EGFR mutation status in a series of Turkish non-small cell lung cancer patients

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Abstract. Epidermal growth factor receptor *(EGFR)* mutations are potential markers driving carcinogenesis, and may alter the response to EGFR tyrosine kinase inhibitors in patients with non-small cell lung cancer (NSCLC). The frequency of *EGFR* mutations in patients with NSCLC differs according to sex, smoking habits and regional-based ethnicity differences. The aim of the present study was to determine the frequency of *EGFR* mutations in Turkish patients with NSCLC to highlight the importance of regional differences, and their associations with patient characteristics. Genomic DNA was extracted from formalin-fixed and paraffin-embedded tumor tissue sections of 409 NSCLC patients. The most common *EGFR* mutations in exons 18, 19, 20 and 21 were detected using BioFilmChip-based microarray assay. The overall *EGFR* mutation frequency was 16.6%, and the highest mutation frequencies were observed in exon 19 (6.4%) and exon 21 (7.3%). There was a higher frequency of *EGFR* mutations in females compared with males and in never-smokers compared with smokers (both $P \leq 0.05$). These results were similar to other European population-based studies, but not consistent Middle-Eastern based studies. The present study may contribute to understanding the gradient frequency of *EGFR* mutation across different ethnicities, and in designing genome wide-based collaborations that may reveal novel decision making and susceptibility mutations in *EGFR* in patients with NSCLC.

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Introduction

Lung cancer, the leading cause of cancer-associated death worldwide, is the most common type of cancer in both sexes (1). According to the latest Cancer Statistics Report published by the Turkey Ministry of Health in 2015, annual lung cancer rates for the Turkish population are 52.5 cases per 100,000 individuals in males, and 9.0 per 100,000 individuals in females (2).

Exposure to various environmental pollutants, such as smoking, radon, asbestos and air pollution, as well as family history and genetic background exert significant effects on the progression of lung cancer (3,4). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 85% of all lung cancer cases, and chemotherapy is the primary means of NSCLC treatment (5). In the past decade, the treatment protocols for patients with NSCLC have received important updates and additions (6). An increased understanding of the signaling pathways involved in NSCLC have shown that epidermal growth factor receptor (*EGFR*) mutations are potential markers driving carcinogenesis, advanced survival and response to definite EGFR tyrosine kinase inhibitors (7,8). EGFR is a transmembrane glycoprotein, and one of four members of the ERBB family of tyrosine kinase receptors. Auto-phosphorylation of receptor tyrosine kinase by EGFR initiates signaling pathways that regulate differentiation, metastasis, survival, angiogenesis and proliferation (8,9). There are several mechanisms for aberrant activation of EGFR such as mutations, overexpression, ligand-dependent receptor dimerization and/or independent activation (10). *EGFR* mutations, primarily located in exons 18, 19, 20 and 21, are widely present in patients with NSCLC, particularly in the adenocarcinoma subtype. The frequency of *EGFR* mutations in NSCLC differs based on sex, tobacco exposure and ethnicity. The frequency of *EGFR* mutations

in the Asian population is 40-60%, which is higher than the 10-30% reported for non-Asian (Caucasian) populations (11). There are extensive amounts of data regarding the frequency of *EGFR* mutations in Asian and Western patients with NSCLC; however, the data available from patients of other ethnicities is not sufficient to evaluate the frequency of *EGFR* mutations based on the ethnicity in patients with NSCLC (7,8,11,12).

Turkey has a multiethnic population due to its geographic location between Europe and Asia continents and its proximity to the Middle-East. The aim of the present study was to determine the frequency of *EGFR* mutation types in Turkish patients with NSCLC to highlight the importance of regional differences and their correlation with patient characteristics.

Materials and methods

Patients and tumor tissue samples. A total of 409 formalin-fixed and paraffin-embedded (FFPE) tumor tissue samples of NSCLC adenocarcinoma patients between November 2012 and November 2017, were included in this retrospective study. All patients were diagnosed, followed-up and *EGFR* mutation testing was performed in the Dokuz Eylul University Hospital. Tumor specimens were evaluated by an experienced pathologist to confirm the NSCLC histology and tumor cell content. The inclusion criteria of the present study were as follows: i) Newly diagnosed and pathologically confirmed non-small cell adenocarcinoma; ii) FFPE tissue section contained ≥75% tumor tissue; iii) there was sufficient tumor tissue sample for molecular testing; iv) the anti-tumor treatment did not begin before sample collection; and v) informed written consent was obtained from patients. Exclusion criteria were as follows: i) Tissue section contained <75% tumor tissue; ii) insufficient tumor tissue samples were excluded; iii) patients were receiving anti-tumor treatment at the time of sample collection; iv) cases with no informed consent. Based on these inclusion and exclusion criteria, 409 patients met the all criteria for inclusion. Tumor sections (10 μm) were sectioned from each FFPE tissue block containing at least 75% tumor tissue for genotyping. Patient demographic data were obtained from the hospital records. This data included the age, sex, smoking status, primary tumor location and metastatic status. The present study was based on pathological archived material and was approved by Dokuz Eylul University, Non-invasive Researches Ethics Committee (Izmir, Turkey) (approval no. 300-GOA, 2011/28-03). Written informed consent was obtained from all patients. The present study also conformed to the principles outlined in the Declaration of Helsinki (13).

Patient characteristics. Based on the Open Source Epidemiologic Statistics for Public Health analysis results, a total of 409 NSCLC adenocarcinoma patients were included in the present study, and the clinicopathological characteristics of these patients are described in Table I. Of these patients, 73.1% (n=299) were male, 26.9% (n=110) of the female. The median age of all the patients was 60 years (range, 23-89 year) and 58.9% of patients had a metastasis at the time of diagnosis. There were marginally more smokers among the patients (60.1%) and 60.6% of patients had tumors localized to the right lung.

The median age of the female patients was 57.7 years (23-89 years) and 53.6% of females were metastatic at the time of diagnosis. In total, 40% of female patients were smokers and 64.5% of female patients had right lung localized tumors. The median age of the male patients was 61 years (31-87 years) and 53.8% of males were metastatic at the time of diagnosis. In total, 67.9% of male patients were smokers and 59.2% of male patients had right lung localized tumors.

DNA extraction. Genomic DNA was extracted from 10-μm thick tumor tissue sections using the QIAamp DNA FFPE Tissue kit (Qiagen, Inc.) according to the manufacturer's protocol. A total of 3-5 sections were used depending on the size of the tumor tissue in the section. Tissues were deparaffinized in xylene, washed with absolute ethanol and air-dried. The lysis process was performed using proteinase K at 56˚C for 1 h. Genomic DNA with a concentration of at least 15 ng/μl was required for genotyping; the quality and quantity of the extracted DNA was determined using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Inc.) (14).

EGFR mutation analysis. The INFINITI *EGFR* assay (AutoGenomics, Inc.) was used to detect the most common *EGFR* mutations in exons 18, 19, 20 and 21 (Table II). The INFINITI method is a BioFilmChip-based microarray assay, and the system is designed to detect fluorescence signals of labeled DNA targets hybridized to the chip. This assay includes several processes: i) DNA extraction from tumor tissue, ii) PCR amplification, iii) specific primer extension with fluorescent labels, iv) hybridization to BioFilmChip, and iv) signal detection using the built-in microscope and results presentation. The 'Zip-code' bound to allele-specific primers can only be extended with fluorescent labels if the specific primer matches with the wild-type or mutated DNA strand. After elongation of the mutated sequences for *EGFR* exons 18, 19, 20 and 21, and also the wild-type DNA strand, all Zip codes were hybridized to the designated area (15,16). Briefly, genomic DNA (15-60 ng) was amplified by using PCR master mix (AutoGenomics, Inc.) in a total volume of 20 μl and purified by using shrimp alkaline phosphatase and exonuclease I treatment SAP/Exo (Affymetrix/USB Products Inc.) on a thermal cycler (BioRad Laboratories, Inc.). The thermocycling conditions used were: 94˚C for 2 min; 10 cycles of 94˚C for 15 sec and 67-57˚C for 15 sec (the temperature was decreased by 1˚C each cycle); followed by 30 cycles of 94˚C for 15 sec and 57˚C for 15 sec. The SAP/Exo procedure was performed as three steps: i) at 37˚C for 60 min; ii) at 94˚C for 20 min; and iii) holding at 4˚C. The samples were analyzed using INFINITI Analyzer (Autogenomics Inc.) according to the manufacturer's protocol. Fluorescently labeled nucleotides were incorporated into the targets via allele-specific primer elongation on the INFINITI® analyzer (Autogenomics Inc.) then samples were hybridized to BioFilm chip microarrays (AutoGenomics Inc.). Scanning, signal detection and analysis were performed using INFINITI Qmatic 6.6 software (Autogenomics Inc.). One microarray chip was used for each case.

Table I. Patient characteristics.

Clinicopathological characteristics	Total	Female	Male
Median age (range), years	60 (23-89)	57.7 (23-89)	61 (31-87)
Smoker, % (n)			
Yes	60.1 (246)	40 (44)	67.9 (203)
No	35.9 (163)	60 (66)	32.1 (96)
Tumor location			
Right lung	60.6 (248)	64.5 (71)	59.2 (177)
Left lung	39.4 (161)	35.5 (39)	40.8 (122)
Age, % (n), years			
≥50	82.2 (336)	77.3 (85)	83.9 (251)
<50	17.8 (73)	22.7 (25)	16.1 (48)
Metastases, % (n)			
Yes	58.9 (241)	53.6 (59)	53.8 (161)
No	41.1 (168)	46.4 (51)	46.2 (138)

Table II. Mutations scanned using the INFINITI analyzer.

Exon	Scanned *EGFR* mutations
Exon 18	Glu709 Ala; Glu709 Gln; Glu709 Gly; Glu709 Lys; Glu709 Val; Gly719 Ala; Gly719 Arg; Gly719 Cys; Gly719 Ser; Ser720 Phe
Exon 19	Lys739_Ile744dup; Lys745_Glu746del; Glu746_Ala750del; Glu746_Thr751delins; Glu746_Thr751delins; Glu746_Ala750delins; Glu746_Ala750del; Glu746 Lys; Glu746_Ala750delins; Glu746_Pro753delins; Glu746_Ser752del; Glu746_Ser752delins; Glu746_Thr751delins; Glu746_Thr751delins; Glu746_Ser752delins; Glu746_Thr751delins; Glu746_Ser752delins; Leu747_Glu749del; Leu747_ Thr751delins; Leu747_Ser752del; Leu747_Ser752delins; Leu747_Pro753delins; Glu746_Glu749del; Leu747_Glu749del; Glu746_Thr751del; Leu747_ Thr751del; Leu747_ Thr751delins; Leu747_ Lys754delins; Leu747_Pro753delins; Leu747_Ala750del; Leu747_ Thr751del; Arg748_Thr751delins; Glu749_Thr751del; Thr751_Ile759delins; Ser752_Ile759del
Exon 20	Glu762insEAFQ; Glu762ins; Val769Met; Val769Leu; Asp770ins; Ala767_Val769dup; Asp770fs; Pro772Arg; Ser768_Asp770dup; His773Arg; His773Leu; Asn771_His773dup; Val774Met; Arg776Cys; Gly779Phe; Thr790Met
Exon 21	Asn826Ser; His835Leu; Leu858Arg; Leu858Met; Leu858Arg; Leu861Gln; Leu861Arg

EGFR, epidermal growth factor receptor.

Statistical analysis. The framework of Cancer Statistics Report of Turkey Ministry of Health, 'Open Source Epidemiologic Statistics for Public Health' (Available from, openepi.com/SampleSize/SSPropor.htm) was used to determine the required case numbers to include in the present study (17).

R version 3.4.3 was used for all statistical analysis (18). Descriptive statistics in R was used to analyze non-categorical clinicopathological characteristics. The frequency of clinicopathological characteristics such as smoking status, location of tumor, age group, metastasis status, *EGFR* mutations in exons 18, 19, 20 and 21, characteristics of patients with *EGFR* mutations and detected *EGFR* mutations according to the exons were calculated in R using the table function. For calculating the percentage of categorical variants, the proportional table function results were multiplied by 100. A Pearson's χ^2 test was used to assess the association between mutation status and clinicopathological characteristics.

Table III. *EGFR* mutation frequency in exons 18, 19, 20 and 21.

Exon	Mutant, % (n)	Wild-type, % (n)
Exon 18	1.2 (5)	98.8 (404)
Exon 19	6.4 (26)	93.6 (383)
Exon 20	3.7 (15)	96.3 (394)
Exon 21	7.3 (30)	92.7 (379)
Multiple exon mutations	2 (8)	98 (401)
Overall	16.6 (68)	83.4 (341)

EGFR, epidermal growth factor receptor.

Table IV. Characteristics of patients with *EGFR* mutations.

Characteristics	Total	Exon 18	Exon 19	Exon 20	Exon 21
Median age (range), years	63 (39-89)	66 (53-76)	59 (39-77)	63 (48-78)	65 (51-78)
Smoker, % (n)					
Yes	47.1 (32)	20 (1)	26.8 (8)	86.7 (13)	50 (15)
No	52.9 (36)	80 (4)	69.2 (18)	13.3 (2)	50 (15)
Sex, % (n)					
Female	38.2 (26)	60 (3)	42.3 (11)	6.7 (1)	50 (15)
Male	61.8 (42)	40 (2)	57.7 (15)	93.3 (14)	50 (15)
Tumor location, % (n)					
Right lung	52.9 (36)	60 (3)	65.4 (17)	33.3 (5)	53.3 (16)
Left lung	47.1 (32)	40 (2)	34.6 (9)	66.7 (10)	46.7 (14)
Age, % (n)					
≥50	88.2 (60)	100 (5)	73.1 (19)	100 (15)	100 (30)
<50	11.8 (8)	0 (0)	26.9 (7)	0 (0)	0 (0)
Metastasis, % (n)					
Yes	51.5 (35)	60 (3)	50 (13)	40 (6)	56.7 (17)
No	48.5 (33)	40 (2)	50 (13)	60 (9)	43.3 (13)

EGFR, epidermal growth factor receptor.

P<0.05 was considered to indicate a statistically significant difference.

Results

In total, 68 patients were detected with an *EGFR* mutation among the 409 patients with NSCLC. The overall frequency of all *EGFR* mutations was 16.6% (Table III). The highest mutation frequencies were detected on exons 19 and 21. The frequency of *EGFR* exon 19 mutations was 6.4% and the exon 21 mutation frequency was 7.3%. The frequency of *EGFR* exon 18 mutations was 1.2% and for exon 20 it was 3.7%. In total 8 patients (2%) had multiple exon mutations; such as deletion of exon 18 and exon 19 mutations in the same patient (Table III). In these multiple exon mutations, exon 21 mutations were predominantly accompanied with other exon mutations. One patient had mutations in exon 18 and exon 19; three patients had mutations in exon 18 and exon 21; one patient had mutations in exon 19 and exon 21; and three patients had mutations in exon 20 and exon 21.

Table IV shows the characteristics of patients with *EGFR* mutations. In terms of smoking, 52.9% of all patients with mutations were non-smokers. The majority of exon 18 and 19 mutations were observed in non-smokers (80 and 69.2%, respectively), but exon 20 mutations were considerably more common in smokers (86.7%). Of the patients with mutations, 61.8% were male, exon 19 and 20 mutations were more commonly observed in male patients (57.7 and 93.3%, respectively); however exon 18 mutations were commonly observed in females (60%). The *EGFR* mutated NSCLC tumors were generally localized in the right lung (52.9%), and exons 18, 19 and 21 mutated tumors were more commonly localized in the right lung (60, 65.4 and 53.3%, respectively).

The presence of mutations were compared with the patient's characteristics. Table V shows the comparison of clinical characteristics of patients with *EGFR*-mutated tumors and *EGFR* wild-type tumors. A significant correlation was found between non-smoking patients and overall *EGFR* mutation presence (P=0.002) and between female patients and the overall presence of *EGFR* mutations (P=0.017). When the presence of exon 18 mutations was compared with patient characteristics, there was no correlation found with any of the other patient characteristics. For exon 19 mutations, *EGFR* mutations were found significantly more often in non-smoking patients compared with smokers (P=0.002). There was a significant correlation found between males and the presence of exon 19 mutations (P=0.047). Except for the primary tumor location, there were no other significant correlations found between exon 20 mutations and any other patient characteristics. Exon 20 mutations were significantly correlated with tumors localized in the left lung (P=0.031). Exon 21 mutations were correlated with sex and age. Female patients had a higher frequency of exon 21 mutations compared with males (P=0.004) and the mutation frequency of exon 21 was significantly higher in patients >50 years (P=0.011)

Different mutation types were observed during the genotyping process (Table VI). For exon 18 and exon 21, only point mutations were detected. For exon 19, deletions were the most common mutation type, but indels were detected in 2 patients. Nearly all mutation types were observed in exon 20, including point mutations, insertions, duplications as well as multiple different types of mutations. The specific mutations observed are listed in Table VI.

Table V. Comparison of EGFR mutation status with patient characteristics.

	Total			Exon 18			Exon 19			Exon 20			Exon 21		
Characteristics	Wild-type	Mutant	P-value	Wild-type	Mutant	P-value	Wild-type	Mutant	P-value	Wild-type	Mutant	P-value	Wild-type	Mutant	P-value
Smoker, % (n)			0.002[b]			0.081			0.002[b]			0.227			0.188
Yes	87.0	13.0		99.6	0.4		96.7	3.3		94.7	5.3		93.9	6.1	
	(214)	(32)		(245)	(1)		(238)	(8)		(233)	(13)		(231)	(15)	
No	77.9	22.1		97.5	2.5		89.0	11.0		98.8	1.2		90.8	9.2	
	(127)	(36)		(159)	(4)		(145)	(18)		(161)	(2)		(148)	(15)	
Sex, % (n)			0.017[a]			0.093			0.047[a]			0.071			0.004[b]
Female	76.4	23.6		97.3	2.7		90	10		99.7	0.3		86.4	13.6	
	(84)	(26)		(107)	(3)		(99)	(11)		(285)	(1)		(95)	(15)	
Male	86.0	14.0		99.3	0.7		95	5.0		88.6	11.4		95.0	5.0	
	(257)	(42)		(297)	(2)		(284)	(15)		(109)	(14)		(284)	(15)	
Tumor location, % (n)			0.213			0.996			0.681			0.031[a]			0.465
Right lung	85.5	14.5		98.8	1.2		93.1	6.9		98.0	2.0		93.5	6.5	
	(212)	(36)		(245)	(3)		(231)	(17)		(243)	(5)		(232)	(16)	
Left lung	80.1	19.9		98.8	1.2		94.4	5.6		93.8	6.2		91.3	8.7	
	(129)	(32)		(159)	(2)		(152)	(9)		(151)	(10)		(147)	(14)	
Age, % (n)			0.121			0.297			0.395			0.254			0.011[a]
>50	82.1	17.9		98.5	1.5		94.3	5.7		95.5	4.5		91.1	8.9	
	(276)	(60)		(331)	(5)		(317)	(19)		(322)	(15)		(306)	(30)	
<50	89.0	11.0		100	0		90.4	9.6		100	0		100	0	
	(65)	(8)		(73)	(0)		(66)	(7)		(72)	(0)		(73)	(0)	
Metastasis, % (n)			0.5596			0.765			0.577			0.29			0.982
Yes	85.5	14.5		98.6	1.4		94.1	5.9		97.3	2.7		92.3	7.7	
	(206)	(35)		(218)	(3)		(208)	(13)		(215)	(6)		(204)	(17)	
No	80.4	19.6		98.9	1.1		93.1	6.9		95.2	4.8		93.1	6.9	
	(135)	(33)		(186)	(2)		(175)	(13)		(179)	(9)		(175)	(13)	

[a]P<0.05, [b]P<0.01. *EGFR*, epidermal growth factor.

Table VI. Frequency of detected *EGFR* mutations in each exon.

Exon	Mutation	Percentage (n)
Exon 18	Gly719Arg[a]	20 (1)
	Gly719Cys[a]	20 (1)
	Gly719Ser[a]	60 (3)
Exon 19	Glu746_Ala750 del[a]	65.4 (17)
	Leu747_Thr751 del	11.5 (3)
	Leu747_Ser752 del	7.7 (2)
	Glu746_Ala750>IP	7.7 (2)
	Glu746-Thr751del	3.8 (1)
	Lys745_Glu746del	3.8 (1)
Exon 20	Glu762ins; Ser768-Asp770dup	6.7 (1)
	Glu762insEAFQ	20 (3)
	Ser768Ile	6.7 (1)
	Ser768-Asp770dup	6.7 (1)
	Ser768-Asp770dup; T790M	13.3 (2)
	Thr790Met[a]	33.3 (5)
	Val769Leu	6.7 (1)
	Val769L; Asn771His773dupAsnProHis	6.7 (1)
Exon 21	Asn826Ser	3.3 (1)
	Leu858Arg[a]	53.3 (16)
	Leu861Gln[a]	43.3 (13)

[a] Most common mutation. *EGFR*, epidermal growth factor.

Discussion

NSCLC is one of the most common types of cancer in the world, including in Turkey, and is the leading cause of cancer-associated deaths in both men and women worldwide (1). The incidence of NSCLC is higher amongst males and smokers (19,20), a pattern which was also observed in the present study. Male patients accounted for 73.1% of all NSCLC patients and smokers accounted for 60.1% of all patients.

EGFR is an important marker driving carcinogenesis and the response to definite EGFR tyrosine kinase inhibitors. *EGFR* mutations are used as predictive biomarkers to understand the clinical response to EGFR tyrosine kinase inhibitors, and 70-80% of patients have benefited from tyrosine kinase inhibitors based therapies. This reveals the clinical importance of *EGFR* mutational status for therapeutic decision-making (7,8). To the best of our knowledge, the present study represents the largest molecular epidemiological dataset of *EGFR* mutation status in a Turkish population. Previous studies have reported that *EGFR* mutation frequency shows variability according to regional differences and ethnicity, and Turkey has a multiethnic population, due to its geographic location between Europe and Asia and its proximity to Middle-East region (21). Therefore, determining the genetic distribution of *EGFR* mutations, may have clinical relevance for therapeutic decision making.

In the present study, *EGFR* mutations were detected in 16.6% of patients, consistent with the frequency of European based studies, such as in Greek, Spain and Poland (22-25), but not consistent with previously published studies from Turkey and the Middle-East (Table VII) (26-30). Previous studies from Turkey, showed higher frequencies (44 and 42.6%, respectively) in terms of overall mutation rates, and the primary reason underlying the difference may be due to the small cohorts used in the previous studies (29,30). The overall *EGFR* mutation frequency was 47.9% in Asian patients and 19.2% in Western patients (8). Asian and Caucasian patients also have different molecular epidemiological data in terms of *EGFR* status (31).

The most common mutations were detected in exon 19 and 21 (6.4 and 7.3%, respectively), consistent with previous studies; however, it has been shown that mutation frequencies can differ in EGFR exons (32,33). The variations in *EGFR* mutation frequencies between countries are likely the result of differences in case selection for testing and case number of tested groups, smoking habits and ethnicity (34).

Detected mutations in exon 18 were all point mutations; Gly719Arg, Gly719Cys and Gly719Ser. All of these Gly719X mutations have been described as drug sensitizing mutations (35,36). For exon 19, deletions were the most common mutations. In the present study, all mutations detected in exon 19 mutated patients were deletions and 2 patients had >1 mutation (indels). Deletions were generally localized to amino acids 745-752, and Glu746_Ala750 deletion was the most common deletion among the patients. Exon 19 deletions are drug sensitizing mutations and the most common type of mutation in exon 19 (35,36). Exon 20, harbored most of the mutation types, including point mutations, duplications and insertions, and point mutations were the most common type of mutation in exon 20. Thr790Met was the most common mutation in exon 20, and it has been previously described as a drug resistant mutation (35,36). In exon 21, only point mutations were detected, and Leu858Arg and Leu861Gln were the most common, which have both been described as drug sensitizing mutations. Leu858Arg was the most common type of mutation in the entire cohort, consistent with previous literature (35,36).

Previous studies have shown a significant association between the clinicopathological characteristics of patients, such as smoking status and sex and the frequency of EGFR mutations in patients with NSCLC (19,37). The present study observed similar results among Turkish patients with NSCLC patients compared with other populations. There was a higher frequency of *EGFR* mutations in females compared with males and in never-smokers compared with smokers. In another Turkish population-based study, *EGFR* mutations were also more common in females and non-smokers (29). The same associations have been observed in different *EGFR* exon mutations. Exon 19 mutation frequency was higher in females compared with males and in non-smokers compared with smokers. Exon 21 mutations were also significantly more common in females compared with males. Overall, *EGFR* mutation patterns and their association with clinicopathological characteristics were similar across most of the common exon mutations (exon 19 and 21). Patients with *EGFR*

Table VII. Frequency of *EGFR* mutation status by different countries.

Author, year	Country	Total patients, n	Overall EGFR mutation, %	Exon 18, %	Exon 19, %	Exon 20, %	Exon 21, %	(Refs.)
Vázquez *et al*, 2016	Spain	184	13.6	0	44	8	48	(24)
Szumera-Ciećkiewicz *et al*, 2013	Poland	273	10.6	3.4	55	10.3	32	(25)
Papadopoulou *et al*, 2015	Greece	1.472	15.83	1.29	67.38	4.29	27.04	(22)
Syrigos *et al*, 2018	Greece	575	15.7	2.2	59.6	11.2	29.2	(23)
Bircan *et al*, 2014	Turkey	25	44	0	32	0	20	(29)
Unal *et al*, 2013	Turkey (Western region)	48	42.6	0	38.9	50	11.1	(30)
Present study	Turkey[a]	409	16.6	7.35	38.23	22.06	44.12	-
Errihani *et al*, 2013	Morocco	137	21	7	69	3	21	(21)
Fakhruddin *et al*, 2014	Lebanon	106	8.5	0	88.9	0	11.1	(26)
Haghgoo *et al*, 2017	Iran	98	37	0	72.2	0	27.8	(27)
Jazieh *et al*, 2015	Saudi Arabia, The United Arab Emirates and Qatar	230	28.7	6.06	54.54	1.5	39.4	(28)

[a]Results from the present study; 8 patients had double mutations. *EGFR*, epidermal growth factor receptor.

mutated tumors were more likely to have a tumor localized to the right lung, although this association was not significant, but there was an association between tumors localized in the left lung tumor and exon 20 mutation frequency. Patients with tumors localized to the left lung had a higher exon 20 mutation frequency. The present study is the first to report this association.

In summary, European based studies showed a mutation frequency of 10-15% in EGFR, Middle Eastern based studies showed a mutation frequency of 21-37%, and Asian population-based studies showed a heterogeneous mutation frequency of 27-62% (38-41). In the present study, the mutation frequency of *EGFR* among the Turkish population was 16.6%, less than that of the Middle Eastern and East Asian based studies; and similar to European based studies. These results may assist in determining the incidence of *EGFR* mutations amongst the different ethnicities present in Turkey, and warrant the design of genome wide-based collaborations that may reveal novel decision-making mutations in *EGFR* in patients with NSCLC. Favorable management of NSCLC includes genetic screening of tumor tissues for informative biomarkers which may be used for targeted therapy, therefore clinical results can be developed with pharmacogenetic screenings that highlight the heterogeneity of NSCLC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GCK and YB conceived and designed the study. GCK and AA performed the experiments. GCK, AA, TS and YB analyzed the data. OUU and IO collected the patient data. DG performed the pathological evaluation and tissue processing. HE analyzed the data. GCK, AA and TS wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was based on pathological archived material and was approved by Dokuz Eylul University, Non-invasive Researches Ethics Committee (Izmir, Turkey) (approval no. 300-GOA, 2011/28-03). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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