

Epidermal Growth Factor Receptor Mutations in 510 Finnish Non–Small-Cell Lung Cancer Patients

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Introduction: Among the driver gene mutations in non–small-cell lung cancer, mutations in epidermal growth factor receptor (EGFR) are the most important because of their predictive role in selecting patients eligible for targeted therapy. Our aim was to study EGFR mutations in a Finnish non–small-cell lung cancer cohort of 528 patients.

Methods: Mutation testing was conducted on DNA extracted from paraffin-embedded, formalin-fixed tumor material using the following real-time polymerase chain reaction-based kits: Therascreen EGFR PCR Kit and cobas EGFR Mutation Test.

Results: EGFR mutation frequency was 11.4% and all positive cases were adenocarcinomas, of which a majority had an acinar predominant pattern. Mutations were seen significantly more often in females and never-smokers than in males and smokers. The most frequent mutations were L858R in exon 21 and deletions in exon 19. Overall survival of the patients, not treated with EGFR inhibitor, did not differ between EGFR mutation-positive and EGFR mutation-negative patients.

Conclusion: EGFR mutation profile in this Finnish non–small-cell lung cancer cohort resembles in many respect with that of other Western European cohorts, even though the overall frequency of mutations is slightly higher. We show the occurrence of EGFR mutations in patients with occupational asbestos exposure and also in those diagnosed with chronic obstructive pulmonary disease who have not been often investigated before.

Key Words: EGFR, Mutations, Frequency, Lung adenocarcinoma, Non–small-cell lung cancer.

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Among the driver gene mutations in non–small-cell lung cancer (NSCLC), mutations in epidermal growth factor

receptor (EGFR) are the most important because of their predictive role in selecting patients eligible for targeted therapy.¹ EGFR mutation frequency and histologic subtype distribution in Finnish NSCLC patients have not been studied earlier. Because of the special heritage history of the Finnish population and the influence of ethnic background on the incidence of EGFR mutations, it is reasonable to study the Finnish population separately. The aim of our study was to examine EGFR mutation frequency in a cohort of 528 consecutive Finnish Caucasian NSCLC patients by using real-time polymerase chain reaction performed on DNA extracted from formalin-fixed, paraffin-embedded tumor material.

MATERIALS AND METHODS

Patients

In total, we collected 613 formalin-fixed, paraffin-embedded specimens, including 610 tumor and 3 pleural effusion specimens, of NSCLC patients treated at the Hospital District of Helsinki and Uusimaa (HUS), Finland, during 2006 to 2012 (primary diagnosis for three patients in 2004). Tumor and pleural effusion specimens were collected upon diagnosis or the surgical operation. In total, 528 specimens were eligible to be tested for EGFR mutation status. Tumor cell content of the specimens ranged from 2 to 98%; in 87% of the samples, tumor cell content was at least 20%. Of the patients with a test obtained, 53% were male, 77% had been diagnosed with adenocarcinoma (ADC), 12% with squamous cell carcinoma, 8% with large cell carcinoma, and 3% had other subtype or not otherwise specified type of NSCLC. The other subtype/not otherwise specified included 10 patients diagnosed with adenosquamous carcinoma, four patients with not otherwise specified NSCLC, and three patients with sarcomatoid carcinoma. Histologic diagnosis was based on pathologist's evaluation according to the World Health Organization criteria. EGFR-positive cases were subtyped according to the updated International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification.² Clinicopathologic characteristics of the patients are presented in Table 1.

DNA Extraction and Mutation Detection

DNA from 496 formalin-fixed, paraffin-embedded tumor tissue specimens of NSCLC patients was extracted using

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QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) as described earlier.³ Tumor DNA samples of 528 NSCLC patients were tested for EGFR mutations using the Therascreen EGFR PCR Kit (Qiagen, Manchester, United Kingdom) according to the manufacturer's protocol, on the ABI7500 platform or the cobas EGFR Mutation Test (Roche Molecular Systems, South Branchburg, NJ; 28 patients) according to the manufacturer's protocol, on the cobas z480 platform.

RESULTS

The total frequency of EGFR mutations was 11.4% (58 of 510). The mean age of patients with EGFR mutation was higher (69.2 ± 6.9 years) compared with patients with wild-type EGFR (65.2 ± 8.8 years, $p = 0.001$). EGFR mutations occurred more often in women (16.6%, 40 of 241) than in men (6.7%, 18 of 269, $p < 0.001$) and in never-smokers (32.8%, 22 of 67) than in ever-smokers (8.2%, 36 of 438, $p < 0.001$). A group of ever-smokers was divided into the following subgroups: light ex-smokers (smoking <20 years, cessation >10 years ago), medium ex-smokers (smoking >20 years, ceased), and current smokers (current smokers, smoking >20 years). Of the EGFR-mutated patients, 38% (22 of 58) were never-smokers, 19% (11 of 58) light ex-smokers,

24% (14 of 58) medium ex-smokers, and 19% (11 of 58) current smokers.

All mutations occurred in patients diagnosed with ADC. Vast majorities, 98%, of EGFR mutation-positive ADCs were invasive ADCs (53 of 54 with sufficient material for reliable reclassification) and one (2%) was diagnosed with nonmucinous minimally invasive ADC. Of the 54 EGFR-positive ADCs, 40 (74%) were acinar predominant, of which 18 were of the mixed acinar type: 10 with papillary (one of these had also micropapillary pattern and one all four patterns), four with lepidic, three with solid pattern, and one with nonmucinous minimally invasive ADC. The other EGFR-positive subtypes of invasive ADCs were lepidic (9%, five of 54), solid (7%, four of 54), micropapillary (4%, two of 54), and papillary predominant (4%, two of 54).

The most frequent mutations were amino acid change L858R in exon 21 and deletions in exon 19, representing 41.4% (24 of 58) and 36.2% (21 of 58) of all mutations, respectively. Other mutations consisted of G719X (8.6%, five of 58), L861Q (3.4%, two of 58), S768I (1.7%, one of 58), and insertions in exon 20 (1.7%, one of 58), together representing 15.5% of all EGFR mutations. Also four double EGFR mutants (6.9%) were detected: two patients with

TABLE 1. Clinicopathologic Characteristics of the Patients Studied

	Total, N (%)*	Tested, N (%)*	Result obtained, N (%)*	EGFR wt, N (%)†	EGFR+, N (%)†	p Value
Total	613	528	510	452	58	
Histology						
ADC	460 (75.0)	411 (77.8)	398 (78.0)	340 (85.4)	58 (14.6)	<0.001
SCC	77 (12.6)	60 (11.4)	60 (11.8)	60 (100)	0	
LCC	43 (7.0)	40 (7.6)	38 (7.5)	38 (100)	0	
NSCLC NOS	33 (5.4)	17 (3.2)	14 (2.7)	14 (100)	0	
Gender						
Male	339 (55.3)	279 (52.8)	269 (52.7)	251 (93.3)	18 (6.7)	<0.001
Female	274 (44.7)	249 (47.2)	241 (47.3)	201 (83.4)	40 (16.6)	
Age, years						
Mean	65.9	65.7	65.7	65.2	69.2	
Range	26–90	26–90	26–90	26–90	55–85	
Smoking						
Never	79 (12.9)	69 (13.1)	67 (13.1)	45 (67.2)	22 (32.8)	<0.001
Light	53 (86.5)	51 (9.7)	51 (10.0)	40 (78.4)	11 (21.6)	
Medium	211 (34.4)	178 (33.7)	173 (33.9)	159 (91.9)	14 (8.1)	
Current	264 (43.1)	225 (42.6)	214 (42.0)	203 (94.9)	11 (5.1)	
Data missing	6	5	5	5	0	
Asbestos						
Exposed	57 (9.3)	48 (9.1)	46 (9.0)	41 (89.1)	5 (10.9)	0.498
Non-exposed	261 (42.6)	226 (42.8)	219 (43.0)	198 (90.4)	21 (9.6)	
No sure information	295 (48.1)	254 (48.1)	245 (48.0)	213 (86.9)	32 (13.1)	
COPD						
Yes	125 (20.4)	113 (21.4)	111 (21.8)	104 (93.7)	7 (6.3)	0.057
No	488 (79.6)	415 (78.6)	399 (78.2)	348 (87.2)	51 (12.8)	

*Proportions calculated from all patients in the group (column) in question.

†Proportions calculated from a total number of variable group (row) in question.

ADC, adenocarcinoma; COPD, chronic obstructive pulmonary disease; LCC, large cell carcinoma; NSCLC NOS, non-small-cell lung cancer.

TABLE 2. Non-Small-Cell Lung Cancer Patients Tested for EGFR Mutations: the Comparison Between Other EGFR Mutation Studies

Study design, patient selection	Caucasian Finnish Patients (Present Study)		Central European, Caucasian Patients ⁶		Norwegian Patients ⁴		West European Dutch Patients ⁵		Spanish Patients ¹³		Korean Patients ¹⁴		Chinese Patients ¹⁵	
	Retrospective, mostly operable patients		Prospective, unselected patients		Operable patients		Patients selected for routine diagnostics		Prospective, unselected patients		Operable patients		Operable patients	
Method	RT-PCR Kit (TheraScreen and cobas)		PCR + sequencing (Pyrosequencing PyroMark)		RT-PCR Kit (TheraScreen) and dHPLC + sequencing		PCR + sequencing/high resolution melting analysis		PCR + 5' nuclease PCR assay (TaqMan), validation by sequencing		PCR + sequencing		RT-PCR	
Number of patients	510		552		240		778		2105		115		208	
ADC	398 (78.0%)		254 (46.0%)		141 (58.8%)		620 (79.7%)		1781 (84.6%)		55 (47.8%)		95 (46.7%)	
SCC	60 (11.8%)		186 (33.7%)		63 (26.3%)		41 (5.3%)		814 (38.7%)		60 (51.2%)		96 (46.2%)	
LCC	38 (7.5%)		79 (14.3%)		23 (9.6%)				287 (13.6%)					
Other NSCLC	14 (2.7%)		33 (6.0%)		12 (5.0%)		117 (15.0%) (inc. LCC)		(37 N/A data)				17 (8.2%)	
SCLC					1 (0.4%)									
Male	269 (52.7%)		352 (63.8%)		124 (51.7%)		421 (54.1%)		1287 (61.1%)		87 (75.7%)		147 (70.8%)	
Female	241 (47.3%)		200 (36.2%)		116 (48.3%)		357 (45.9%)		814 (38.7%)		28 (24.3%)		61 (29.2%)	
Ever-smokers	438 (85.8%) (5 missing data)				225 (93.8%)		259/288 (90.0%)		1382 (65.7%) (111 N/A data)		92 (80%)		130 (62.5%)	
EGFR + from all studied	58 (11.4%)		27 (4.9%)		18 (7.5%)		71 (9.1%)		350 (16.6%)		20 (17.4%)		51 (24.5%)	
L858R from EGFR+ cases	24 (41.4%)		8 (29.7%)		5 (27.8%)		21 (28.4%)				10 (50.0%)		22 (43.1%)	
Exon 19 del from EGFR+ cases	21 (36.2%)		15 (55.6%) [‡]		8 (44.4%)		39 (52.7%)				9 (45.0%)		20 (39.2%)	
Other mutations from EGFR+ cases	9 (15.5%) [†]		3 (11.1%)		5 (27.8%)		11 (14.7%)				1 (5.0%)		3 (5.9%)	
Compound mutations from EGFR+ cases	4 (6.9%); G719X + Ins [n = 2]; G719X + S768I; L858R + S768I				1 (5.5%); E709A + G719C		3 (4.1%); T790M + del19; T790M + L858R [n = 2]						6 (11.8%); P843L + L858R; L861Q + G719A; G724S + P769L; L858R + S768I; L833P + H835L; del19 + A750P	
EGFR+/women	40 of 241 (16.6%)*		17 of 200 (8.5%)		14 of 116 (12.0%)		48 of 357 (13.4%)		244 of 814 (30.0%)		13 of 24 (54.2%) from ADC subtype		29 of 61 (47.5%)	
EGFR+/men	18 of 269 (6.7%)		10 of 352 (2.8%)		4 of 124 (3.2%)		23 of 421 (5.5%)		106 of 1287 (8.2%)		7 of 31 (22.6%) from ADC subtype		22 of 147 (15.0%)	
EGFR+/ADC	58 of 398 (14.6%)*		22 of 254 (8.7%)		16 of 141 (10%)		66 of 620 (10.6%)		317 of 1781 (17.8%)		20 of 55 (36.4%)		42 of 95 (44.2%)	
EGFR+/non-ADC	0 of 112 (0%)		5 of 298 (1.7%); 2 SCC; 2 LCC; 2 ADSQ		2 of 99 (2.0%); 2 SCC		5 of 158 (3.2%); 3 LCC; 1 ADSQ; 1 SC		33 of 324 (10.2%)		0 of 60 (0%)		9 of 113 (8.0%); 3 ADSQ; 5 SCC; 1 LCC	

(Continued)

TABLE 2. (Continued)

	Caucasian Finnish Patients (Present Study)	Central European, Caucasian Patients ⁶	Norwegian Patients ⁴	West European Dutch Patients ⁵	Spanish Patients ¹³	Korean Patients ¹⁴	Chinese Patients ¹⁵
EGFR+/ever-smokers	36 of 438 (8.2%); LS 11 of 51 (21.6%) MS 14 of 173 (8.1%) CS 11 of 214 (5.1%); 5 N/A data)	9 of 27 (33%) from EGFR+ tumors	10 of 225 (4.4%) FS 8 of 83 (9.6%) CS 2 of 142 (1.4%)	17 of 259 (6.6%) FS 10 (8.5%) CS 7 (4.9%)	FS 91 (9.5%) CS 25 (5.8%)	7 of 33 (21.2%) from ADC subtype	18 of 130 (13.9%)
EGFR+/never-smokers	22 of 67 (32.8%); 5 N/A data)*	18 of 27 (67%) from EGFR+ tumors	8 of 15 (53.3%)	14 of 29 (48.3%)	231 (37.7%)	13 of 22 (59.0%) from ADC subtype	33 of 78 (42.3%)
EGFR+/asbestos exposed	5 of 46 (10.9%)						
EGFR+/non-exposed	21 of 219 (9.6%)						
EGFR+/COPD	7 of 111 (6.3%)						
EGFR+/no COPD	51 of 399 (12.8%)						

*Statistically significant.

†Mutations detected in our study: G719X (n = 5); L861Q (n = 2); insertion (n = 1); S768I (n = 1).

‡Included 10 delE746-A750 and five other mutations in exon 19.

ADC, adenocarcinoma; ADSQ, adenosquamous carcinoma; CS, current smoker; COPD, chronic obstructive pulmonary disease; FS, former-smoker; LCC, large cell carcinoma; MS, medium smoker; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; NS, never-smoker; PCR, polymerase chain reaction; SC, sarcomatoid carcinoma; SCC, small cell lung cancer.

G719X and an insertion in exon 20, one patient with G719X and S768I, and one patient with L858R and S768I. Results are presented in Table 2.

EGFR mutations were found in 10.9% (five of 46, $p = 0.498$) of those patients who had been exposed to asbestos. These patients had a history of occupational exposure to asbestos, but no diagnosis of pleural plaques or asbestosis or any other asbestos-related disease. In total, 6.3% (seven of 111) of those NSCLC patients diagnosed with chronic obstructive pulmonary disease (COPD) harbored EGFR mutation. EGFR mutations occurred less frequently, but not significantly so, in patients with COPD ($p = 0.057$).

There was no statistical difference in overall survival between ADC patients with wild-type EGFR and ADC patients with mutated EGFR (not treated with EGFR-TKI; Fig. 1). Similarly, no statistical differences were seen in OS between EGFR mutation-positive and EGFR mutation-negative patients when only ADC, nonsmoker patients were included in the analyses (Table 3). Overall survival data for patients tested for EGFR mutations and treated with EGFR TKIs (gefitinib or erlotinib) are presented in Supplementary Table 1 (Supplemental Digital Content, <http://links.lww.com/JTO/A540>).

DISCUSSION

In this study, EGFR mutations were detected in 11.4% of all NSCLC patients. The frequency is slightly higher than previously reported in Northern/Western Europeans.⁴⁻⁶ The small difference might be explained by the high proportion of ADC patients in our cohort and the testing method used. As in many other previous studies, EGFR mutations detected in this study were limited to the ADC subtype. A majority, 74 %, of the tumors had predominant acinar subtype. Our findings are supported by a number of studies.⁷ In our study, EGFR mutations were most frequent in never-smokers, as expected. Notable is, however, that the frequency was very similar in all smokers regardless of current (heavy) or light smoking history. In contrast, others have shown that EGFR mutations reduce in frequency with increasing cigarette consumption.⁸ Our results suggest that EGFR mutations also occur in heavy-smokers and that smoking status should not be considered as a strong indicator for EGFR testing.

EGFR mutations type and their distribution were very similar to that reported previously.¹ The most common mutations were deletions in exon 19 and mutation L858R in exon 21 which represented 77.6% (45 of 58) of all the mutations. In recent studies on European patients, the frequencies vary from 72.2%⁶ to 85.1%.⁴

We found EGFR double mutations in 0.8% (four of 510) of the patients. Characteristics of the patients which harbored compound mutations are presented in Table 4. G719A + S768I and L858R + S768I have also been detected previously.⁹ The proportion of compound EGFR mutations has been quite low (Table 2), but frequencies of up to 9%¹⁰ have been found in studies on Asian NSCLC patients.

Of the patients whose occupational asbestos exposure was established based on patient interviews, EGFR

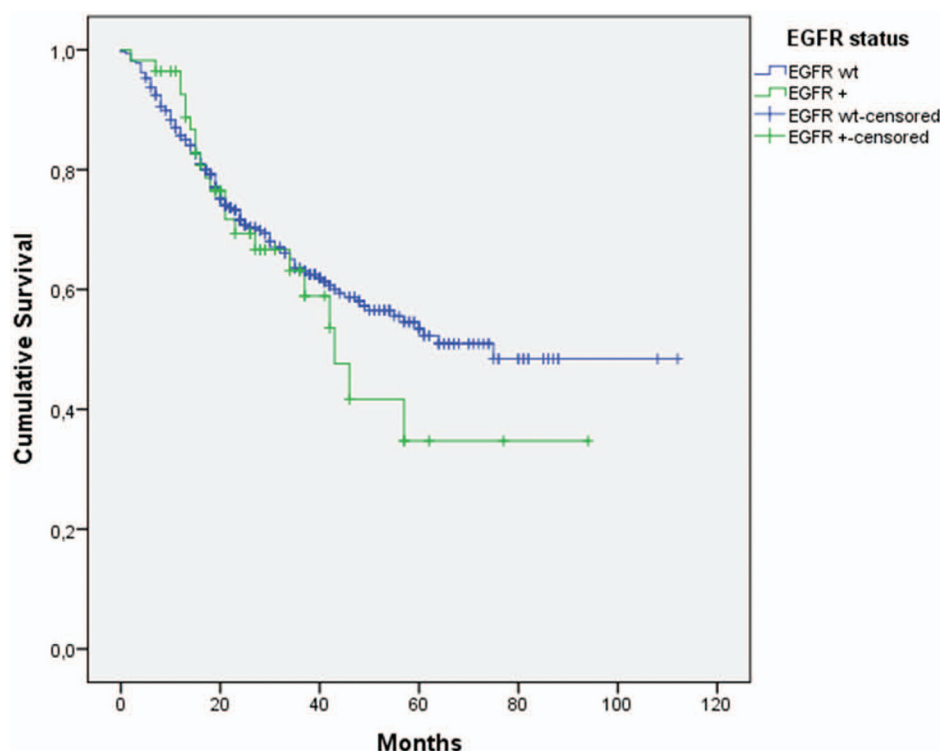


FIGURE 1. Kaplan-Meier curve for all lung adenocarcinoma patients with known EGFR status and not treated with TK inhibitors. Mean overall survival for wt EGFR patients was 68.6 ± 3.1 (95% confidence interval 62.6–74.7) and EGFR+ patients 52.2 ± 6.1 (95% confidence interval 40.2–64.2) months, $p = 0.460$.

mutation was found in 11% (five of 46). The patients with EGFR mutation and a history of asbestos exposure were diagnosed with ADC: three with acinar, one with lepidic, and one with solid predominant pattern. These patients were not diagnosed with asbestos-related diseases, that is asbestosis or pleural plaques. All five patients were men and also had a history of smoking. Further large scale studies are needed to study an influence of asbestos exposure on EGFR mutations.

We found EGFR mutations in seven patients diagnosed with COPD. All of them were current smokers. Predominant

growth patterns were acinar in five, lepidic in one, and solid in one case. However, mutations occurred more often in patients without COPD (Tables 1 and 2).

In this study, no difference in the survival rates between EGFR-negative and EGFR-positive patients was seen in patients not treated with EGFR TKIs. The prognostic value of EGFR mutation status remains debatable: many studies have shown a difference in survival between EGFR mutation-positive versus EGFR mutation-negative patients,¹¹ whereas many other studies have not seen a difference.¹² Similarly, a prognostic role for smoking history among EGFR mutants is somewhat contradictory, although in many of the studies never-smokers and/or light-smokers have been shown to have a better prognosis compared with ever-smokers and/or heavy-smokers.¹¹

In conclusion, EGFR mutation frequency observed in our Finnish NSCLC patient cohort is slightly higher than that reported in recent studies on patients of European origin. This may most likely be because of the relatively high proportion of ADC subtype in our patient cohort, although the type of mutation test used may also affect the results. Statistical significance was observed for differences in EGFR mutation occurrence in ADC subtype, women, and never-smokers compared with other subtypes, men, and smokers. Many of the mutations occurred in the acinar predominant pattern of ADC. Overall, our EGFR mutation testing results are concordant with the results reported from other European populations.

TABLE 3. Overall Survival Estimates by Kaplan-Meier Analysis and p Value of Log Rank Test: No Statistical Difference Was Seen

	Mean OS \pm sem (95% confidence interval)	p Value
ADC cases		
EGFR+	52.2 ± 6.1 (40.2–64.2)	0.460
EGFR–	68.6 ± 3.1 (62.6–74.7)	
Never-smokers	53.3 ± 5.1 (43.3–63.2)	0.838
Ever-smokers	70.7 ± 3.2 (64.5–76.9)	
EGFR+ cases		
Never-smokers	52.2 ± 9.3 (34.0–70.4)	0.891
Ever-smokers	46.9 ± 6.1 (34.9–58.9)	

ADC, adenocarcinoma; OS, overall survival.

TABLE 4. Characteristics of the Non–Small-Cell Lung Cancer Patients with Compound EGFR Mutations

Mutation	Gender	Age	Predominant Subtype	Survival (m)	Metastasis or Recurrence	Smoking Status	COPD	Asbestos Exposure
G719X, Ins 20	M	63	Acinar	13	Yes	CS	No	Yes
G719X, S768I	M	71	Solid	14	Yes	MS	No	Yes
L858R, S768I	M	79	Acinar + lepidic	26+	No	NS	No	N/A
G719X, Ins 20	F	71	MIA	46+	No	NS	No	N/A

COPD, chronic obstructive pulmonary disease; CS, current smoker; MIA, minimally invasive adenocarcinoma; MS, medium smoker; NS, never-smoker.

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