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The Difference of Clinical Characteristics Between Patients With Exon 19 Deletion and Those With L858R Mutation in Nonsmall Cell Lung Cancer

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Abstract: Recent studies have demonstrated that exon 19 deletion (19 Del) and exon 21 L858R mutation (L858R) are 2 different types of sensitive epidermal growth factor receptor (EGFR) mutations in nonsmall cell lung cancer (NSCLC). However, whether there are some differences between those 2 groups in baseline clinical characteristics is still unclear.

We enrolled consecutive 1271 NSCLC patients detected with either 19 Del or L858R and collected their baseline clinical characteristics including age, sex, comorbidity, smoking and drinking status, body mass index (BMI), TNM stage, histologic type, differentiation, tumor maximum diameter (TMD), and CEA level. χ^2 test and multivariate logistic regression analysis were used to compare the difference.

We found a higher percentage of 19 Del in younger patients group ($<=50$ yr) than L858R ($P<0.001$) through χ^2 test. Besides, patients with 19 Del have higher risk of lymph node metastasis ($P<0.001$). However, there were no significant differences in other items of clinical characteristics between 19 Del and L858R. Multivariate analysis showed similar significant results. Subgroup analysis in different age groups (10 yr as an interval) and N stages (stratified by N0, N1, N2, and N3) also indicated above-mentioned trends.

NSCLC patients with 19 Del are more likely to be young and have lymphatic metastasis than those with L858R. Age and N stage might be considered in predicting EGFR mutation type in NSCLC.

(*Medicine* 94(44):e1949)

Abbreviations: 19 Del = exon 19 deletion, ALK = anaplastic lymphoma kinase, BMI = body mass index, CEA = carcinoembryonic antigen, EGFR = epidermal growth factor receptor, EGFR-TKI = EGFR tyrosine kinase inhibitor, KRAS = kirsten rat sarcoma, L858R = exon 21 L858R mutation, NSCLC = nonsmall-cell lung cancer, OR = odd ratio, TMD = tumor maximum diameter.

INTRODUCTION

Nonsmall-cell lung cancer (NSCLC) is the predominant form of lung cancer, the leading cause of cancer-related mortality worldwide, and patients are usually diagnosed in the advanced stages of disease.[1–4] NSCLC could be caused by the accumulation of genetic alterations. The most common one is kirsten rat sarcoma viral oncogene (KRAS) mutations (22%), followed by epidermal growth factor receptor (EGFR) mutations (17%), and anaplastic lymphoma kinase (ALK) rearrangement (7%).[5] EGFR mutations can be divided into common EGFR mutations (19 Del/ L858R) and rare EGFR mutations. While common EGFR mutations considering EGFR tyrosine kinase inhibitors (EGFR-TKIs) as first-line treatment,[6] platinum-based chemotherapy should be a first-line treatment for rare EGFR mutation.[7–9]

However, 19 Del and L858R are 2 different types of sensitive EGFR mutations in NSCLC. Recent studies have reported that 19 Del and L858R have different responses to EGFR-TKIs. EGFR-TKIs treatment is more effective than chemotherapy in 19 Del patients. But for the patients with L858R, EGFR-TKIs treatment and chemotherapy have similar effect and chemotherapy might even be better.[10,11] This breaks the previous idea that EGFR mutation patients should use TKI as possible. It also indicated that the 2 population, 19 Del patients and L858R ones, are different. However, whether there were any differences between those 2 groups in clinical characteristics is still unclear.

Therefore, we sought to conduct a retrospective study to assess the difference of clinical characteristics between patients with 19 Del and those with L858R in nonsmall cell lung cancer in southern Chinese.

Editor: Yinyuan Wu.
Received: August 1, 2015; revised: October 6, 2015; accepted: October 7, 2015.
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YZ, DH, and WF contributed equally to this work.
All the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.
This study was supported by the following funds: (1) National High Technology Research and Development Program of China (Grant No. 2012AA02A502); (2) Innovative drug R&D center based on real-time high-throughput cell-based screening platform and large capacity compound library (Grant No. 2013ZX09401003-002); (3) National Natural Science Funds of China (Grant No. 81372502); (4) Natural Science Foundation of Guangdong (Grant No. S2013010016564); (5) Specialized Research Fund for the Doctoral Program of Higher Education (20120171120116); (6) Young Teacher Training Program of Sun Yat-Sen University (14ykpy38); (7) Outstanding Young Talent Cultivation Project of Sun Yat-Sen University Cancer Center (04140701); (8) Wu Jieping Medical Foundation Project (Grant No. 320.6750.131).
The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001949

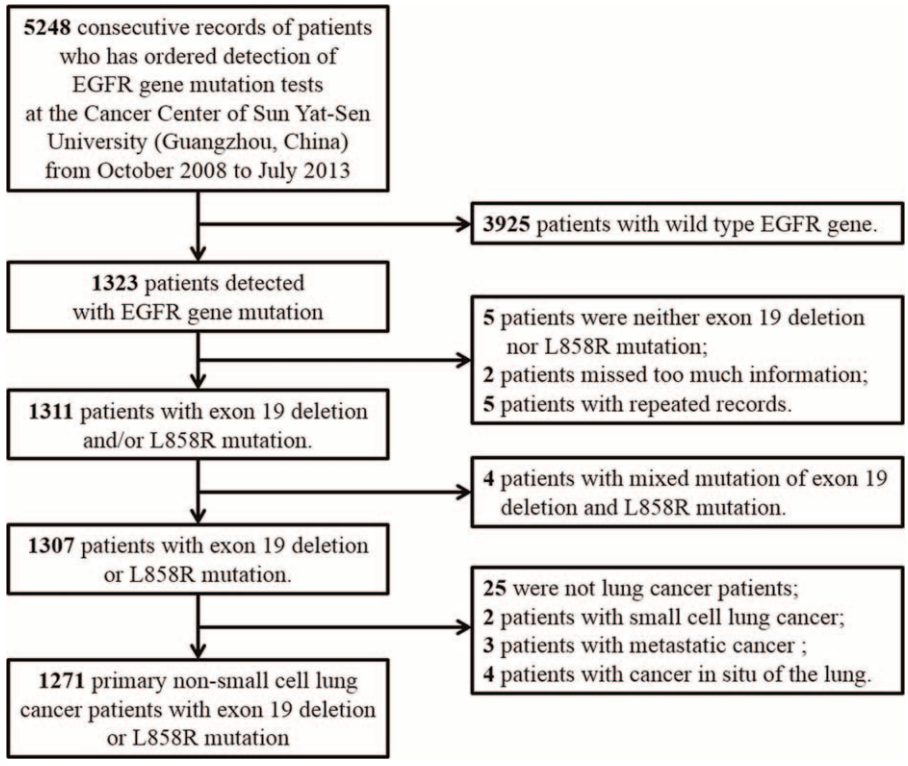


FIGURE 1. Flow chart of the enrollment.

MATERIALS AND METHODS

Study Population

We identified 1323 records of patients who had EGFR gene mutation positive in 5248 patients from October 2008 to April 2014 at Sun Yat-Sen University Cancer Center, Guangzhou, China. The study was approved by the Institutional Review Board of Sun Yat-Sen University Cancer Center. All the patients had provided written informed consent before samples were collected. In these records, 5 are neither 19 Del nor L858R. Two records are missing too much information while another 5 are repeat records. Therefore, we included 1311 records of patients. Figure 1 summarizes the flow chart. Among these patients, 4 are mixed mutation of 19 Del and L858R. Two are small cell lung cancer patients and 25 of them are not lung cancer patients. In addition, 3 patients' lung cancer is metastatic. Another 4 patients are cancer in situ of the lung with T-stage marking Tis. The above patients were excluded and finally 1271 patients were included in the study. The clinicopathological features of the patients included age, sex, and comorbidity, smoking history, drinking history, body mass index (BMI), TNM stage, histologic type, differentiation, tumor maximum diameter (TMD), and carcino-embryonic antigen (CEA) level.

Categories of each characteristic were divided as follows: for age, patients more than 50 years old were considered the older group. Smoking, alcohol history, and comorbidities were noted as yes or no. BMI equal to or larger than 24 kg/m^2 was considered overweight group and the rest as normal group. Histologic type is divided into adenocarcinoma and non-adenocarcinoma. Differentiation is considered high, moderate, and low. High CEA level was defined, if CEA level in serum was >5 ng/mL. Similarly, large tumor was defined when the TMD was >3 cm. For N stage, we considered N0 as ''without lymphatic metastasis'' group and N1, N2, N3 as ''with lymphatic metastasis'' group. Similarly, M0 was defined as non-metastasis group and M1 as metastasis group.

EGFR Mutation Detection

EGFR mutations were detected using PCR-based direct sequencing of exons 18–21. The method is briefly introduced as follows.

First, genomic DNA was extracted from tumors embedded in paraffin blocks or fresh frozen tumors. Then, use Hot Star Taq DNA polymerase (Qiagen Inc, Valencia, CA) to complete PCR amplification with a forward primer (5′-GGATCGGCCTCTT-CATGC-3′) and a reverse primer (5′-TAAAATTGATTC-CAATGCCATCC-3′). Sequencing was performed by ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using Applied Biosystems PRISM dye terminator cycle sequencing method (Perkin-Elmer Corp., Foster City, CA) directly on PCR products. Any in-frame deletions in exon 19 or point mutations in exon 21 (L858R substitutions), which confer sensitivity to EGFR-TKIs therapy, were considered EGFR mutant.

Statistical Analyses

SPSS 16.0 software was used for the statistical analysis. Continuous variables were divided into different categories as mentioned above. All the cut-off values were obtained by X-tile software (Version 3.6.1, Yale University, New Haven, CT), taking clinical expertise into consideration. Further investigations of multivariable analyses were performed by Cox

regression for factors which were significantly associated in univariate survival analyses. Results were reported with odd ratio (OR), corresponding 95% confidence intervals (CI). A P value <0.05 was considered statistically significant.

RESULTS

Clinicopathological Characteristics of the Patients

A total of 1271 NSCLC patients were enrolled in this study. Nineteen Del and L858R accounted for 49.7% (632/1271) and 50.3% (639/1271), respectively. The gender distribution was 579 males and 692 females. There was no difference in the distribution of other basic characteristics (all P value ≥ 0.05), except age and N stage. Clinicopathological features of the patients are presented in Table 1.

Higher Percentage of 19 Del in Younger Patients Group (<= 50 yr) Than L858R

We sought to find out the age difference between 19 Del and L858R patients. The percentages of older group (>50 yr) and younger group (<= 50 yr) were 68.4% (432/632) and 31.6% (200/632) in 19 Del and 79.8% (510/639) and 20.2% (129/639) in L858R, respectively. Univariate analyses indicated that the higher percentage of 19 Del in younger patients group (<= 50 yr) than L858R was significant ($P < 0.001$). Multivariate analysis also showed similar significant result ($P = 0.011$) (Table 2)

We also conducted a subgroup analysis in different age groups. Age group was classified as 21–30, 31–40, 41–50, 51–60, 61–70, 71–80, and 81–90 yr (10 yr as an interval). Nineteen Del patients showed higher percentage than L858R patients in all age groups below 61 yr, including 21–30, 31–40, 41–50, 51–60 yr, while lower percentage in all age groups above 60 yr, including 61–70, 71–80, and 81–90 yr (Figure 2A). This indicated same trends we found in univariate and multivariate analyses.

Patients With 19 Del Have Higher Risk of Lymph Node Metastasis Than L858R

We assessed the lymph node metastasis rate of 19 Del and L858R patients. Lymphatic metastasis rates of 19 Del and L858R were 46.8% (296/632) and 39.6% (253/639), respectively. In univariate analysis, significant difference was observed in distribution of ''without lymphatic metastasis'' group (N0) and ''with lymphatic metastasis'' group (N1, N2, and N3) of each mutation. Patients with 19 Del had higher risk of lymph node metastasis than L858R ones ($P < 0.001$). Multivariate analysis showed the same result ($P = 0.002$) (Table 2).

We also conducted subgroup analysis in N stages (stratified by N0, N1, N2, and N3). The result showed that the percentages of N1, N2, and N3 in 19 Del were 9.7%, 23.6%, and 13.6%, respectively. And in L858R those numbers were 6.6%, 20.3%, and 12.7%, respectively. In each N stage, 19 Del had a higher percentage than L858R, except N0 where the percentage of 19 Del was 19.9% and L858R 29.0% (Figure 2B).

No Significant Differences in Other Items of Clinical Characteristics Between 19 Del and L858R

There was no statistical difference in T or M or TNM stages between 19 Del and L858R patients in univariate analyses. Subgroup analysis in metastatic sites was performed and no statistical difference was found (Figure 2C). Similarly, no statistical significance was found in histologic types or differentiation as well as in tumor size and CEA level (Table 2).

DISCUSSION

NSCLC Patients With 19 Del Are More Likely to Be Young and Have Lymphatic Metastasis Than Those With L858R

NSCLC, as one of the leading causes of cancer-related mortality around the globe, could be caused by the accumulation of genetic alterations. EGFR mutations, usually 19 Del or L858R, count for the second common type of genetic alteration in NSCLC. EGFR tyrosine kinase inhibitors (EGFR-TKIs) were considered to be the first-line therapy for advanced NSCLC patients harboring EGFR 19 Del and L858R.[12–15] Although 19 Del and L858R are both common-type EGFR mutations and under the same first-line therapy recommendation, recent studies have shown that 19 Del patients benefit more from EGFR-TKI therapy than L858R patients.[10,11,16] However, few studies reported the difference of clinical characteristics between 19 Del and L858R patients.

Based on these investigations, we hypothesized that there are some differences between the clinical characteristics of 19 Del and L858R patients. In our study, NSCLC patients with 19 Del did show differences with those L858R patients. NSCLC patients with 19 Del were more likely to be young. Besides, they are more likely to have lymphatic metastasis than L858R patients. We confirmed our findings by multivariate analysis. Subgroup analysis was also performed. Multivariate analysis showed the same trends above, as well as further subgroup analysis. However, there was no statistically significant difference among other aspects we observed in this study.

The difference between 19 Del and L858R may help explain, in some aspects, why 19 Del patients benefit more from EGFR-TKI therapy than L858R patients. The reasons, to some degree, might lay to the young age of 19 Del patients and the inhibition of lymphatic metastasis of EGFR-TKI. Nineteen Del patients are more likely to be younger than L858R patients. Therefore, their basic conditions are better than L858R patients which could help in better prognosis. In addition, EFGR-TKI might inhibit the lymphatic metastasis of NSCLC patients and the inhibition effect would be greater in 19 Del patients because they are more likely to have lymphatic metastasis. The reason why 19 Del patients tend to be younger and have lymphatic metastasis is worthy of further research.

Therefore, we concluded that NSCLC patients with 19 Del are more likely to be young and have lymphatic metastasis than those with L858R.

Age and N Stage Might Be Considered in Predicting EGFR Mutation Type in NSCLC

Although testing of the mutations in EGFR, KRAS, and ALK is the today's standard of care,[15,17] a recent study has reported that the detection rate of epidermal growth factor receptor (EGFR) mutation in NSCLC patients in China was only 9.6% because of the limited prevalence of testing technology and that EGFR-TKIs were used more frequently as salvage rather than upfront therapy.[18] This indicated the

TABLE 1. Baseline Characteristics of Enrolled 1271 Nonsmall-Cell Lung Cancer Patients With Either Exon 19 Deletions or Exon 21 L858R Mutations

Parameter	Exon 19 Deletion (N = 632)	L858R Mutation (N = 639)	*P*
Age, yr			
Available patients (N = 1271)	632	639	
Mean	55.98	59.53	
Range	57 (27–84)	56 (28–84)	
Sex			
Male	288	291	
Female	344	348	0.992
Comorbidity			
No	374	354	
Tuberculosis	1	1	1.000
Hepatitis B	24	23	0.967
Hepatitis C	19	26	0.233
Diabetes	0	2	0.237
Hypertension	86	93	0.425
Coronary heart disease	7	11	0.295
Other	14	19	0.314
NA	107	110	0.593
Smoking			
No	386	402	
Yes	136	127	0.444
NA	110	110	0.790
Drinking			
No	442	452	
Yes	80	75	0.618
NA	110	112	0.977
BMI, kg/m^2			
Available patients (N = 944)	466	478	
Mean	23.029	23.0195	
Range	19.57 (14.57–31.14)	20.34 (12.77–33.11)	
T-stage			
1a	32	39	
1b	25	26	0.666
1a/1b	35	32	0.400
2a	115	143	0.941
2b	15	13	0.446
2a/2b	92	79	0.216
3	31	40	0.866
4	87	77	0.261
NA	200	190	0.336
N-stage			
0	126	185	
1	61	42	0.001
2	149	130	0.002
3	86	81	0.021
NA	210	201	0.005
M-stage			
0	259	280	
1a	82	79	0.521
1b	142	126	0.187
1a/1b	4	0	0.038
NA	145	154	0.902
TNM-stage			
IA	41	49	
IB	58	97	0.211
IIA	32	13	0.005
IIB	13	16	0.945
IIIA	73	70	0.475
IIIB	41	28	0.083

Parameter	Exon 19 Deletion (N = 632)	L858R Mutation (N = 639)	*P*
IV	226	204	0.227
NA	148	160	0.677
Histologic type			
Adenocarcinoma	480	490	
Large cell carcinoma	12	14	0.714
Adenosquamous carcinoma	1	1	1.000
Squamous cell carcinoma	15	9	0.219
other	7	6	0.772
NA	111	119	0.675
Differentiation			
High	63	73	
Moderate	182	193	0.659
Low	226	206	0.223
NA	161	167	0.588
TMD, cm			
Available patients (N = 537)	254	283	
Mean	2.939	2.84	
Range	14.5 (0.5–15.0)	7.6 (0.4–8.0)	
CEA, ng/mL			
Available patients (N = 783)	388	395	
Mean	77.4243	127.33	
Range	4839.8 (0.2–4840)	15262.626 (0.374–15263)	

BMI = body mass index, CEA = carcinoembryonic antigen, NA = not available, TMD = tumor maximum diameter.

TABLE 2. The Difference of Baseline Characteristics Between NonSmall-Cell Lung Cancer Patients With Exon 19 Deletions and Those With Exon 21 L858R Mutations in Univariate and Multivariate Logistic Regression Analysis

			Univariate Analysis				Multivariate analysis* (N = 853)			
Parameter	Exon 19 Deletion	L858R Mutation	OR	LL (95% CI)	UL (95% CI)	*P* Value	OR	LL (95% CI)	UL (95% CI)	*P*
Age, yr										
>50	432	510	0.546	0.423	0.706	0.000	0.663	0.482	0.911	0.011
< = 50	200	129	1							
Sex										
Male	288	291	1.001	0.803	1.249	0.992				
Female	344	348	1							
Comorbidity										
Yes	151	175	0.817	0.629	1.061	0.130	0.885	0.657	1.192	0.422
No	374	354								
Smoking										
Yes	136	127	1.115	0.843	1.475	0.444				
No	386	402								
Drinking										
Yes	80	75	1.091	0.775	1.534	0.618				
No	442	452								
BMI, kg/m^2										
> = 24	165	175	0.949	0.728	1.238	0.700				
<24	301	303								
T-stage										
1	92	97								
2	222	235	1.191	0.784	1.811	0.413				
3	31	40	1.196	0.837	1.710	0.326				
4	87	77	1.458	0.832	2.553	0.187				
T-stage										
1–2	314	332								

Parameter	Exon 19 Deletion	L858R Mutation	Univariate Analysis OR	LL (95% CI)	UL (95% CI)	P Value	Multivariate analysis* (N = 853) OR	LL (95% CI)	UL (95% CI)	P
3–4	118	117	1.066	0.791	1.437	0.673				
N-stage										
0	126	185				0.001				
1	61	42	1.559	1.068	2.276	0.022				
2	149	130	0.731	0.445	1.201	0.216				
3	86	81	0.926	0.631	1.360	0.696				
N-stage										
0	126	185								
1234	296	253	1.718	1.296	2.277	0.000	1.665	1.210	2.291	0.002
M-stage										
0	259	280								
1	228	205	1.202	0.933	1.549	0.154	0.742	0.432	1.275	0.280
TNM-stage										
I	99	146								
II	45	29	1.634	1.189	2.245	0.002				
III	114	100	0.714	0.431	1.181	0.190				
IV	226	204	0.972	0.700	1.350	0.864				
TNM-stage										
I-IIIA	217	247								
IIIB-IV	267	232	1.310	1.017	1.688	0.037	1.223	0.706	2.120	0.473
Histologic type										
Adenocarcinoma	486	490								
Non-adenocarcinoma	35	30	1.176	0.711	1.946	0.527				
Differentiation										
High	63	73								
Moderate	182	193	1.271	0.864	1.871	0.223				
Low	226	206	1.163	0.882	1.535	0.284				
TMD, cm										
<=3	179	196								
>3	75	88	0.933	0.645	1.349	0.713				
CEA, ng/mL										
<=5	165	171								
>5	223	227	1.018	0.767	1.351	0.901				

BMI = body mass index, CEA = carcinoembryonic antigen, CI = confidence interval, LL = lower limit, OR = odd ratio, TMD = tumor maximum diameter, UL = upper limit.

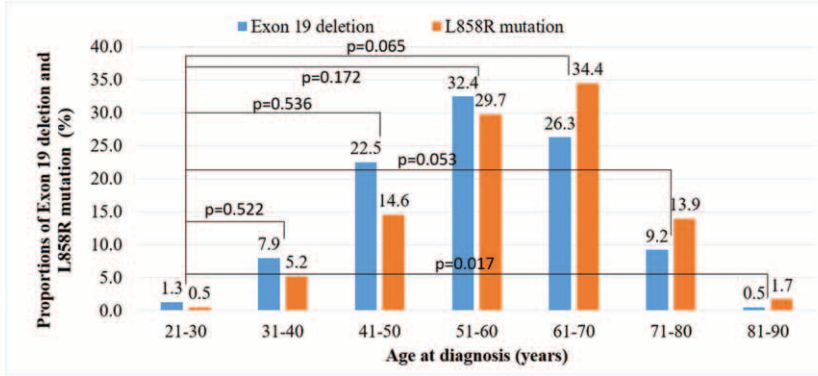
* Only item in univariate analysis with a P value <= 0.200 will be selected for multivariate analysis.

importance of predicting EGFR mutation status and type in NSCLC patients. Previous studies had revealed that Asian nonsmoking women with adenocarcinoma is the population with higher EGFR mutation possibility but not specific to the type of EGFR mutation.[19–22] This population also showed greater benefit in EGFR-TKI therapy.[23–25] However, some recent studies have shown that 19 Del and L858R have different responses to EGFR-TKIs and chemotherapy. Patients with 19 Del benefited more from EGFR-TKIs treatment than chemotherapy, while for the patients with L858R, EGFR-TKIs treatment and chemotherapy have similar effect and chemotherapy might be even better.[10,11] Therefore, prediction specific to the type of EFGR mutation is essential for treatment selection and additional factors for EGFR mutation type prediction are needed.

In our study, we observed that 19 Del patients presented higher percentage of in younger group (<= 50 yr) than L858R, as well as higher lymphatic metastasis risk. It suggested that NSCLC patients with 19 Del are more likely to be young and have lymphatic metastasis than those with L858R. Subgroup analysis performed for age and N-stages also indicates the same conclusion. The above data suggested that EGFR mutation type might be predicted by the patient's age and lymphatic metastasis condition. Young patients with lymph node metastasis at diagnosis would probably be 19 Del rather than L858R. However, the efficiency of this prediction needs further investigation.

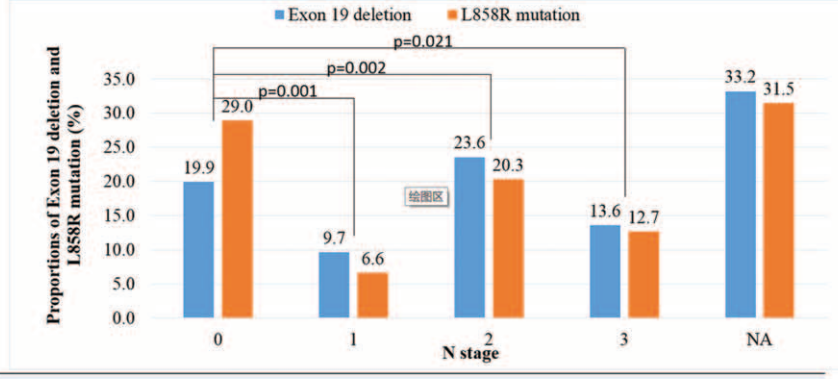
Summary

In conclusion, our investigation suggested that NSCLC patients with 19 Del are more likely to be young and have lymphatic metastasis than those with L858R. It is worthy of further investigation on the underlying mechanism. Besides, age and N stage might be considered in predicting EGFR mutation type in NSCLC, which might help in choosing the initial therapy for EGFR mutation NSCLC.



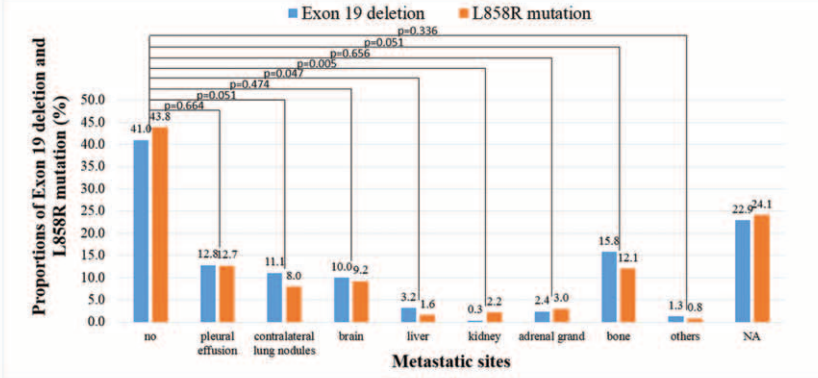
Age at diagnosis		21-30	31-40	41-50	51-60	61-70	71-80	81-90
Exon 19 deletion	N=632	8	50	142	205	166	58	3
L858R mutation	N=639	3	33	93	190	220	89	11

A



N stage		0	1	2	3	NA
Exon 19 deletion	N=632	126	61	149	86	210
L858R mutation	N=639	185	42	130	81	201

B



Metastatic sites		no	Pleural effusion	Contralateral lung nodules	Brain	Liver	Kidney	Adrenal grand	Bone	Others	NA
Exon 19 deletion	N=632	259	81	70	63	20	2	15	100	8	145
L858R mutation	N=639	280	81	51	59	10	14	19	77	5	154

C

FIGURE 2. The incidence of exon 19 deletion and exon 21 L858R mutation in nonsmall-cell lung cancer patients according to different age groups (A), different N-stage groups (B), and different M-stage groups (C).

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