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RESEARCH

Molecular Spectrum of Somatic EGFR and KRAS Gene Mutations in non Small Cell Lung Carcinoma: Determination of Frequency, Distribution Pattern and Identification of Novel Variations in Indian Patients

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Abstract Somatic mutations of EGFR and KRAS gene represent the most common alterations currently known in NSCLC patients. This study explored the frequency, distribution pattern of EGFR and KRAS mutations in Indian patients. The frequencies of EGFR and KRAS mutations were 29 % (116/400) and 4.5 % (6/132) respectively. Both EGFR and KRAS mutations were prevalent in females, and a trend towards higher mutation frequency was seen in patients under≥60 years age. The presence of EGFR and KRAS mutations were higher in adenocarcinomas in comparison to other histological subtype. Sequencing analysis of EGFR exon 18 revealed Inframe deletion (G709_T710>A) and missense mutation (K713R). Among exon 19 positive cases, 49.3 % (37/75) were in-frame deletions, of which E746_A750del was frequent. Similarly, ~47 % (35/75) cases showed complex mutation involving indel. Among mutations in exon 20 (N= 9), 8 were substitutions, one showed duplication, while all exon 21 mutations were of the missense types with L858R as the most recurrent type. Sequencing analysis of KRAS exon 1 revealed three different types codon 12 substitutions resulting in c34G>T (G12C) (n=4), c.35G>A (G12D) (n=1), and c.35G>T (G12V) (n=1). In conclusion, the present study is an example of molecular diversity of EGFR and KRAS gene in Indian patients and further confirms that the frequency of EGFR and KRAS mutations varies considerably globally. To the best of our knowledge, this is the first Indian study to evaluate KRAS mutation. The current study also served to identify novel variations that added new insights into the genetic heterogeneity of NSCLC.

Keywords EGFR mutation · Lung cancer · Novel mutation · India

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Introduction

Carcinoma of lung is one of the leading causes of cancer associated deaths worldwide. In India, the incidence of lung cancer is rising at alarming rates accounting for 63,000 newly diagnosed cases each year with 52,000 deaths thereby contributing to 8 % of all cancer associated deaths [1, 2]. Studies from India have demonstrated that even with the modern chemotherapy; the median survival of unresectable NSCLC is between 23 to 40 weeks, mainly due to dropouts, because of the high costs and side effects [3, 4]. Recent studies in the last few years have shown some improvement in terms of understanding the biology of lung cancer with substantial incremental advances in therapeutic strategies, but unfortunately the outcomes are still fairly grim. The successes of the ABL tyrosine kinase inhibitor imatinib in the treatment of chronic myeloid leukemia (CML) [5] and Trastuzumab in breast cancer [6] has already been well established, and have demonstrated the effectiveness of identifying and targeting the critical genetic lesion that promotes proliferative signals in cancer cells. Similarly, in order to further improve treatment outcomes in lung cancer patients, new strategies targeting molecular genomic abnormalities are under intensive investigation. The epidermal growth factor receptor (EGFR) and KRAS gene plays a considerable role in various cancers through their

involvement in cell proliferation, apoptosis, enhanced cell motility, and neoangiogenesis [7].

Early 2004 witnessed the first landmark study on activating mutations in the tyrosine kinase domain of the EGFR gene which was found to be the underlying responsiveness of NSCLC to gefitinib [8]. Since then a wealth of data has been published worldwide, reporting EGFR mutation in approximately 10–20 % in white patients and more than 30-70 % of East Asian patients with NSCLC [9–11]. Majority of the EGFR mutations clusters around the tyrosine kinase domain mostly within exons 18–21 of the EGFR gene, of which in-frame deletions in exon 19 and point mutation L858R in exon 21 together accounts for 80-85 % of the EGFR mutations in lung cancer [8, 12, 13]. Most of the patients with these two mutations respond well to the anti EGFR therapy, while another mutation T790M is associated with resistance to therapy [14]. More recently, the American Society for Clinical Oncology (ASCO) recommends that patients with NSCLC who are being considered for first-line therapy with an EGFR TKI should have their tumor tested for EGFR mutations to determine whether an EGFR TKI or chemotherapy is the appropriate first-line therapy [15]. Therefore, detection of the EGFR mutation is becoming an important predictive biomarker for drug response, and efficient detection of the EGFR mutation is expected to be highly helpful for increase of survival rate of patients with lung cancer.

Mutation of the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), is the second most common recurrent genetic abnormality after EGFR mutations. KRAS mutation is generally reported in 10–30 % of NSCLC clustering most commonly around codons 12/13 in exon 2 and rarely in codon 61 of exon 3 [16]. Unlike EGFR mutation, there is increasing evidence that presence of KRAS mutation may be associated with resistance to TKIs therapy in patients with NSCLC [17], even though it is still debatable whether KRAS can actually predict resistance to TKIs [18, 19]. Interestingly, the fact that EGFR and KRAS mutations were found to be mutually exclusive in a series of NSCLC patients fits well with this concept [9]. Notably, the increased complexity of the EGFR and KRAS mutations due to varying degree of ethnicity, sex and smoking history, further adds major challenges for the evaluation of efficacy of the TKI treatment in NSCLC patients [20]. Therefore determination of frequency and genetic heterogeneity associated with EGFR and KRAS mutation is an important aspect for adoption of targeted based therapy in any given population. Furthermore, most of the available reports on EGFR mutation come from western world [8, 21–23], and Asian countries [24–26], including India [27–30], however no precise published data is available with respect to KRAS mutation in lung cancer from India. Therefore, in the present study, we report mutational spectrum of EGFR and KRAS mutation from Indian NSCLC patients and set out to evaluate their frequencies, distribution pattern and association with the clinicopathological characteristics in Indian NSCLC patients.

Materials and Methods

The present study was conducted at the Research and Development Division of SRL Ltd., Mumbai, India. The study included 400 formalin-fixed paraffin-embedded (FFPE) tumor samples from lung lesions, bronchial biopsies, metastatic lymph nodes, etc. derived from lung cancer patients. The study population consisted of 253 males (63.2 %) and 147 females (36.8 %) with median age 61 years, ranging from 27 to 88 years [Table 1]. Distributions across various histological subtypes were as follows: adenocarcinoma: 339 (84.8 %), squamous cell carcinoma: 43 (10.7 %), adenosquamous: 14

Table 1 Clinicopathological details of 400 NSCLC patients

Parameters	Total (%)	EGFR mt n(%)	EGFR wt n (%)	p value	Total (%)	KRAS mt n(%)	KRAS wt n(%)	p value
Total cases								
400	400 (100)	116 (29)	284 (71)		132(100 %)	6(4.5 %)	126(95.5 %)	
Gender								
Male	253 (63.2)	61 (24.1)	192 (75.9)	0.005	85(64.4 %)	3(3.5 %)	82(96.5 %)	0.451
Female	147 (36.8)	55 (37.4)	92 (62.6)		47(35.6 %)	3(6.4 %)	44(93.6)	
Age								
≥60	199 (49.7)	67 (33.7)	132 (66.3)	0.041	64(48.5 %)	5(7.8)	59(92.2)	0.08
<60	201 (50.3)	49 (24.3)	152(75.7)		68(51.5 %)	1(1.47 %)	67(98.53 %)	
Median (Range)	61 (27–88)				61(27–88)			
Histological type								
Adenocarcinoma	339 (84.8)	105 (31)	234 (69)	0.218	109(82.57 %)	6(5.5 %)	103(94.5 %)	
Squamous cell carcinoma	43 (10.7)	7 (16.2)	36 (83.8)		14(10.6 %)	0(0 %)	14(100 %)	0.723
Adenosquamous carcinoma	14 (3.5)	3 (21.4)	11(78.6)		8(6.06 %)	0(0 %)	8(100 %)	
Large cell carcinoma	4 (1)	1 (25)	3 (75)		1(0.75 %)	0(0 %)	1(100 %)	

(3.5 %), and large cell carcinoma: 4(1 %). The study is in accordance with the declaration of Helsinki and approved by Institute ethics committee. Treatment and outcome were not analyzed. The details of the clinical characteristics of all patients are depicted in Table 1.

Genomic DNA Extraction

Genomic DNA was extracted from FFPE tissue using Qiagen extraction kit as per manufacturer's instruction with slight modification. Prior to DNA extraction, separate hematoxylin and Eosin (HE) slides were reviewed by a pathologist to assure greater than 50 % tumor content as suitable for DNA extraction. At least, five FFPE sections of 5 μm thickness were processed for genomic DNA extraction.

Screening of the EGFR Exon 18, 19, 20 and 21 Mutation

Genomic DNA was amplified to detect EGFR mutation using PCR sequencing approach. Briefly, we used nested PCR approach by designing outer primers for exons 18 to 21 while the inner primers were similar to those reported earlier [31]. The outer primer sequences were as follows: Exon 18 outer forward 5′-gcactgctttccagcatggtga-3′, Exon 18 outer reverse 5′- catgagaggccctgcggccca-3′; Exon 19 outer forward 5′-tggtaacatccacccagatcac-3′; Exon 19 outer reverse 5′-cagctgccagacatgagaaaag-3′; Exon 20 outer forward 5′-ggtccatgtgcccctccttctgg-3′; Exon 20 outer reverse 5′-atgtgaggatcctggctcctta-3′; Exon 21 outer forward 5′-catgaacatgaccctgaattcg-3′; Exon 21 outer reverse 5′-ctggtccctggtgtcaggaaaatg-3′. The first and second round of PCR was performed in a 25 μL volume containing 50 ng of starting genomic DNA, 1.5 mmol/L $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer, and 1.5 unit of Taq polymerase (Invitrogen). The PCR conditions consisted of an initial heating step at 95 °C for 5 min followed by 30 cycles at 94 °C for 30 sec, 60 °C for 45 s, 72 °C for 45 s, and a final step at 72 °C for 5 min. In the second round, 1 ul of first round PCR product was added for amplification, followed by checking of amplified product on 2 % agarose gel.

Screening of the KRAS Codon 12 and 13 Gene Mutation

The exon 1 of KRAS gene was amplified using nested PCR approach as per previous report [32]. The primer sequences were as follows: KRAS outer forward 5′-aggcctgctgaaaatgactgaata-3′, KRAS outer reverse 5′-ctgtatcaaagaatggtcctgcac-3′; KRAS inner forward 5′-aaaatgactgaatataaacttgtgg-3′; KRAS inner reverse 5′-ctctattgttggatcatattcgtc-3′. Briefly, first round PCR was performed in a total volume of 25 μL containing 50 ng of starting genomic DNA using HotStarTaq® Master Mix (Qiagen). The PCR conditions consisted of an initial heating step at 95 °C for 15 min followed by 35 cycles at 94 °C for 20 s, 60 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. In the second round, 1 ul of first round PCR product was added for amplification using the same condition, followed by verification of the amplified product on 2 % agarose gel.

Sequencing Analysis

Amplified products for EGFR and KRAS gene were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced in both the direction by Automated ABI prism 3100 Avant Genetic Analyzer (Applied Biosystems Inc., Foster city, CA) using ABI prism BigDye terminator kit (version 3.1). Abnormal sequencing results were confirmed by at least two independent PCR reactions right from initial amplification and the results were reproducible in all the abnormal cases. Furthermore, a widltype sequencing control was run for comparison of abnormal results and dbSNP was consulted to verify that the novel mutations were not known SNPs.

Statistical Analysis

The data were analyzed using $\chi 2$ test or Fisher exact test to calculate the significance of association between EGFR, KRAS mutations and other discrete variables among subgroup of patients. P value <0.05 was considered to be significant.

Result

Clinical Characteristics of NSCLC Patients

In the current study, we investigated 400 tumor tissues from patients with NSCLC. As shown in Table 1, the frequency of NSCLC was more preponderant in males (63.2 %) in comparison to females (36.8 %). There was a gradient increase in the frequency of lung cancer with the increase in age group (< 30 years: 1.2 %, 30 to 60 years: 48.5 % and >60 years: 50.3 %). Histopathological classification revealed that adenocarcinoma was the most frequently observed subtype (84.8 %), followed by squamous cell carcinoma (10.7 %), adenosquamous (3.5 %), and large cell carcinoma (1 %).

Frequency of EGFR Mutation and its Relation to Clinico-Pathological Features

Molecular screening of the exons 18–21 which encodes tyrosine kinase domain of the EGFR gene was performed by direct sequencing. Among the 400 NSCLC cases studied, 116 cases (29 %) showed the presence of 126 mutations in

the EGFR gene, while the remaining cases (284, 71 %) showed normal wild type alleles [Table 1]. Interestingly, among these 116 mutations, 13 (11.2 %) were novel variations that has not yet been reported in the literature [Table 2].

The frequency of EGFR mutations were significantly more prevalent in females in comparison to their male counterparts (37.4 %, 55/147 vs. 24.1 %, 61/253; P=0.005). The level of significance was consistent even in multivariate analysis after adjustment for other covariates. Interestingly, a significant increased frequency of EGFR mutations were noted in patients with less than 60 years in comparison to those patients with more than 60 years (33.7 %, 67/199 vs. 24.3 %, 49/201; p=0.041)[Table 1]. This indicates that that EGFR mutation is lesser in older age groups in comparison to those patients in the first six decade of their life. The median age of patients with EGFR mutations were lower than those without mutation (59 years vs. 62 years; P=0.046 at 95 % CI). The presence of EGFR mutations were predominantly observed in adenocarcinomas (31 %, 105/339), followed by large cell carcinomas (25 %, 1/4), adenosquamous carcinomas (21.4 %, 3/14), and squamous cell carcinomas (16.2 %, 7/43). Interestingly, the frequency of EGFR mutations were significantly higher in adenocarcinomas when compared to other histological subtype (31 %, 105 of 339 vs. 18 %, 11 of 61; P=0.04). However, no statistical differences in the mutation frequencies were observed amongst other histological subtypes (P>0.05).

EGFR Mutation Types

Sequencing analysis of the EGFR exons 18,19,20 and 21 revealed that patients with isolated exon 18 mutation was found in only 1 case (0.8 %), isolated exon 19 mutation in 71 (61.3 %) cases, isolated exon 20 mutation in 2 cases (1.7 %) and isolated exon 21 mutation in 32 cases (27.6 %). It is interesting to note that 10 (8.6 %) cases had concurrent mutations in more than one exon [Fig. 1].

Both the mutations in exon 18 [Table 2] were new variations, wherein one case harbored in frame deletion resulting in G709_T710>A [Fig. 2a] while the other case showed missense mutation (K713R) [Fig. 2b]. Among all mutations in exon 19 (N=75), 49.3 % (37/75) were typical in-frame deletions around c.2230–2250, of which the most common mutation type was E746_A750del (86.4 %, 32/37) [Table 3]. Similarly, ~47 % (35/75) cases showed complex mutation mainly involving insertion and deletions together, wherein E746_S752>V (17.1 %, 6/35) mutations were more frequent. In contrast to in-frame deletions or indels, substitution mutations were less prevalent in exon 19 (4 %, 3/75). To the best of our knowledge, 6 of these exon 19 mutations were novel variation which is yet to be seen in NSCLC [Fig.3a—f] [Table 2]. Among mutations in exon 20 (N=9), 8 were of the substitution type while one showed duplication. Notably, F795S substitution mutation has not yet been observed earlier [Fig. 2c]. As shown in Tables 2 and 3, all mutations in exon 21 (N=40) were of the missense types with L858R as the most recurrent type observed in 31 cases. It is worth noting that 4 of the 40 mutations, namely; A840V [Fig. 2d], K852R [Fig. 2e], L862P [Fig. 2f] and K867N [Fig. 2g] were novel findings in this study. Thus, despite genetic heterogeneity, all EGFR gene mutations result in a distinct sequence affecting the tyrosine kinase domain of the EGFR receptor protein.

Frequency of KRAS Mutation, Mutation Type and its Relation to Clinico-Pathological Features

Among the 284 EGFR widltype cases, sufficient DNA was available for 132 cases, hence we evaluated KRAS codon 12 and 13 mutation in these cases. Of the total 132 cases, KRAS mutation was observed in 6 (4.6 %) cases while remaining 126

Table 2 Summary of patients harboring 13 novel EGFR mutations

Sr No	Age	Sex	Histology	Exon	Alteration	Amino acid
1	55	F	Adenocarcinoma	19	Substitution	K740E
2	55	F	Adenocarcinoma	21	Substitution	A840V
3	61	F	Adenocarcinoma	19	Indel	E746_P753>LQSA
4	62	F	Adenocarcinoma	18	Del	G709_T710>A
5	75	M	Large cell carcinoma	18+21	Substitution	K713R, L858R
6	37	M	Adenocarcinoma	19	Indel	E746_T751>AA
7	52	M	Squamous cell carcinoma	21	Substitution	K867N
8	56	M	Adenocarcinoma	20	Substitution	F795S
9	59	M	Adenocarcinoma	21	Substitution	L862P
10	65	M	Adenocarcinoma	19	Indel	L747_A755>SMS
11	65	M	Squamous cell carcinoma	19	Substitution	E749K
12	67	M	Adenocarcinoma	19	Indel	A750_I759>PT
13	81	M	Adenocarcinoma	21	Substitution	K852R

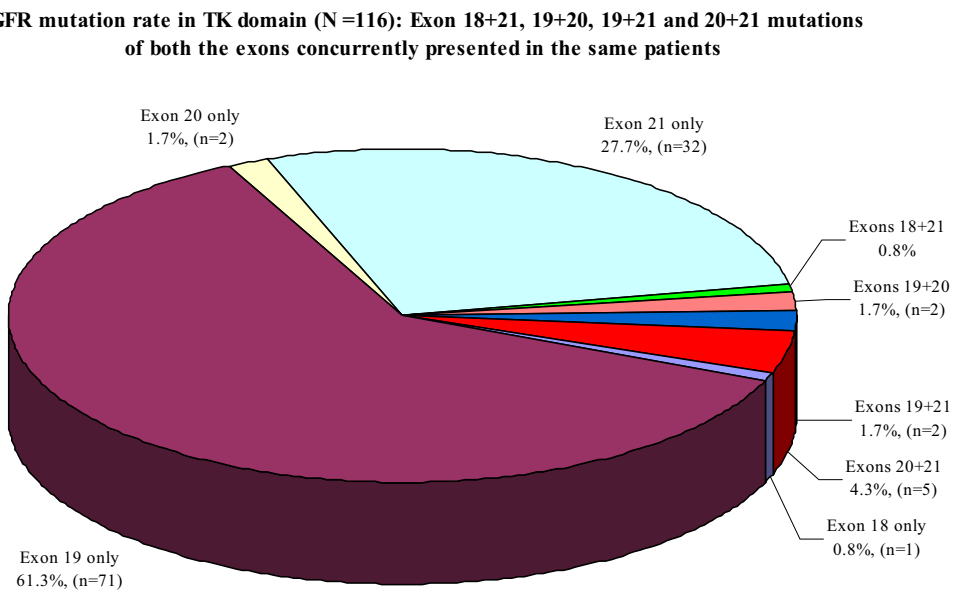

Fig. 1 Distribution of EGFR mutation rate in 116 positive cases

(95.4 %) showed normal wild type alleles [Table 1]. Although KRAS mutations were more preponderant in females than their male counterparts, this difference was not significant (6.5 %, 3/46 vs. 3.5 %, 3/85, p=0.434). Similarly, KRAS mutations were more frequent in patients with age≥60 years than patients with<60 years (8 %, 5/63 vs. 1.4 %, 1/68, p= 0.077). It is interesting to note that all the six KRAS mutations were found exclusively adenocarcinomas in comparison to other histological subtype (p=0.666). Sequencing analysis revealed that only codon 12 of the KRAS gene was found to be mutated while codon 13 mutations were not observed in this cohort. Of the six cases, c34G>T (G12C) mutation was more frequent, which was seen in four cases, c35G>A (G12D) in one case, while another case showed c35G>T (G12V) mutation [Fig.4a—c].

Discussion

Lung cancer is one of the leading causes of cancer associated mortality, and remains a major health related concern worldwide. Although tremendous progress has been made in therapeutic strategies for lung cancer in recent times, but the 5-year survival rate is still fairly grim accounting for only about 15 % [33, 34]. Analyses of molecular lesion of predictive nature among patients with locally advanced or metastatic NSCLC is important for the selection of targeted therapy, and can prove to be a milestone in patient management. In this context, two gene mutations namely EGFR and KRAS mutations have been widely studied for predicting response to anti-EGFR therapy. While detection of mutations in the EGFR gene has dramatically changed the treatment strategies in NSCLC, and that the patients with mutated EGFR proteins are susceptible to inhibition by tyrosine kinase inhibitors (TKIs), mutations in the KRAS gene is an important predictor of resistance to therapy with no response to TKIs [17].

In the current study, we assessed the frequency and distribution pattern of EGFR (n=400) and KRAS mutation (n=132 EGFR negative cases) in Indian NSCLC patients. Although EGFR mutations in lung cancer are extensively studied worldwide including India [Table 4][27—30, 35—67], to the best of our knowledge, there is no report of KRAS mutation from India, our study being the first. The frequency of EGFR mutations varies considerably across different parts of the globe, with reported incidence in about 14 to 75 %, 7 % to 27 %, and 10 % to 59 % of Asians, Europeans, and Americans respectively [Table 4]. Similarly, the reported frequency of KRAS mutation varies between 20 % to 30 % for the western countries and 5 % to 16 % for Asian population [Table 4]. In this comprehensive analysis of EGFR and KRAS mutations, we have found the frequency of 29 % and 4.5 % respectively.

The frequency of EGFR mutation in the current study is comparable to those published from Korea, Japan, Italy and USA (25-29 %) [41, 48, 56, 59] higher than those from Canada, France, Brazil and Japan (5–21 %) [38, 47, 51, 53], while lower in comparison to Taiwan, China and Mexico (33–54 %) [43, 54, 57]. In comparison to recent Indian studies, our frequency was pretty much similar to some studies 23–32 % [27, 65, 66], though few studies even reported a much higher mutation rate varying between 39–52 % [28, 64, 67] which could be attributed to small sample size, and or clinically selected patients. In contrast to EGFR mutation, the frequency of KRAS mutation in our study is lower then recent reports from western countries (15–30 %) [35, 38, 50], however, it is comparable to recent reports from Asia (3.5–5.2 % [55, 57,

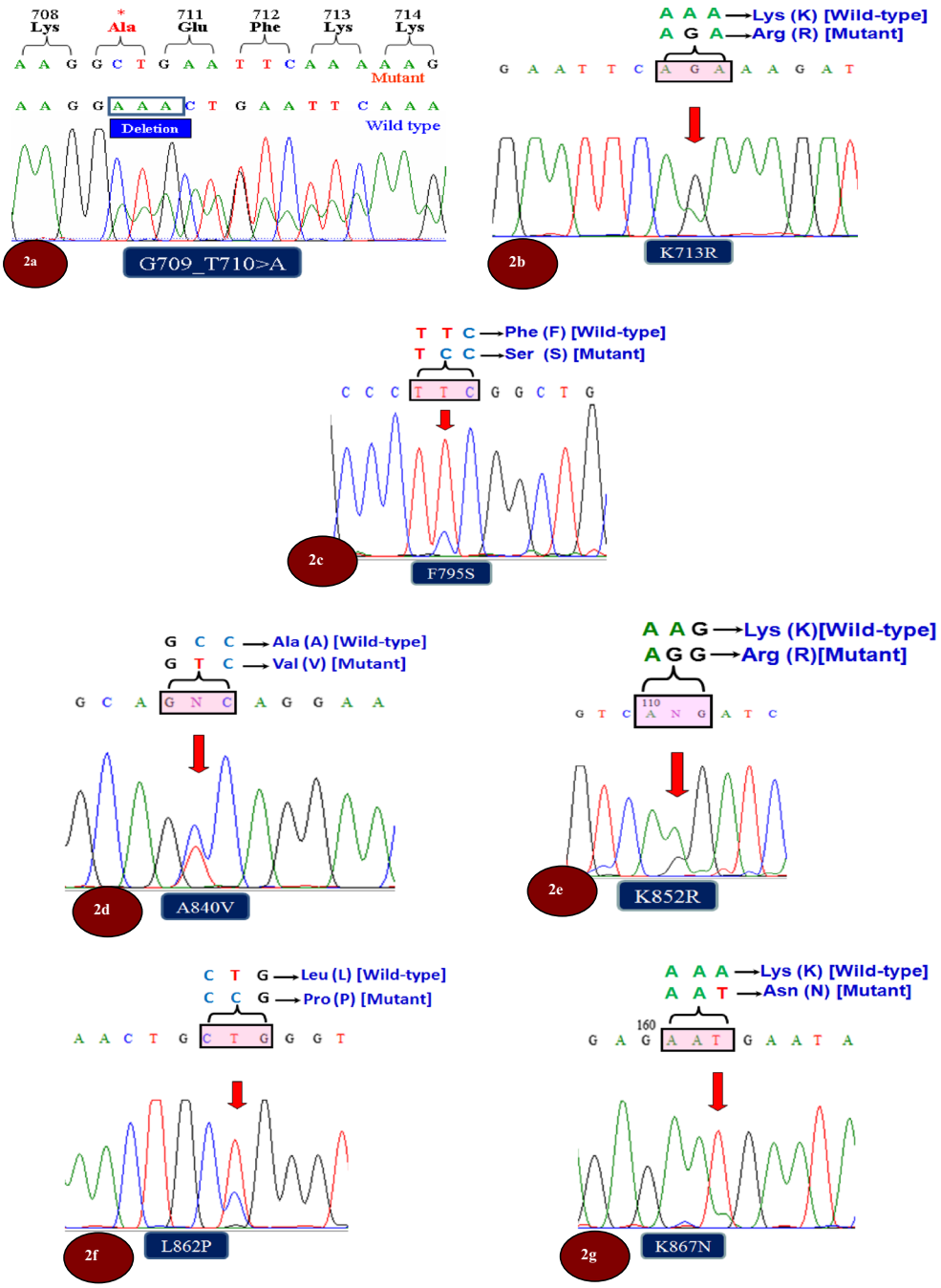

Fig. 2 Partial electropherograms of novel variations: 2a and 2b: exon 18 mutations showing G709_T710>A and K713R respectively; 2c: F795S in exon 20; 2 (d-g) mutations in exon 21 demonstrating A840V, K852R, L862P and K867N respectively

60]. Infact most of the Asian studies have consistently reported frequency lesser than 10 % which is lesser than most of the western studies (15–30 %) [Table 4]. The differences in the frequencies of both of these mutations can be attributed to ethnicity, geographical distribution as well as use of sensitive techniques across some studies [34, 64].

In the current study, EGFR mutations were significantly more frequent in female patients when compared to their male counterpart, which is in agreement with recent studies [27, 64]. To determine the association with age, patients were divided into two groups (≥ 60 and<60 years). Interestingly, EGFR mutations were significantly more frequent in patients with age≥60 years indicating that EGFR mutations are more preponderant in those patients who are younger than 60s. In contrast to this, no significant association of KRAS mutation between these two age groups were observed. Furthermore, the current report as well as previous studies has consistently demonstrated high prevalence of EGFR and KRAS mutation in adenocarcinomas [27, 45, 68]. In case of adenosquamous carcinomas, our EGFR mutation rate is in line with several recent studies (15 % to

Table 3 Distribution pattern of different types of EGFR mutation detected in 116 cases

EGFR exon (N)	Alterations (n)	Amino acid change	Total No
18 [*N*=2]*	Substitution (*n*=1)	K713R	1
	del (*n*=1)	G709_T710>A	1
19 [*N*=75]*	Inframe deletions (*n*=37)	E746_A750del	32
		E746_T751del	1
		L747_P753del	3
		L747_S752del	1
	Indel (*n*=35)	A750_I759>PT	1
		E746_ S752>V	6
		E746_A750>A	1
		E746_E749>A	1
		E746_P753>LQSA	1
		E746_S752>A	1
		E746_T751>A	3
		E746_T751>AA	1
		E746_T751>D	1
		E746_T751>Q	2
		E746_T751>V	1
		L747 _A750>P	5
		L747_A755>SMS	1
		L747_P753>L	1
		L747_P753>S	3
		L747_T751>A	1
		L747_T751>P	4
		R748_P753>S	1
	Substitution (*n*=3)	L739E	1
		I744M	1
		E749K	1
20 [*N*=9]*	Substitution (*n*=8)	T790M	5
		F795S	1
		R776C	2
	duplication (*n*=1)	A767_V769dup	1
21 [*N*=40]*	Substitution (*n*=40)	A840V	1
		K846R	1
		P848S	1
		K852R	1
		L858R	31
		L861R	1
		L862P	1
		K867N	1
		T847A	1
		V851A	1

"*" indicates total number of cases which includes isolated as well as double mutation of two exons in the same case

27 %) [69, 70], though a higher prevalence of these mutations in adenosquamous carcinomas has also been reported from Asia [71]. Furthermore, it is interesting to note although a previous study from Italy reported no EGFR mutation among 454 patients with squamous cell carcinoma, as well as the NCCN guidelines not recommending EGFR mutation testing in squamous cell carcinoma [22, 72], we noticed frequent mutations in squamous cell carcinomas (16.2 %) which is even higher than recent Indian studies (4-10 %) [27, 64].

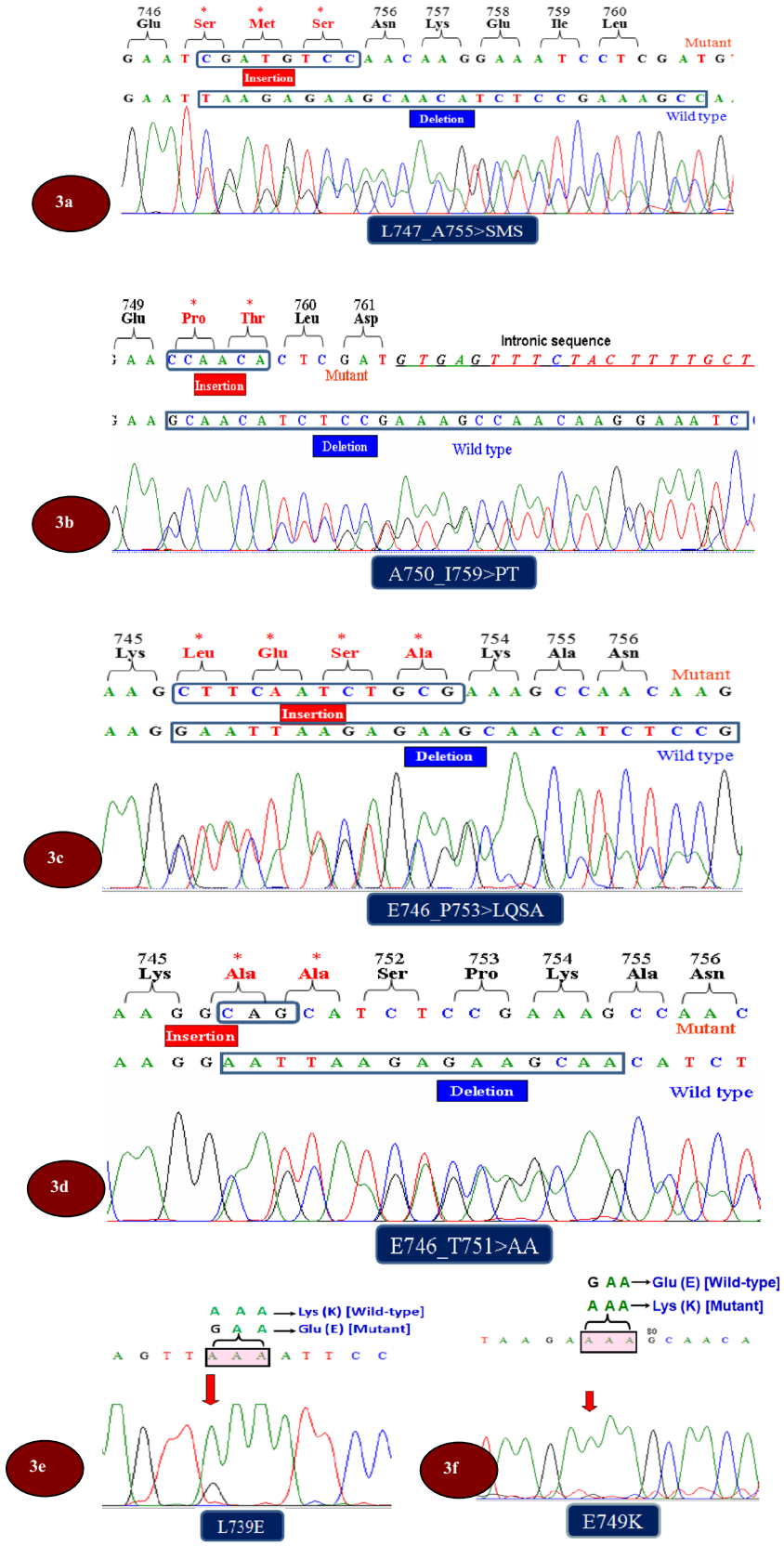

Fig. 3 Partial electropherograms of novel variations in exon 19: 3a-3d mutations showing complex mutations involving simultaneous indels resulting in L747_A755>SMS, A750_I759>PT, E746_P753>LQSA, and E746_T751>AA respectively. 3e and 3f shows two substitution mutations resulting in L739E and E749L respectively

Our study is in agreement with another recent report from Korea wherein, 6 out of 54 patients (11.1 %) with squamous cell carcinoma demonstrated EGFR mutation [73]. Albeit, larger prospective studies are warranted to

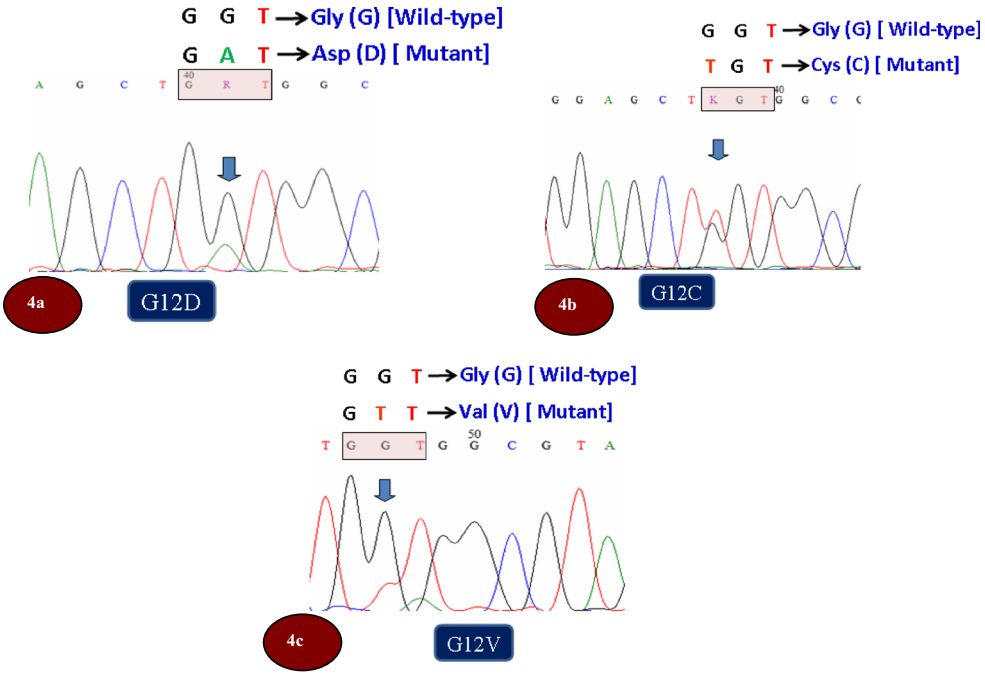

Fig. 4 Partial electropherograms of KRAS mutation: 4a. KRAS c.35G>A (G12D)

better characterize the prevalence of EGFR mutation status and its effect in the clinical outcome of patient with squamous cell lung carcinoma. Nevertheless, none of the other histological tumor type demonstrated KRAS mutation indicating the less role of KRAS mutation in this cohort of patients.

Direct sequencing remains one of the gold standard techniques for mutation detection because it enables to visualize the exact nucleotide change thereby revealing both known as well as identify novel variations which cannot be detected by probe based mutation detection such as those by real time PCR despite the fact that real time PCRs are more sensitive. Sequencing analysis revealed that most of the EGFR mutations in our study clustered overwhelmingly in exon 19 (n=75), followed by exon 21 (n=40), exon 20 (n=9) and least in exon 18 (n=2). The frequency of exon 18 mutation in the current study is nearly comparable to some studies (0.8 % to 0.9 %) [22, 74, 75] while it is lower than other reports (1.9 % to 4 %) [21, 64, 76]. As far as the alteration of exon 19 is concerned, it was the most frequently mutated exon observed in ~19 % (75/400) of the cases indicating that the frequency of this mutation varies globally, with some studies having a lower frequency (4.8 % to 12.5 %) [22, 74, 75], some higher (27.5 % to 48.3 %) [77–79], and some having almost the same frequency as ours (~15 %) [80]. In this study, in agreement with data from others [12, 23], the majority of EGFR mutations were in-frame deletions (49.3 %, 37/75) wherein E746_A750del was the most common type of deletions [Table 4]. Similarly, ~47 % (35/75) cases showed complex indel mutations, wherein E746_S752>V (17.1 %, 6/35) indels were more common. Nevertheless, substitution mutations were less frequently noticed in exon 19 (4 %, 3/75). Strikingly, to our knowledge, six cases showed novel variation in exon 19 which is not yet reported in the literature [Table 2].

Mutations in the exon 20 are infrequent with reported frequency ranging between 0.9 % to 6 %, thereby suggesting that our frequency of 2.2 % tallies with previous reports [75, 79]. The T790M point mutation was the most recurrent genetic alteration detected in five cases, and this mutation has been demonstrated to result in EGFR TKI resistance in previous study [14]. All the mutation in exon 21 were of missense types with L858R as the most frequent type (n=31/40). The frequency of exon 21 mutation in the current study (10 %) is similar to some studies (9.3 % to 15 %) [77, 80, 81], higher than other research groups (2 % to 5.7 %) [22, 24], while was lower than few reports (17 % to 19 %) [21, 64, 76].

As reported earlier that KRAS mutations can be clinically useful for the selection of patients for EGFR-directed TKIs and other targeted therapies, particularly in EGFR wild type cases [17]. Sequencing analysis of such cases revealed G12C, G12D and G12V mutation of codon 12 while codon 13 mutations were not observed suggesting that codon 13 mutations are less frequent in NSCLC patients which is in

Table 4 Comparison of worldwide incidence of EGFR and KRAS mutations from various countries

Geographical region	Country	Year	Total patients	EGFR mt %	KRAS mt %	References
Western countries	USA	2005	274	13	21	35
	USA	2006	159	8.8	11.3	36
	USA	2007	71	9.8	22.8	37
	Canada	2008	206	17	15	38
	Austria	2009	96	7	38	39
	USA	2010	297	15	18 (49/275)	40
	Italy	2010	67	26.7	17.9	41
	UK	2011	126	10.3	17.5	42
	Mexico	2011	1150	33.2	16.6	43
	USA	2011	175	19.4	23.6	44
	Germany	2011	493	11 (49/437)	18	45
	USA	2012	344	17	24	46
	France	2012	307	14	14	47
	USA	2013	49	29	21	48
	Czech Republic	2013	223	7.2	7.4	49
	Netherland	2013	368	10.9	30	50
	Brasil	2014	88	3.4	5.7	51
Asian Countries	Japan	2004	277	40	13	52
	Japan	2005	617	21	8	53
	China	2006	215	53.4	9.8	54
	Korea	2007	115	17.4	5.2	55
	Korea	2007	133	24	12	56
	Taiwan	2008	237	40.8	3.8	57
	Korea	2009	104	24	9.6	58
	Japan	2012	77	27	1	59
	Korea	2012	229	48	3.5	60
	China	2013	251	55.8	7.2	61
	Japan	2014	58	45	19	62
	Japan	2014	411	35	8.5	63
Indian Studies	India	2011	220	51.8	Not Done	64
	India	2013	367	32	Not Done	65
	India	2013	1018	25	Not Done	66
	India	2013	907	23	Not Done	27
	India	2013	106	39.6	Not Done	28
	India	2013	166	25.9	Not Done	29
	India	2013	111	35.1	Not Done	30
	India	2013	1036	40.3	Not Done	67
	India	2014	400	29	4.5 (6/132)	Present Study

agreement with previous findings [82]. Our study being the first to evaluate the presence of KRAS mutation in NSCLC patients highlights the fact that more ongoing larger studies are warranted to evaluate the true clinical utility of KRAS mutation in Indian NSCLC patients.

In conclusion, the current study highlights the frequency and distribution pattern of EGFR and KRAS mutation in Indian cohort. The current study identified several novel variations that added now insights into the genetic heterogeneity of NSCLC patients. Furthermore, this is the first study to report the presence of KRAS mutation in Indian patients. Similarities and dissimilarities of the present findings with those of other researchers may be attributed to the influence of different technologies, differences in ethnic origins as well as differential environmental exposure to unknown carcinogenic agents.

Conflict of Interest None

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