Thorac Res Pract. 2024; 25(3): 102-109 DOI: 10.5152/ThoracResPract.2024.23067

Original Article

The Epidermal Growth Factor, Anaplastic Lymphoma Kinase, and ROS Proto-oncogene 1 Mutation Profile of Non-Small Cell Lung Carcinomas in the Turkish Population: A Single-Center Analysis

Eylül Gün[1], İzzetiye Ebru Çakır[2], Hasan Ersöz[3], Utku Oflazoğlu[4], Bünyamin Sertoğullarından[5]

[1]Department of Cellular Pathology, Basildon & Thurrock University Hospital, Mid and South Essex NHS Foundation Trust, Basildon, Essex, United Kingdom
[2]Department of Pathology, İzmir Katip Çelebi University Atatürk Training and Research Hospital, İzmir, Türkiye
[3]Department of Thoracic Surgery, İzmir Katip Çelebi University Atatürk Training and Research Hospital, İzmir, Türkiye
[4]Department of Medical Oncology, İzmir Katip Çelebi University Atatürk Training and Research Hospital, İzmir, Türkiye
[5]Department of Thoracic Diseases, İzmir Katip Çelebi University Atatürk Training and Research Hospital, İzmir, Türkiye

Cite this article as: Gün E, Çakır İE, Ersöz H, Oflazoğlu U, Sertoğullarından B. The epidermal growth factor, anaplastic lymphoma kinase, and ROS proto-oncogene 1 mutation profile of non-small cell lung carcinomas in the Turkish population: A single-center analysis. *Thorac Res Pract.* 2024;25(3):102-109.

Abstract

OBJECTIVE: The management of non-small cell lung carcinomas (NSCLC) has changed with the identification of molecular pathways. We aimed to reveal the 3-year epidermal growth factor (*EGFR*), anaplastic lymphoma kinase (ALK), and ROS proto-oncogene 1 (*ROS1*) mutation profile in the Turkish population.

MATERIAL AND METHODS: The histopathological and molecular data of all NSCLC cases from our department between May 2019 and April 2022 were evaluated.

RESULTS: Molecular testing was performed in 197 NSCLC cases, and results were obtained in 182 (92.4%) (M/F: 144/38, aged 39-86). Of these, 121 were diagnosed with adenocarcinoma, 36 with squamous cell carcinoma, and 25 with NSCLC-not otherwise specified. The *EGFR* mutation was seen in 21 (11.5%) cases (6 exon 19 deletions, 3 exon 18 [all codon 719], 2 exon 20, 8 exon 21 point mutations, 1 concurrent exon 19 deletion and exon 20 codon 790 M point mutation, and 1 concurrent exon 19 deletion and exon 21 point mutation). The double mutation rate of *EGFR* was 1.1%. The mean age of these patients was 63.4 (40-79), with 24% of all females (n = 9) and 8.3% of all males (n = 12). The *ALK* mutation was detected in 6 (3.3%) patients (M/F: 4/2, aged 45-82), whereas the *ROS1* mutation was detected in 3 (1.7%) (M/F: 2/1, aged 40-64).

CONCLUSION: It is well established in the literature that *EGFR*-activating mutation rates vary depending on regions and ethnic groups. We concluded that the *EGFR*-activating mutation rates of the Turkish population are similar to the European molecular data instead of the Asian. The *ALK* and *ROS1* mutation rates also seem concordant with the literature.

KEYWORDS: ALK, EGFR, non-small cell lung cancer, mutation profile, ROS1

Received: *July 27, 2023* ***Revision Requested:*** *October 10, 2023* ***Last Revision Received:*** *October 30, 2023*
Accepted: *January 31, 2024* ***Publication Date:*** *February 8, 2024*

INTRODUCTION

Lung cancer is the second most common cancer in the United States, according to Cancer Statistics 2022 data, and constitutes the most common cause of cancer-related death in both genders.[1] Global Cancer Statistics 2020 data estimated around 2.2 million new cases, representing 10% of all cancer cases, and 1.8 million new deaths.[2] The latest Cancer Statistics report published by the Turkish Ministry of Health in 2021 depicted the annual lung cancer rates as 56.7 cases per 100 000 individuals in men, making it the most common cancer in men, and 11.1 per 100 000 individuals in women, ranking fourth in women.[3]

The histopathological classification of lung cancer is divided into 2 broad categories: small cell and non-small cell lung cancer (NSCLC), with the latter representing approximately 85% of all lung cancer cases.[4] The NSCLC has several subtypes, such as adenocarcinoma, squamous cell carcinoma, large cell carcinoma, etc. The management of NSCLCs has changed with the identification of the molecular pathways that are now used in targeted therapies. Testing the presence of driver mutations in specific genes in NSCLCs has affected the clinical management and outcomes of the disease, with recent reports about lung cancer becoming seemingly more favorable in all stages.[5,6]

After the first discovery of overexpression and aberrant activation of the epidermal growth factor receptor (EGFR) in patients with lung adenocarcinoma and several activating mutations of the tyrosine kinase domain of the *EGFR* gene, biological agents called "tyrosine kinase inhibitors" have started to be used in the treatment.[7] Thus, a new era began

Corresponding author: Eylül Gün, e-mail: dreylulgun@gmail.com

in personalized medicine for NSCLC patients. It is estimated that up to 69% of advanced NSCLC patients have druggable mutations in numerous genes such as *EGFR*, anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), Kirsten rat sarcoma virus (*KRAS*), V-raf murine sarcoma oncogene homolog B1 (*BRAF*), *MET*, human epidermal growth factor receptor (*HER2*), etc.[8] In current practice, predictive testing for *EGFR* mutations, *ALK, ROS1,* NTRK fusions, and BRAF mutations is recommended to be performed in lung adenocarcinoma regardless of the different risk factors that these patients carry, as only the drugs targeting these molecular alterations have been approved for clinical use.[5]

The incidence rates of these specific mutations vary among different geographical areas, populations, and genders. There have been many reports from Asian and European populations regarding the frequency of these mutations. Türkiye is a country with a unique geographical location between Asia and Europe, and with its multicultural structure, it is home to various ethnic groups. We aimed to reveal the 3-year *EGFR, ALK*, and *ROS1* mutation profiles in the Turkish population in this series and to identify the clinicopathologic characteristics of the mutated cases.

MATERIAL AND METHODS

All NSCLC cases that were evaluated histopathologically and by molecular testing in our department between May 2019 and April 2022 were included in the study. The demographic data of the patients, the type of specimen in which the molecular testing was done (cell block, biopsy, resection), the localization (primary/metastatic) of the specimen in which the molecular testing was done, the histopathological diagnosis, and the mutation rates were noted.

Molecular Analysis

After marking the tumor-rich areas on the hematoxylin-eos in-stained slides, DNA was extracted from 5 paraffin sections (10 µm) representative of the marked tumor tissue using the GeneJET Thermo Scientific extraction kit according to the manufacturer's protocol. The PNAClamp™ *EGFR* Mutation Detection kit (PANAGENE Inc., Korea) was used to detect *EGFR* mutations by real-time polymerase chain reaction (PCR). Real-time PCR reactions of PNA-mediated clamping PCR were performed using an Applied Biosystems™ 7500 Fast and 7500 Real-Time PCR. The *EGFR* mutation types were detected using PNA-mediated real-time PCR. The efficiency of PCR clamping was determined by measuring the threshold cycle (Ct) value. The target somatic mutations included E19 deletions, E21 L858R and L861Q mutations, E18 G719X mutation, E20 S768I mutation, E20 insertions, and E20 T790M mutation.

The fluorescence in situ hybridization (FISH) method was used for ALK and ROS1 translocation using the ZytoLight SPEC ALK and ROS1 Dual Color Break Apart Probe (ZytoVision GmbH, Bremerhaven, Germany). The FISH signal abnormalities were confirmed using a fluorescence microscope with appropriate channels. The signal pattern was evaluated in at least 50 tumor cells as per the standard procedure. If ≥15% split signals and/or loss of green for ALK/loss of orange for ROS1 signals were detected, the test was considered to have yielded a positive result.

This study was performed in line with the principles of the Declaration of Helsinki, and approval was granted by the Ethics Committee of İzmir Katip Çelebi University (Approval number: 2021-GOKAE-0460, date: 2021). Informed consent was obtained from the patients prior to the study.

Statistical Analysis

The results were analyzed using Statistical Package for the Social Sciences Statistics software, version 24.0 for Windows (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as the median, and categorical data as percentages. Besides descriptive statistics, the categorical data were compared using the chi-square test. Differences in continuous measurements between the 2 groups were examined by the Student's *t*-test. A *P*-value of <.05 was considered statistically significant.

RESULTS

A total of 197 NSCLC cases underwent molecular analysis. The molecular test results were obtained in 182 (92.4%) of them, with 119 primary lung carcinomas and 63 metastases. There were 144 males (79.1%) and 38 females (20.9%), with a median age of 64 (39-86 years). Molecular testing was not successful in 15 cases because of the inability to retrieve sufficient DNA. These consisted of 7 bone biopsies, 6 cytological specimens, and 2 tissue biopsies with low cellularity.

The molecular testing was performed on biopsies in 106 cases, on cell blocks obtained from cytological specimens in 56 cases, and on resections in 20 cases. The histopathological subtype was adenocarcinoma in 121 cases (66.5%), squamous cell carcinoma (SCC) in 36 cases (19.8%), and NSCLC-Not otherwise specified (NOS) in 25 cases (13.7%).

The *EGFR* mutation was detected in 21 cases, with an overall rate of 11.5% and a median age of 67 (40-79). It was seen more frequently in females with a rate of 23.7% (n = 9), whereas this rate was 8.3% in males (n = 12), and this

Main Points

- The study investigates the molecular mutation profile of non-small cell lung carcinomas (NSCLC) in the Turkish population, focusing on epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (ALK), and ROS oncogene 1 (ROS1) mutations.
- The *EGFR*-activating mutation rates in the Turkish population were found to be similar to European data rather than Asian, with an overall rate of 11.5%.
- The *ALK* and *ROS1* mutation rates in Turkish NSCLC cases were consistent with the literature, with rates of 3.3% and 1.7%, respectively.
- Adenocarcinoma was the most common histological subtype associated with *EGFR* mutations, and *EGFR* mutations were more prevalent in females compared to males.
- These findings underscore the importance of molecular profiling in NSCLC management and highlight the need for personalized therapies targeting specific mutations for improved patient outcomes.

Table 1. Relationship Between Case Characteristics and *Epidermal Growth Factor Receptor* Gene Mutation

			EGFR Gene Mutation Status		
Characteristics		**n (%)**	**Mutated (%)**	**Wild Type (%)**	***P***
Gender	Female	38 (20.9)	9 (23.7)	29 (76.3)	**<.05**
	Male	144 (79.1)	12 (8.3)	132 (91.7)	
Age	Mean ± SD	182 (100)	63.38 ± 9.19	63.71 ± 12.2	.907
	Median (min-max)		67 (40-79)	64 (39-87)	
Histologic diagnosis	Adenocarcinoma	121 (66.5)	19 (15.7)	102 (84.3)	**<.05**
	SCC	36 (19.8)	1 (2.8)	35 (97.2)	
	NSCLC, NOS	25 (13.7)	1 (4)	24 (96)	
Specimen type	Cell blocks (cytology)	56 (30.8)	7 (12.5)	49 (87.5)	.102
	Biopsies	106 (58.2)	9 (8.5)	97 (91.5)	
	Resections	20 (11)	5 (25)	15 (75)	
Sample location	Primary	119 (65.4)	13 (10.9)	106 (89.1)	.722
	Metastatic	63 (34.6)	8 (12.7)	55 (87.3)	
Total		182 (100)	21 (11.5)	161 (88.5)	

EFGR, epidermal growth factor receptor; NOS, not otherwise specified; NSCLC, non-small cell lung carcinoma; SCC, squamous cell carcinoma.

difference was statistically significant (P = .008). The mean age for *EGFR*-mutated cases did not differ statistically from the *EGFR* wild-type cases (63.38 ± 9.19 vs. 63.38 ± 12.20, P = .907).

The mutations were observed in 10.9% (13/106) of primary tumor samples and 12.7% (8/55) of metastatic tumor samples. There was no statistically significant relationship between the tumor samples and *EGFR* mutation presence (P = .722). Also, no significant difference was found between the specimen types (cell blocks, biopsies, and resections) and *EGFR* mutation (P = .102).

The histological subtypes of *EGFR*-mutated cases were as follows: 19 (90.4%) adenocarcinomas, 1 (4.8%) SCC with exon 20 T790M mutation, and 1 (4.8%) NSCLC-NOS with concurrent exon 19 deletion and exon 21 L858R point mutation. The *EGFR* mutation prevalence was significantly higher in

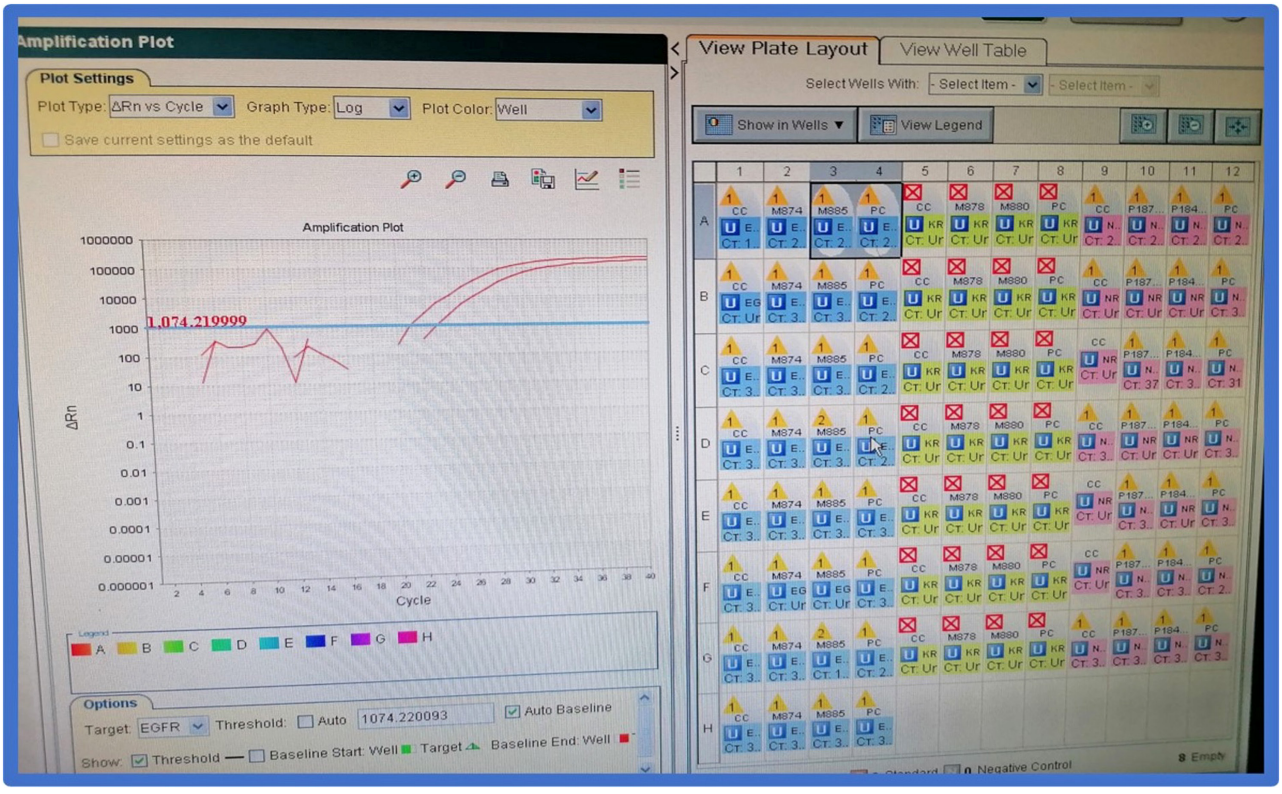

Figure 1. Cycle threshold curve in the real-time polymerase chain reaction analysis result of the case with epidermal growth factor receptor exon 20 T790M mutation.

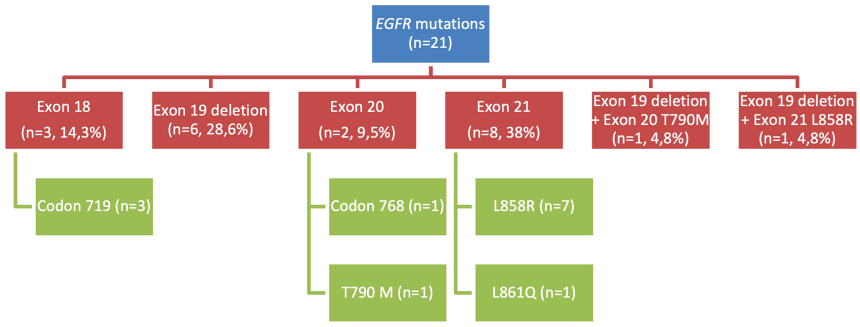

Figure 2. The details and frequencies of *EGFR* gene mutations in each exon. EGFR, epidermal growth factor receptor.

adenocarcinoma cases ($P = .046$). The rate of *EGFR*-mutated adenocarcinomas was 15.7% among all adenocarcinoma cases. The relationship between case characteristics and *EGFR* mutation is given in Table 1.

The most common *EGFR* mutation was detected in exon 21 in 8 cases (38%). The point mutation in this exon was seen in L858R in 7 cases and L861Q in 1 case. This was followed by the exon 19 deletion in 6 cases (28.6%). Other point mutations were detected in exon 18 codon 719 of the gene in 3 cases (14.3%), in exon 20 in 2 cases (9.5%) with a point mutation in codon 768 in 1 case, and in exon 20 T790M in 1 case (Figure 1).

Apart from classical mutation patterns, double mutations were detected in 2 cases (1,1%). Of these, 1 case was diagnosed with NSCLC-NOS, with an exon 19 deletion and an exon 21 codon L858R point mutation. The other case showed an exon 20 codon 790M point mutation with an exon 19 deletion. This case was diagnosed as pulmonary adenocarcinoma metastasis in the liver, and molecular studies were applied to a liver needle biopsy. A case with the EGFR Exon 20 T790M mutation was diagnosed as "NSCLC-primarily compatible with squamous cell carcinoma" from the cell block of a lung transthoracic needle biopsy. The details and the frequencies of *EGFR*-mutated cases in each exon are given in Figure 2.

The *ALK* rearrangement was detected in 6 cases, with an overall rate of 3.3% (Figure 3).

Four of these patients were male, whereas 2 of them were female, and the age range was 45-82. The histological diagnosis was adenocarcinoma in 5 of these cases and NSCLC and NOS in 1. Among the six patients showing rearrangement of the ALK gene, three received the diagnosis from bronchoscopic biopsy, one from endobronchial ultrasound (EBUS) bronchoscopy material, one from transthoracic needle biopsy, and the 48-year-old female ex-smoker patient was

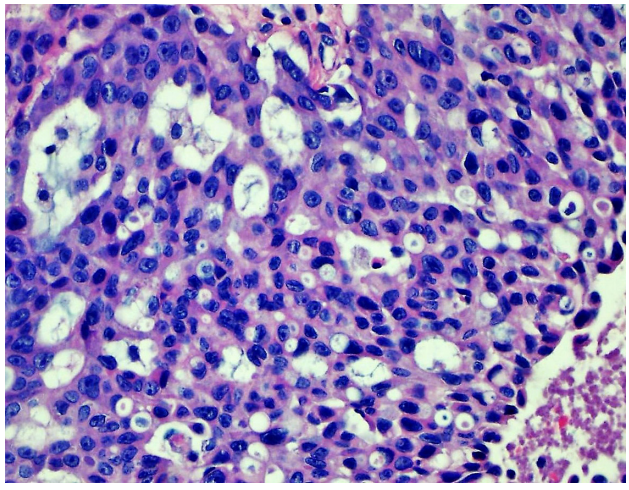

Figure 4. Adenocarcinoma in solid and cribriform pattern in the case showing *anaplastic lymphoma kinase* mutation that was diagnosed from brain metastasis (H&E, X200)

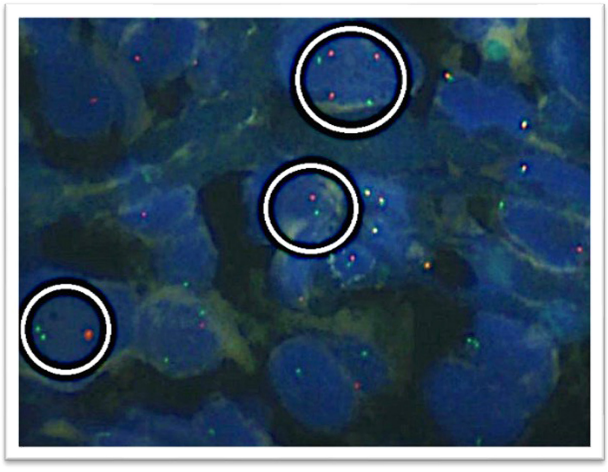

Figure 3. Anaplastic lymphoma kinase fluorescence in situ hybridization-positive case showing split red and green signals detected with anaplastic lymphoma kinase break-apart probe.

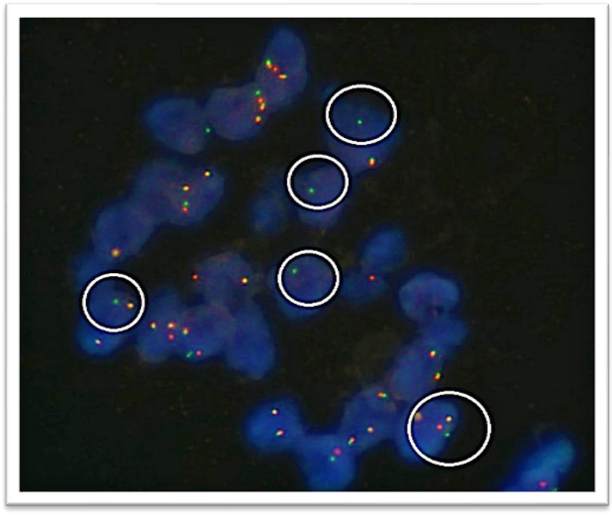

Figure 5. *ROS1* FISH-positive case showing split signals and loss of red signals detected with *ROS1* break-apart probe (*ROS1*: ROS proto-oncogene 1, FISH: Fluorescence in situ hybridization)

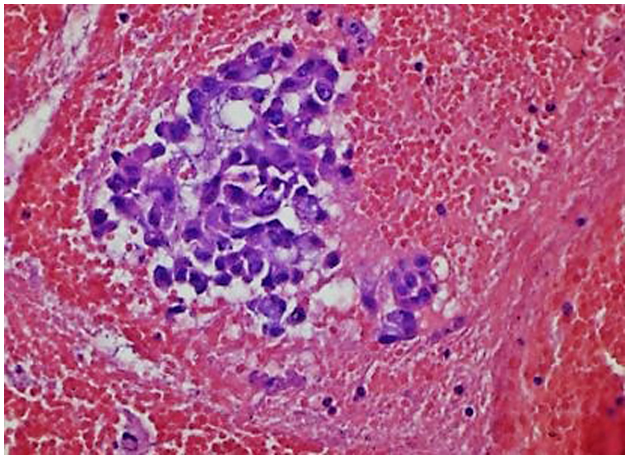

Figure 6. Adenocarcinoma in the cell block in the case showing *ROS proto-oncogene 1* mutation diagnosed from endobronchial ultrasound bronchoscopy material (H&E, X200).

diagnosed with the metastasis specimen from the brain. In the brain metastasis case, the tumor exhibited a solid and cribriform pattern (Figure 4). Three patients were ex-smokers, two were non-smokers, and one was a smoker. ALK gene rearrangement rates in the FISH analysis were observed between 20%-45% (mean 32%).

Three patients harbored *ROS1* rearrangement at an overall rate of 1.7% (Figure 5). Two of them were male, and 1 was female, aged between 40 and 64 years. Two of these tumors were adenocarcinomas, and 1 of them was SCC. The diagnosis of these 3 patients was made from a cell block from EBUS material (Figure 6) in one, an incisional biopsy material from a supraclavicular lymph node in one, and from bronchoscopic biopsy material in one patient. Two patients were diagnosed with adenocarcinoma and one with SCC. In FISH analysis, ROS1 gene rearrangement rates ranged from 25% to 60% (mean 37%). The patient diagnosed with SCC was a 64-year-old male who was diagnosed from a bronchoscopic biopsy. Among the cases, one was an ex-smoker, one was a non-smoker, and one was a smoker patient.

DISCUSSION

As one of the most commonly diagnosed cancers, lung cancer remains the leading cause of death from cancer worldwide. With recent developments regarding the identification of sensitizing mutations in NSCLCs, patients with metastatic disease who did not have a chance at longer survival before might now have more favorable outcomes with the use of targeted, personalized therapies.[9] With this aim, molecular profiling is recommended for all NSCLC patients to be able to decide if they can be candidates for therapies with the so-called "smart drugs."

The *EGFR* is a cell surface protein that encodes a transmembrane protein with an extracellular component that serves as ligand-binding sites. It promotes the autophosphorylation of its tyrosine kinase domain after ligand binding, leading to a molecular cascade of events. The gene is observed to be involved in cell proliferation, decreased apoptosis, angiogenesis, metastasis, and chemoresistance in NSCLCs.[10]

The incidence of *EGFR* mutation depends on different characteristics such as gender, tumor type, smoking history, and ethnic background.[11] The prototype for this is non-smoking, East Asian women with adenocarcinomas, as previously reported by many studies.[12-14] Even though NSCLC incidence is reported to be higher in males, almost all studies that have been published to date have reported a higher frequency of *EGFR* mutation in females. Zhang et al[14] reported the overall female prevalence to be 43.7%, whereas the male prevalence was 24%. In the latest series reported from Türkiye, this rate was observed as 30.5% in females and 7.5% in males.[15] Consistent with these findings, our study revealed a statistically significant difference in terms of gender in *EGFR*-mutated cases, with a female prevalence of 23.7% and a male prevalence of 8.3%.

The *EGFR* mutation prevalences vary between different populations, ethnic groups, and geographic regions. The highest prevalence worldwide has been shown in East Asian populations (up to 59%), whereas the lowest has been reported in European populations (around 10%).[14,16-24] The causes of this discrepancy are still obscure and yet to be defined. With a unique geographical location between Asia and Europe, Türkiye is home to many ethnic groups and populations. The studies from Turkish patient groups have shown a big variation ranging from 4% to 48.1% throughout the years.[15,25-28] The reason for this variation might be the small sample size in the early studies and the use of different methods for the detection of the *EGFR* gene mutation. In the study by Özçelik et al[29] that evaluated the regional distributions of mutations in lung cancer, even though the highest mutation prevalence was observed in the Mediterranean region, the differences were not found to be statistically significant. As a tertiary reference center in the Aegean region in Western Türkiye, the prevalence from our hospital was 11.5%, which was similar to the results of Diniz et al[30] (11.6%) but slightly lower than the results of Calibasi-Kocal et al[25] (16.6%) who reported the prevalence from the same region. The most recent studies with the biggest sample sizes from the central region of Türkiye reported this prevalence to be 16.7% and 16.6%.[15,28] Along with our study, these latest results show that the *EGFR*-activating mutation rates of the Turkish population are similar to the European molecular data instead of the Asian.

In terms of the histological tumor type, *EGFR* mutations are reported to be present in up to 78% of adenocarcinomas in East Asian populations, as opposed to only 10-16% of adenocarcinomas in other ethnicities.[14,16-24] This rate was 20.3% in a comprehensive study from Türkiye that evaluated 959 NSCLC cases.[28] Zhang et al[14] state in their review that adenocarcinoma patients were more likely to harbor *EGFR* mutations, with Asians showing a more striking prevalence than Caucasians. There were a total of 121 adenocarcinoma cases in our series, and 15.7% (n = 19) of them showed an *EGFR* mutation with a statistically significant difference from the other histological subtypes, validating our results being close to the European data once more.

With the extensive research that focused on the activating mutations within the *EGFR* gene, it is now known that

mutations in NSCLCs are limited to the first 4 exons (18-21), and most of the mutations are either point mutations that lead to amino acid substitutions (exons 18 and 21) or in-frame deletions (exon 19).[31] In-frame deletions in exon 19 and L858R point mutations in exon 21 constitute the most powerful predictive biomarkers of response to EGFR tyrosine kinase inhibitor therapy, whereas exon 20 mutations were shown to be associated with a poor response to therapy and increased mortality.[32] In the present study, the most commonly detected mutation type was point mutations in exon 21 (n = 8; 38%) followed by an in-frame deletion in exon 19 (n = 6; 28.6%), which comprised the majority (66.6%) of the mutations in this population. It has been shown that these mutation frequencies can differ in *EGFR* exons owing to the fact that patient populations vary in terms of ethnicity and smoking habits. Exon 20 codon 790M point mutation was observed in 1 of our cases diagnosed with squamous cell carcinoma from a lung transthoracic needle biopsy. The *EGFR* mutations are reported at a rate of 2%-10% in Asian series in squamous cell carcinomas. On a case-by-case basis, adenocarcinomas transforming into squamous cell carcinoma after tyrosine kinase treatment and the presence of EGFR mutations in squamous cell carcinoma in these cases have been reported.[33] However, our case underwent the initial diagnosis and treatment stages in an external center, and the clinical history could not be reached; therefore, no interpretation of the transformation could be made.

Double mutation patterns have also been shown in NSCLC patients, with prevalence rates ranging from 0.47% to 7%.[32,34] Even though the data on the clinical characteristics of these cases are limited in the literature, in the study by Wei et al[32] that assessed 32 patients with double mutations in the *EGFR* gene, the response rate to therapy was found to be lower compared to patients who showed single mutations. The double mutation rate in our study was 1.1%, and the mutated genes were exon 19 deletion and exon 21 L858R in 1 case, and exon 19 deletion and exon 21 L858R point mutation in the other.

Due to the increase in genetic alterations with age, oncogenic mutations are more likely to be seen in the elderly. In patients with tumors harboring *EGFR* mutations, the mean age is reported to be around 60-65 years. Older age has been associated with a higher EGFR mutation rate in NSCLCs.[28,34] The median age in our study was 64, and we could not demonstrate a significant relationship between the presence of an *EGFR* mutation and age.

e *ALK* gene encodes a tyrosine kinase transmembrane protein and consists of 30 exons mapping to the long arm of chromosome 2. After the discovery of *EGFR* mutations, a novel transforming fusion gene resulting from the linkage between the echinoderm microtubule-associated protein-like 4 (EML4) and *ALK* genes in NSCLCs has been identified. It was elucidated that the resulting chimera proteins from this fusion have accelerated tyrosine kinase activity, which results in potent oncogenic effects. This fusion is reported to be seen at varying rates in NSCLCs throughout the world, ranging from 1% to 10%, is more prevalent in younger adults (in the fourth or fifth decades) compared to *EGFR* mutations, and is typically detected in adenocarcinomas with solid patterns.[9] Our study's ALK rearrangement rate was 3.3% with 6 cases (5 adenocarcinomas, 1 NSCLC, NOS). Two of the patients were under 50 years old, 3 were ex-smokers, and 2 were non-smokers. In the majority of cases (5 out of 6), the histopathological type was adenocarcinoma, consistent with the literature. In the patient diagnosed with brain metastasis, the metastatic pattern was solid and cribriform.

ROS1 is a gene that acts as an orphan tyrosine kinase receptor, and even though it has functional and structural similarities to the *ALK* gene, it has a unique extracellular domain, and little is known about its function. Its frequency is similar worldwide, with an estimated overall prevalence of 1.9% ranging from 0.9% to 2.6% in different studies. Just like *ALK*, rearrangements in this gene are seen more frequently in women, non-smokers, and younger ages.[9] We detected 2 cases with *ROS1* rearrangement, with an overall rate of 1.7%, which is consistent with the literature data. The youngest patient was a 40-year-old female who was an ex-smoker. Two patients had adenocarcinoma, whereas unexpectedly, the other case was a SCC. The SCC diagnosis was given from a bronchoscopic biopsy and the patient was a 64-year-old male. In the literature, rearrangements of the ROS1 gene are reported as quite rare in SCCs. Since our case received the diagnosis from a bronchoscopic biopsy, the possibility of an accompanying adenocarcinoma component cannot be ruled out.

Molecular profiling in NSCLCs is performed routinely in Türkiye in most centers. However, in 5%-25% of the cases retrieved for molecular studies, poor-quality biopsies and an insufficient amount of tumor cells cause the testing to be unsuccessful.[35] This rate was 7.8% in our study with 15 cases. To obtain better results in molecular testing, tissue processing and handling should be done very carefully.

A limitation of our study is that, because our hospital is a tertiary reference center, most of the cases were brought from other centers only for molecular testing. Therefore, the data on the smoking history and clinical symptoms and signs could not be obtained in most of them, and the association between smoking and mutation presence could not be assessed. However, detailed and good-quality molecular analysis along with confirmed histopathological diagnoses constitute the strong point of our study.

In conclusion, it is well established in the literature that *EGFR*-activating mutation rates vary depending on regions and ethnic groups. Our study showed that the *EGFR*-activating mutation rates of the Turkish population are similar to the European molecular data instead of the Asian. Comprehensive studies on mutation incidences report a substantial lack of data from several large geographic regions of the world, including our region, and we believe that our findings will contribute to the literature in this context.

Ethics Committee Approval: This study was approved by Ethics committee of İzmir Katip Çelebi University (Approval number: 2021-GOKAE-0460, date: 2021).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.G., I.E.C.; Design – E.G., I.E.C.; Supervision – I.E.C.; Resource – I.E.C., U.O., H.E., B.S.; Materials – I.E.C., U.O., H.E., B.S.; Data Collection and/or Processing – E.G., I.E.C., U.O., H.S., B.S.; Analysis and/or Interpretation – I.E.C., B.S.; Literature Search – E.G., I.E.C.; Writing – E.G., I.E.C.; Critical Review – I.E.C.

Acknowledgments: The preliminary results of this study were presented as an oral presentation at the 33rd European Congress of Pathology, which was held virtually between August 29, 2021 and August 31, 2021 (The EGFR, ALK, and ROS1 mutation profile of non-small cell lung carcinomas in the Turkish population: a single-centre analysis. E. Gun, I.E. Cakir. VIRCHOWS ARCHIV 479, OFP-16-007).

The authors thank Biologist Aylin Şahin for her dedicated and valuable work during the molecular analysis of the cases.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This study received no funding.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33. [CrossRef]
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3): 209- 249. [CrossRef]
3. *Turkey Ministry of Health Cancer Statistics-2017 Report*. Available at: https://hsgm.saglik.gov.tr/depo/birimler/kanser-db/istatistik/Turkiye_Kanser_Istatistikleri_2017.pdf
4. Rolfo C, Mack PC, Scagliotti GV, et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): A statement paper from the IASLC. *J Thorac Oncol*. 2018;13(9):1248-1268. [CrossRef]
5. WHO Classification of Tumors Editorial Board. *Thoracic tumours* (WHO classification of tumours series, 5th ed.; vol. 5). Lyon (France): International Agency for Research on Cancer; 2021. Available at: https://publications.iarc.fr/595.
6. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med*. 2018;142(3):321-346. [CrossRef]
7. Yamamoto H, Toyooka S, Mitsudomi T. Impact of EGFR mutation analysis in non-small cell lung cancer. *Lung Cancer*. 2009;63(3):315-321. (doi: [CrossRef])
8. Wheeler DA, Wang L. From human genome to cancer genome: the first decade. *Genome Res*. 2013;23(7):1054-1062. [CrossRef]
9. Fois SS, Paliogiannis P, Zinellu A, Fois AG, Cossu A, Palmieri G. Molecular epidemiology of the main druggable genetic alterations in non-small cell lung cancer. *Int J Mol Sci*. 2021;22(2):612. [CrossRef]
10. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer*. 2006;118(2):257-262. [CrossRef]
11. Cheng L, Zhang S, Alexander R, et al. The landscape of EGFR pathways and personalized management of non-small-cell lung cancer. *Future Oncol*. 2011;7(4):519-541. [CrossRef]
12. Sasaki H, Endo K, Konishi A, et al. EGFR Mutation status in Japanese lung cancer patients: genotyping analysis using LightCycler. *Clin Cancer Res*. 2005;11(8):2924-2929. [CrossRef]
13. Shi Y, Au JS-K, Thongprasert S, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non–small-cell lung cancer of adenocarcinoma histology (Pioneer). *J Thorac Oncol*. 2014;9(2):154-162. [CrossRef]
14. Zhang YL, Yuan JQ, Wang KF, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget*. 2016;7(48):78985-78993. [CrossRef]
15. Musayeva M, Sak SD, Özakıncı H, Boyacıgil Ş, Coşkun Ö. Evaluation of epidermal growth factor receptor mutations and thyroid transcription factor-1 status in Turkish non-small cell lung carcinoma patients: A study of 600 cases from a single center. *Turk Gogus Kalp Damar Cerrahisi Derg*. 2020;28(1):143-150. [CrossRef]
16. Tanaka T, Matsuoka M, Sutani A, et al. Frequency of and variables associated with the EGFR mutation and its subtypes. *Int J Cancer*. 2010;126(3):651-655. [CrossRef]
17. Wei WE, Mao NQ, Ning SF, et al. An analysis of EGFR mutations among 1506 cases of non-small cell lung cancer patients in Guangxi, China. *PLoS One*. 2016;11(12):e0168795. [CrossRef]
18. Lai Y, Zhang Z, Li J, et al. EGFR mutations in surgically resected fresh specimens from 697 consecutive Chinese patients with non-small cell lung cancer and their relationships with clinical features. *Int J Mol Sci*. 2013;14(12):24549-24559. [CrossRef]
19. Zhang XC, Wang J, Shao GG, et al. Comprehensive genomic and immunological characterization of Chinese non-small cell lung cancer patients. *Nat Commun*. 2019;10(1):1772. [CrossRef]
20. Chougule A, Prabhash K, Noronha V, et al. Frequency of EGFR mutations in 907 lung adenocarcioma patients of Indian ethnicity. *PLoS One*. 2013;8(10):e76164. [CrossRef]
21. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic review and global map by ethnicity (mutMapII). *Am J Cancer Res*. 2015;5(9):2892-2911. [CrossRef]
22. Benbrahim Z, Antonia T, Mellas N. EGFR mutation frequency in Middle East and African non-small cell lung cancer patients: a systematic review and meta-analysis. *BMC Cancer*. 2018;18(1):891. [CrossRef]
23. Skov BG, Høgdall E, Clementsen P, et al. The prevalence of EGFR mutations in non-small cell lung cancer in an unselected Caucasian population. *APMIS*. 2015;123(2):108-115. [CrossRef]
24. Esteban E, Majem M, Martinez Aguillo M, et al. Prevalence of EGFR mutations in newly diagnosed locally advanced or metastatic non-small cell lung cancer Spanish patients and its association with histological subtypes and clinical features: the Spanish REASON study. *Cancer Epidemiol*. 2015;39(3):291-297. [CrossRef]
25. Calibasi-Kocal G, Amirfallah A, Sever T, et al. EGFR mutation status in a series of Turkish non-small cell lung cancer patients. *Biomed Rep*. 2020;13(2):2. [CrossRef]. Epub 2020 Jun 2.
26. Unal OU, Oztop I, Calibasi G, et al. Relationship between epidermal growth factor receptor gene mutations and clinicopathological features in patients with non-small cell lung cancer in western Turkey. *Asian Pac J Cancer Prev*. 2013;14(6):3705-3709. (doi: [CrossRef])

27. Bircan S, Baloglu H, Kucukodaci Z, Bircan A. EGFR and KRAS mutations in Turkish non-small cell lung cancer patients: a pilot study. *Med Oncol*. 2014;31(8):87. (doi: [CrossRef])
28. Güler Tezel G, Şener E, Aydın Ç, Önder S. Prevalence of epidermal growth factor receptor mutations in patients with non-small cell lung cancer in Turkish population. *Balk Med J*. 2017;34(6):567-571. [CrossRef]. Epub 2017 Aug 23.
29. Özçelik N, Aksel N, Bülbül Y, et al. Regional distribution of genetic mutation in lung cancer in Turkey (REDIGMA). [Türkiye'de akciğer kanserinde genetik mutasyonların bölgesel dağılımı (REDIGMA)]. *Tuberk Toraks*. 2019;67(3):197-204. [CrossRef]
30. Diniz G, Komurcuoglu B, Ozyilmaz B, Ozguzer A, Yucel N, Kirbiyik O. ALK, ROS1 and EGFR status of lung cancers in the Aegean Region of Turkey. *Indian J Pathol Microbiol*. 2022;65(2):305-310. [CrossRef]
31. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res*. 2004;64(24):8919-8923. [CrossRef]
32. Wei Z, An T, Wang Z, et al. Patients harboring epidermal growth factor receptor (EGFR) double mutations had a lower objective response rate than those with a single mutation in non-small cell lung cancer when treated with EGFR-tyrosine kinase inhibitors. *Thorac Cancer*. 2014;5(2):126-132. [CrossRef]
33. Joshi A, Zanwar S, Noronha V, et al. EGFR mutation in squamous cell carcinoma of the lung: does it carry the same connotation as in adenocarcinomas? *Onco Targets Ther*. 2017;10:1859-1863. [CrossRef]
34. Ke L, Xu M, Jiang X, Sun X. Epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase/oncogene or C-Ros oncogene 1 (ALK/ROS1) fusions inflict non-small cell lung cancer (NSCLC) female patients older than 60 years of age. *Med Sci Monit*. 2018;24:9364-9369. [CrossRef]
35. Ryska A, Berzinec P, Brcic L, et al. NSCLC molecular testing in Central and Eastern European countries. *BMC Cancer*. 2018;18(1):269. [CrossRef]