 (31.6)  | 0.02                |
| Nonsmoker       | 9 (90)         | 1 (10)            | 0         |                     |
| **COPD**        | Exon 19del (%) | p.(L858R) (%)     | Other (%) |                     |
| Yes             | 5 (55.6)       | 3 (33.3)          | 1 (11.1)  | 0.60                |
| No              | 11 (55)        | 4 (20)            | 5 (25)    |                     |

* One patient had an exon 19del and p.(T790M),
† One patient had a p.(L858R) and p.(T790M),
‡ One patient had a p.(G719S) and p.(S768I) and another patient had a p.(G719C) and an exon 20 insertion.

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COPD. In other studies no relation between smoking and the presence of KRAS mutations have been observed in lung cancer patients [25, 30–32]. These differences may be caused by differences in selecting study groups, ethnicity, number of patients and smoking status. The lack of an association with COPD is in concordance with the results reported in a recent study [33].

Although smoking females were younger and lighter smokers based on pack years than the males, we noticed that KRAS mutations were more common in smoking females than in smoking males with NSCLC. This supports an increased susceptibility of females to cigarette carcinogens as reported previously [34]. Moreover, these results are also consistent with a previous study showing that females had a higher OR for lung cancer at every level of tobacco exposure [35]. This elevated vulnerability to smoking may be caused by the higher expression levels of genes encoding tobacco carcinogen-metabolizing enzymes, such as CYP1A1 and CYP1B1, in normal lung tissue of female smokers in comparison to male smokers [36]. Uppstad and colleagues [37] also showed higher expression of CYP1A1 in cell lines derived from lung adenocarcinoma of female compared to cell lines derived from adenocarcinomas of male patients.

Although we observed the smoking related p.(G12C) KRAS mutation at the same frequency in both genders, smoking related transversions, i.e. G>T and G>C, were significantly more common in females than in males. In a previous study with a sample size of over 2,500 patients, the c.34G>T; p.(G12C) KRAS mutation occurred more frequent in females and current or past smokers, while the c.35G>A; p.(G12D) KRAS mutations were more frequent in never smokers [20]. This suggests again that females are more susceptible of cigarette smoke related KRAS mutations compared to males.

We showed that EGFR activating mutations were more common in females, non-smokers and in non-COPD NSCLC patients. In a recent study, EGFR mutations were seen in 12.8% (51/399) of lung cancer patients without COPD and in 6.3% (7/111) of patients with COPD [38]. Suzuki and colleagues [39] identified EGFR mutations in 32% (56/177) of the non-COPD and in 8% (4/52) of the COPD NSCLC patients. Lim and colleagues [33] found EGFR mutations in 37.3% (91/244) of non-COPD and in 16% (17/106) of COPD patients. They also found an inverse association between the presence of EGFR mutation with severity of airflow obstruction. The finding that EGFR mutations are more common in non-COPD lung cancer patients might indicate that lung cancer development is dependent on activating EGFR mutations in non-COPD patients.

Chronic pulmonary diseases, such as severe asthma and COPD, cause an increased activation of the epithelial growth factor receptor (EGFR) [12, 40]. Moreover, COPD is characterized by epithelial inflammatory reactions and many pro-inflammatory chemokines and growth factors are induced by transcription factor Nuclear Factor kB (NFkB). This transcription factor can be activated via physical and chemical stress such as smoke [41]. In addition, increased activation of EGFR by oxidative stress, which is involved in pathogenesis of COPD, or cigarette smoke can occur in human bronchial epithelial cells [14–15, 42]. All together suggesting that EGFR activation in COPD is induced by smoking, oxidative stress and subsequently by inflammation probably via NFkB.

In conclusion, KRAS mutations were more common in females and smokers, but are not associated with COPD-status in NSCLC patients. EGFR mutations are more common in females and non-smoking NSCLC patients.

Supporting Information

S1 Table. Patients data used for statistical analysis.

(XLSX)
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Author Contributions
Conceived and designed the experiments: HJMG AvdB. Performed the experiments: AS TJNH GSMAK. Analyzed the data: TJNH AS GSMAK MvdB. Contributed reagents/materials/analysis tools: AS AJvdW GSMAK MvdB WT ES AtE AvdB TJNH HJMG. Wrote the paper: AS AJvdW GSMAK MvdB WT ES AtE AvdB TJNH HJMG.

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