Histological subtype and smoking status, but not gender, are associated with *epidermal growth factor receptor* mutations in non-small-cell lung cancer

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Abstract. Mutations in *epidermal growth factor receptor* (*EGFR*) commonly occur in non-small-cell lung cancer (NSCLC) patients characterized by female gender, never-smoker status and adenocarcinoma histology. The aim of this study was to determine whether gender is a confounding factor for *EGFR* mutations in NSCLC. To elucidate the confounding effect, Pearson's χ^2 test and logistic regression models were used to correlate these characteristics with *EGFR* mutations in 426 NSCLC patients treated at our institutes. Of those 426 NSCLC patients, 47% were females, 57% were non-smokers and 84% had adenocarcinomas. The multivariate logistic regression analysis demonstrated that never-smoker status [odds ratio (OR)=3.49, 95% confidence interval (CI): 1.99-6.13; P<0.001)] and adenocarcinoma (OR=9.43, 95% CI 3.62-24.56; P<0.001) were associated with *EGFR* mutations; however, gender was not (OR=1.25, 95% CI: 0.73-2.15; P=0.416). Furthermore, gender was not associated with *EGFR* mutation subtypes (OR=1.19, 95% CI: 0.56-2.50; P=0.650). The frequency of *EGFR* mutations among females and males was not different in non-smokers (64.8 vs. 55.8%, P=0.204) or ever-smokers (27.8 vs. 24.2%, P=0.775). Therefore, if the assessment for *EGFR* mutation status was limited to non-smoking females with adenocarcinoma, up to 40% of the patients harboring *EGFR* mutations would be precluded from the benefit of *EGFR* inhibitor therapy. Our results indicated that gender is a confounding factor for *EGFR* mutations in NSCLC and suggested that gender may not be associated with tumorigenesis in NSCLC-harboring *EGFR* mutations.

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Introduction

Smoking, the major risk factor for lung cancer, is associated with all major histological subtypes, particularly small-cell and squamous cell lung carcinoma. By contrast, adenocarcinoma is the predominant subtype of lung cancer encountered in never-smokers (1-3). Global statistics estimated that 15% of lung cancer cases in males and 53% in females are not associated with smoking. Furthermore, among the East Asian populations, 11-23% of male and 61-90% of female lung cancer patients are never-smokers (1-7).

In lung cancer patients from Asia-Pacific countries, mutations in the *epidermal growth factor receptor* (*EGFR*) are the most common genetic aberrations (8-12) and are significantly associated with a good response to treatment with *EGFR* tyrosine-kinase inhibitors (TKIs). First-line *EGFR*-TKI therapy with gefitinib or erlotinib (13-17) in patients with lung adenocarcinoma harboring *EGFR* mutations may yield a response rate of ~70% and achieve a longer progression-free survival (PFS) (8-11,17). *EGFR* mutations are commonly associated with female gender, adenocarcinoma histology, never-smoker status and Asian ethnicity (9,13,14,18-21). Therefore, these characteristics have been integrated into clinical practice to guide treatment selection for patients with advanced lung cancer (13,14,22-24).

However, these *EGFR* mutation-associated clinicopathological characteristics are probably mutually interactive. For

Table I. Association of clinicopathological characteristics of NSCLC patients with *EGFR* mutations.

Characteristics	Total (n=426)	*EGFR* mutations		P-value
		Present (n=197)	Absent (n=229)	
Age (years)				0.094
≥60	294 (69)	128 (44)	166 (56)	
<60	132 (31)	69 (52)	63 (48)	
Gender				<0.001
Male	226 (53)	74 (33)	152 (67)	
Female	200 (47)	123 (62)	77 (39)	
Smoking status				<0.001
Smokers	183 (43)	45 (25)	138 (75)	
Non-smokers	243 (57)	152 (63)	91 (37)	
Histology				<0.001
Adenocarcinoma	359 (84)	192 (53)	167 (47)	
Non-adenocarcinoma[a]	67 (16)	5 (7)	62 (93)	
Stage				0.842
Local and advanced	352 (83)	162 (46)	190 (54)	
Early	74 (17)	35 (47)	39 (53)	

[a]Includes squamous cell carcinoma, adenosquamous carcinoma and large-cell carcinoma. NSCLC, non-small cell carcinoma; *EGFR*, *epidermal growth factor receptor*. Parenthetical data represent percentage values.

example, in Asian countries, the majority of female lung cancer patients are never-smokers and have tumors of the adenocarcinoma subtype, which makes it difficult to determine whether gender, smoking history or histological subtype is the decisive factor for *EGFR* mutations. The identification of the decisive factor associated with *EGFR* mutations is essential for designing preventive strategies and therapeutic interventions and for the elucidation of the processes underlying tumorigenesis in lung cancer. Therefore, it is critical to determine whether these well-established clinicopathological characteristics associated with *EGFR* mutations in NSCLC patients are confounded with each other. Thus, we retrospectively analyzed data retrieved from our institutes to elucidate the association between *EGFR* mutations and clinicopathological characteristics.

Patients and methods

Patients and variables. We retrospectively analyzed the clinicopathological characteristics of NSCLC patients who had adequate tumor tissue for *EGFR* mutation analyses of exons 18-21, between January, 2006 and August, 2011. Variables including age, gender, histological subtype, smoking history, disease stage and the presence and location of *EGFR* mutations were collected and analyzed. A patient was classified as a non-smoker if he/she had never smoked or had smoked <100 cigarettes during their lifetime. By contrast, an ever-smoker was defined as one who had smoked >100 cigarettes over their lifetime. For histological classification, we grouped the histology as adenocarcinoma and non-adenocarcinoma. Among the non-adenocarcinomas, 32 were squamous cell carcinomas, 28 were NSCLCs not otherwise specified, 4 were adenosquamous carcinomas and 1 was a large-cell carcinoma.

This study was approved by the Joint Institutional Review Board of Taipei Medical University (Taipei, Taiwan). Informed patient consent was obtained.

EGFR mutation analysis. *EGFR* mutations were determined using either direct sequencing or other previously described methods (25,26).

Statistical analysis. The association between *EGFR* mutation status and age, gender, histological subtype, smoking status and disease stage were evaluated using the Pearson's χ^2 test or the Fisher's exact test when appropriate. Univariate (unadjusted) and multivariate logistic regression models (ULR and MLR, respectively) were used to delineate the effects of these established clinicopathological characteristics on *EGFR* mutations and the results were described as odds ratio (OR) with a 95% confidence interval (CI) and P-value. Furthermore, patients were stratified by smoking status to elucidate the association between *EGFR* mutations and the variables mentioned above. P<0.05 was considered to indicate a statistically significant difference. Data analyses were conducted using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics and EGFR mutation status. We analyzed the clinical characteristics and *EGFR* mutation status of 426 NSCLC patients (Table I). Of the 426 patients, 359 (84%) had adenocarcinoma, 67 (16%) had non-adenocarcinoma

Table II. Logistic regression analysis of clinicopathological characteristics as predictors of the presence of *EGFR* mutations.

	Univariate analysis			Multivariable analysis		
Predictor	OR	95% CI	P-value	OR	95% CI	P-value
Age (≥60 years)	0.70	0.47-1.06	0.095	0.84	0.53-1.33	0.452
Gender (female)	3.28	2.20-4.89	<0.001	1.25	0.73-2.15	0.416
Smoking (non-smokers)	5.12	3.35-7.84	<0.001	3.49	1.99-6.13	<0.001
Histology (adenocarcinoma)	14.26	5.60-36.30	<0.001	9.43	3.62-24.56	<0.001
Stage (local and advanced)	0.95	0.57-1.57	0.842	0.84	0.47-1.49	0.545

EGFR, *epidermal growth factor receptor*; OR, odds ratio; CI, confidence interval. Valid n=426.

Table III. Association of clinicopathological characteristics with *EGFR* L858R vs. del 19.

		EGFR mutation type		Univariate analysis	Multivariate analysis[a]		
Characteristics	Total (n=185)	del 19 (n=82)	L858R (n=103)	P-value	OR	95% CI	P-value
Age (years)				0.143			
≥60	119 (64)	48 (40)	71 (60)		1.52	0.82-2.80	0.184
<60	66 (36)	34 (52)	32 (48)		Reference		
Gender				0.642			
Male	71 (38)	33 (46)	38 (54)		Reference		
Female	114 (62)	49 (43)	65 (57)		1.19	0.56-2.50	0.650
Smoking status				0.863			
Smokers	44 (24)	20 (45)	24 (55)		Reference		
Non-smokers	141 (76)	62 (44)	79 (56)		0.89	0.37-2.11	0.789
Histology				0.172[b]			
Adenocarcinoma	180 (97)	78 (43)	102 (57)		4.89	0.52-45.98	0.165
Non-adenocarcinoma[c]	5 (3)	4 (80)	1 (20)		Reference		
Stage				0.918			
Local and advanced	154 (83)	68 (44)	86 (56)		1.13	0.51-2.49	0.760
Early	31 (17)	14 (45)	17 (55)		Reference		

[a]Valid n=185. [b]Fisher's exact test. [c]Includes squamous cell carcinoma, adenosquamous carcinoma and large-cell carcinoma. *EGFR*, *epidermal growth factor receptor*; OR, odds ratio; CI, confidence interval. Parenthetical data represent percentage values.

NSCLC, 47% were females, 57% were non-smokers and 43% were ever-smokers. Of the 226 male patients, 73% were ever-smokers, whereas only 8% of the 200 female patients were ever-smokers. In total, 197 (47%) of the 426 patients were found to harbor *EGFR* mutations. Using the χ^2 test, our results demonstrated that female gender, adenocarcinoma and non-smoking status were significantly associated with *EGFR* mutations in NSCLC (Table I). In detail, female patients had a higher *EGFR* mutation rate compared to males (62 vs. 33%, P<0.001) and non-smokers had a higher *EGFR* mutation rate compared to ever-smokers (63 vs. 25%, P<0.001). Patients with adenocarcinoma were more likely to harbor *EGFR* mutations compared to those with non-adenocarcinoma NSCLC (53 vs. 7%, P<0.001). In conclusion, our data were consistent with those reported by previous studies (9,13,14,18-21), demonstrating that gender, histological subtype and smoking are associated with *EGFR* mutations in a ULR analysis (Table II).

To eliminate the possible confounding effect, MLR analysis was used to evaluate the complex associations between these factors in the presence of *EGFR* mutations in NSCLC patients and demonstrated that gender was not an independent factor statistically associated with the presence of *EGFR* mutations in NSCLC patients (OR=1.25, P=0.416) (Table II). However, adenocarcinoma and never-smoker status were independently associated with the presence of *EGFR* mutations (OR=9.43, P<0.001 and OR=3.49, P<0.001, respectively).

EGFR mutation subtypes. In our study, 185 (93.9%) of the 197 *EGFR* mutations were either a deletion in exon 19 (del 19) (41.6%) or a single amino acid substitution in exon 21

Table IV. Association of clinicopathological characteristics with the presence of *EGFR* mutations in patients classified by smoking status.

	Smokers (n=183)			Non-smokers (n=243)		
Characteristics	*EGFR*$^+$ (n=45)	*EGFR*$^-$ (n=138)	P-value	*EGFR*$^+$ (n=152)	*EGFR*$^-$ (n=91)	P-value
Age (years)			0.337			0.611
≥60	31 (23)	105 (77)		97 (61)	61 (39)	
<60	14 (30)	33 (70)		55 (65)	30 (35)	
Gender			0.775[a]			0.204
Male	40 (24)	125 (76)		34 (56)	27 (44)	
Female	5 (28)	13 (72)		118 (65)	64 (35)	
Histology			<0.001			<0.001
Adenocarcinoma	42 (32)	90 (68)		150 (66)	77 (34)	
Non-adenocarcinoma[b]	3 (6)	48 (94)		2 (13)	14 (87)	
Stage			0.700			0.494
Local and advanced	35 (24)	111 (76)		127 (62)	79 (38)	
Early	10 (27)	27 (73)		25 (68)	12 (32)	

[a]Fisher's exact test. [b]Includes squamous cell carcinoma, adenosquamous carcinoma and large-cell carcinoma. *EGFR*, *epidermal growth factor receptor*; *EGFR*$^+$, presence of *EGFR* mutation; *EGFR*$^-$, absence of *EGFR* mutation. Parenthetical data represent percentage values.

Table V. Association of clinical characteristics with the presence of *EGFR* mutations in patients with adenocarcinoma.

		EGFR mutations		Univariate analysis	Multivariate analysis[a]		
Characteristics	Total (n=359)	Present (n=192)	Absent (n=167)	P-value	OR	95% CI	P-value
Age (years)				0.439			
≥60	242 (67)	126 (52)	116 (48)		0.92	0.57-1.47	0.731
<60	117 (33)	66 (56)	51 (44)		Reference		
Gender				<0.001			
Male	172 (48)	71 (41)	101 (59)		Reference		
Female	187 (52)	121 (65)	66 (35)		1.19	0.68-2.08	0.553
Smoking status				<0.001			
Smokers	132 (37)	42 (32)	90 (68)		Reference		
Non-smokers	227 (63)	150 (66)	77 (34)		3.77	2.10-6.77	<0.001
Stage				0.606			
Local and advanced	297 (83)	157 (53)	140 (47)		0.78	0.43-1.41	0.407
Early	62 (17)	35 (56)	27 (44)		Reference		

[a]Valid n=359. [b]Fisher's exact test. *EGFR*, *epidermal growth factor receptor*; OR, odds ratio; CI, confidence interval. Categorical parenthetical data represent percentage values.

(L858R, 52.3%). To elucidate whether the previously described clinicopathological factors were preferentially associated with specific *EGFR* subtypes, we performed logistic regression analyses in patients with L858R vs. those with del 19. We observed no preferential association of del 19 or L858R with any of the clinicopathological factors using either ULR or MLR analyses (Table III).

Patient stratification by smokimg status. To elucidate the complex effects of smoking and gender in the presence of *EGFR* mutations, we stratified all 426 NSCLC patients into non-smokers and ever-smokers (Table IV). Although 65% of the 182 non-smoking female patients were found to harbor *EGFR* mutations, which was a higher incidence compared to that observed among non-smoking male patients (56%), this

Table VI. Association of clinical characteristics with the presence of *EGFR* mutations in patients with lung adenocarcinoma stratified by smoking status.

	Smokers (n=132)			Non-smokers (n=227)		
Characteristics	*EGFR*$^+$ (n=42)	*EGFR*$^-$ (n=90)	P-value	*EGFR*$^+$ (n=150)	*EGFR*$^-$ (n=77)	P-value
Age (years)			0.925			0.739
≥60	30 (32)	65 (68)		96 (65)	51 (35)	
<60	12 (32)	25 (68)		54 (68)	26 (33)	
Gender			1.000[a]			0.377
Male	38 (32)	80 (68)		33 (61)	21 (39)	
Female	4 (29)	10 (71)		117 (68)	56 (32)	
Stage			0.417			0.642
Local and advanced	32 (30)	74 (70)		125 (65)	66 (35)	
Early	10 (38)	16 (62)		25 (69)	11 (31)	

[a]Fisher's exact test. *EGFR*, epidermal growth factor receptor; *EGFR*$^+$, presence of *EGFR* mutation; *EGFR*$^-$, absence of *EGFR* mutation. Parenthetical data represent percentage values.

Table VII. Patient populations tested and *EGFR* mutations precluded under different mutation testing strategies according to clinicopathological characteristics.

	Patients tested		*EGFR* mutations detected		*EGFR* mutations precluded	
Population tested	No.	%	No.	%	No.	%
Adenocarcinoma only	359	85	192	97.5	5	2.5
Non-smokers only	243	57	152	77.2	45	22.8
Females only	200	47	123	62.4	74	37.6
Non-smoking females	182	43	118	59.9	79	40.1
All patients	426	100	197	100	0	0

EGFR, epidermal growth factor receptor.

difference was not statistically significant (P=0.204). Among ever-smokers, *EGFR* mutations were detected in 24% of male and 28% of female patients, although this difference was also not statistically significant (P=0.775). However, adenocarcinoma histological subtype was significantly associated with *EGFR* mutations in ever- and non-smokers (32 vs. 6%, P<0.001 and 66 vs. 13%, P<0.001, respectively) compared to non-adenocarcinoma NSCLC.

To confirm that gender is a confounding factor associated with *EGFR* mutations, we analyzed the 359 patients with lung adenocarcinoma using an MLR model and determined that smoking, but not gender, was significantly associated with *EGFR* mutations in patients with lung adenocarcinoma (Tables V and VI).

EGFR mutation testing. It was suggested that *EGFR* mutation testing is routinely performed in patients with lung adenocarcinoma in most countries (27-29). However, a number of NSCLC patients harboring *EGFR* mutations, who would potentially benefit from *EGFR*-TKI treatment (gefitinib or erlotinib) may be precluded if the decision to perform *EGFR* mutation testing is based solely on histological subtype, gender and smoking status. Our analysis demonstrated that 2.5-40.1% of NSCLC patients harboring *EGFR* mutations may not be identified if testing is selectively performed based upon these clinicopathological phenotypes (Table VII). In particular, if never-smoking status, female gender or never-smoking female patients with adenocarcinoma were used as the selection criteria for *EGFR* mutation testing, 22.8, 37.6 and 40.1% of NSCLC patients with *EGFR* mutations, respectively, would be precluded from *EGFR*-TKI treatment.

Discussion

In this study, we investigated the different clinicopathological factors that are widely considered to be critically associated with *EGFR* mutations in NSCLC patients. We observed that, as opposed to the conclusions reported by previous studies,

gender is not independently associated with the frequency of *EGFR* mutations in NSCLC patients (Tables II, V and VI) or with *EGFR* mutation subtypes (Table III). Furthermore, the current guidance of routine *EGFR* mutation analysis in NSCLC patients was validated.

Our conclusion seems to be discordant with the results of the majority of earlier studies (5,9,12,15). The univariate analysis with Pearson's χ^2 test or Fisher's exact test, or the ULR model used in the present and the majority of previous studies (5,9,12,15), led to the conclusion that NSCLC patients characterized by female gender, never-smoking status and adenocarcinoma histology were more likely to harbor *EGFR* mutations (Table I). However, particularly in East Asian populations, the majority of female NSCLC patients have no history of smoking and their lung tumors are of the adenocarcinoma subtype, making the determination of the critical factors for *EGFR* mutations challenging. An MLR analysis may better elucidate the critical risk factors. In accordance with our results, supporting that gender is not associated with the frequency of *EGFR* mutations in NSCLC patients (Tables II and IV-VI), scattered pilot studies using MLR analyses to eliminate the confounding effects of these clinicopathological characteristics demonstrated that only adenocarcinoma and smoking history are significantly associated with *EGFR* mutations in NSCLC (30-32). Consistent with our findings (Tables V and VI), a recent study in the USA that enrolled 2,142 patients with lung adenocarcinoma revealed that the *EGFR* mutational frequency among female and male patients was not significantly different when patients were stratified into never- and ever-smokers (33). The results mentioned above collectively suggest that gender is not an independent factor associated with *EGFR* mutations in NSCLC patients.

Our findings may provide a rationale and clinical evidence to support the use of routine *EGFR* mutation testing prior to the administration of either *EGFR*-TKIs or chemotherapy as first-line treatment for lung adenocarcinoma, which is endorsed by the majority of available guidelines (27-29). Furthermore, our data demonstrated that 7% of non-adenocarcinoma NSCLC patients also harbor *EGFR* mutations. As shown in Table VII, up to 40.1% of NSCLC patients harboring *EGFR* mutations would be precluded from the significant benefit of *EGFR*-TKI treatment if mutational testing was restricted to female non-smokers with adenocarcinoma. Of equal importance, up to 32% of female non-smokers with adenocarcinoma (Table VII), who are commonly considered candidates for treatment with *EGFR*-TKIs, had no detectable *EGFR* mutations, indicating that first-line *EGFR*-TKI treatment would be harmful, rather than beneficial, to these patients. *EGFR*-TKI therapy in this population yielded meager response rates and a shorter PFS compared to those who received conventional platinum-based doublet chemotherapy (34), which may achieve a response rate of 23.5-31% (8,35).

Due to the significant differences in the percentage of *EGFR* mutations among East Asian and non-Asian NSCLC patients (~30 vs. 8%, respectively), ethnicity is considered to be significantly associated with *EGFR* mutation status (9,18). However, this observation may be confounded by clinicopathological parameters, such as histological subtype and smoking behavior. Our analysis (Table VIII) suggested that smoking status, but not ethnicity, is the major determinant for *EGFR* mutations in lung cancer among different populations. Additionally, the contribution from the cumulative smoking dose to the *EGFR* mutation frequency should also be considered. Recent studies demonstrated that the cumulative smoking dose is inversely correlated with the presence of *EGFR* mutations in lung adenocarcinoma (32,33), ranging from 4 to 34% in smokers with various numbers of smoking pack years (33). Therefore, the difference in the percentage of *EGFR* mutations among smokers (Table VI) may be attributed to the cumulative smoking dose.

Table VIII. Comparison of the presence of *EGFR* mutations in patients with adenocarcinoma stratified by smoking status.

	Ever-smokers		Non-smokers	
Study group	*EGFR*$^+$ (%)	*EGFR*$^-$ (%)	*EGFR*$^+$ (%)	*EGFR*$^-$ (%)
Current study (n=359)	31	69	66	34
USA study (n=2,142)[a]	13	87	52	48

[a]Data were derived from reference no. 33 with the permission of the American Society of Clinical Oncology. *EGFR*, *epidermal growth factor receptor*; *EGFR*$^+$, presence of *EGFR* mutation; *EGFR*$^-$, absence of *EGFR* mutation; USA, United States of America.

Another factor that may contribute to the differences in the incidence of *EGFR* mutations in never-smokers among different ethnic groups may be cumulative exposure to environmental tobacco smoke (ETS). Recently, household and workplace ETS, in particular, were reported to be inversely associated with the frequency of *EGFR* mutations in non-smokers with NSCLC (36). Specifically, the presence of *EGFR* mutations in lung adenocarcinoma was significantly different in never-smokers with and without ETS exposure (40.5 and 64.1%, respectively; P=0.015). Therefore, the difference in the percentage of *EGFR* mutations in either never- or ever-smokers among the two study groups (Table VIII) may correlate with the cumulative effect of direct or indirect exposure to smoking, suggesting that ethnicity may not be a crucial factor associated with *EGFR* mutations in NSCLC patients.

However, there were several limitations to this study. One limitation was the relatively small sample size, particularly the number of female smokers, which may affect our analyses. Since <10% of women are smokers and only 6.4% of female lung cancer patients are smokers in Taiwan (6), it is difficult to recruit an adequate number of female smokers in lung cancer studies. Furthermore, we were unable to present a comprehensive image on the *EGFR* mutation status of all the NSCLC patients, since not every patient had adequate tissue for mutation testing. Therefore, our findings require validation by further large-scale studies. The elucidation of the association between these factors and *EGFR* mutations may help identify factors that increase lung cancer susceptibility in the never-smoking population and design optimal preventive strategies.

In conclusion, this study demonstrated that gender is a confounding factor, whereas histological subtype and smoking status are independently and significantly associated with *EGFR* mutations in NSCLC patients. These findings support the current guidance that *EGFR* mutation testing should be routinely performed in patients with lung cancer of the adenocarcinoma subtype, regardless of their gender, and may indicate that gender may not be associated with tumorigenesis in NSCLC patients harboring *EGFR* mutations.

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