Original Article

Distinct Clinical Features and Outcomes in Never-Smokers With Nonsmall Cell Lung Cancer Who Harbor *EGFR* or *KRAS* Mutations or *ALK* Rearrangement

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BACKGROUND: The objectives of this study were to determine the proportions of major oncogenic alterations and to examine survival in genotype-specific subsets of never-smokers with nonsmall cell lung cancer (NSCLC). **METHODS:** The authors concurrently analyzed mutations in the epidermal growth factor receptor (*EGFR*) and v-Ki-*ras*2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes and investigated anaplastic lymphoma kinase (*ALK*) gene rearrangements in samples from 229 never-smokers with NSCLC. *ALK* rearrangements were identified by fluorescent in situ hybridization and were confirmed by immunohistochemistry. Mutations in *EGFR* (exons 18 to 21) and *KRAS* (codons 12 and 13) were determined by direct sequencing. **RESULTS:** Of 229 tumors, the frequency of *EGFR* mutations, *ALK* rearrangements, *KRAS* mutations, and no mutations (wild type [WT]) in any of the 3 genes (*WT/WT/WT*) was 48%, 8.3%, 3.5%, and 40.2%, respectively. All genetic alterations were mutually exclusive. The median progression-free survival after treatment with EGFR tyrosine kinase inhibitors (TKIs) was 12.8 months, 6.3 months, 2.1 months, and 1.6 months in patients with *EGFR* mutations, the *WT/WT/WT* genotype, *KRAS* mutations, and *ALK* rearrangements, respectively. In a Cox regression model, the adjusted hazard ratio for the risk of disease progression after treatment with EGFR TKIs was 0.59 (95% confidence interval [CI], 0.40-0.87; $P = .008$) for patients with *EGFR* mutations, 4.58 (95% CI, 2.07-10.15; $P < .001$) for patients with *ALK* rearrangements, and 4.23 (95% CI, 1.65-10.8; $P = .003$) for patients with *KRAS* mutations. Overall survival also differed significantly among genotypes. **CONCLUSIONS:** To the authors' knowledge, this was the largest comprehensive and concurrent analysis to date of 3 major oncogenic alterations in a cohort of East Asian never-smokers with NSCLC. Because survival outcomes differed among genotypes, and drugs that target specific alterations currently are available, genetic profiling to identify genotype-specific subsets can lead to successful treatment with appropriate kinase inhibitors. ***Cancer*** **2012;118:729-39.** © *2011 American Cancer Society.*

KEYWORDS: *EGFR*, *KRAS*, *ALK*, never-smoker, nonsmall cell lung carcinoma.

INTRODUCTION

Lung cancer is a leading cause of cancer mortality worldwide, and the majority of lung cancers are caused by tobacco smoking.[1] However, approximately 25% of lung cancers occur in lifelong never-smokers, and the proportion of never-smokers with lung cancer has been increasing over time.[1-4] The development of lung cancer in never-smokers (LCINS) is of great concern in East Asian countries, and some estimates suggest that up to 70% of these cancers in women may be unrelated to tobacco smoking.[1,5-7] Currently, LCINS is regarded as a distinct disease entity with a unique tumorigenic pattern, clinicopathologic features, and natural history.[8,9]

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LCINS is more likely to be addicted to signaling from a single oncogene rather than widespread genetic and epigenetic changes, like those observed in lung cancers observed in smokers.[10] It is noteworthy that mutations in the epidermal growth factor receptor (*EGFR*) and v-Ki-*ras*2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes and anaplastic lymphoma kinase (*ALK*) rearrangements are 3 major recurrent oncogenic alterations associated with LCINS.[11] *EGFR* mutations are the most frequently encountered mutation type in LCINS. *EGFR* mutations have been reported in approximately 50% of never-smoker lung cancer patients compared with 10% of smoker lung cancer patients.[2,12-14] The high frequency of *EGFR* mutations in never-smoker patients is associated with dramatic and durable responses to *EGFR* tyrosine kinase inhibitors (TKIs).[2,15-20]

ALK rearrangement, which results from a small inversion within chromosome 2p, is a newly identified driver oncogene in nonsmall cell lung cancer (NSCLC).[21-23] Results from a recent clinical trial of crizotinib, an inhibitor of the met proto-oncogene (hepatocyte growth factor receptor [*MET*]) and *ALK*, demonstrated a remarkable response rate and benefit of progression-free survival in *ALK*-positive patients.[24] In an unselected NSCLC population, the frequency of *ALK* fusion gene ranged from 1.5% to 7.5%.[21-23,25-29] Recently, Shaw et al[25] reported a significantly higher rate (22%) of *ALK* rearrangements in never/light smokers with NSCLC, suggesting a strong association between *ALK* rearrangements and a never/light smoking history. However, because most studies, including that by Shaw et al, investigated predominantly smokers, the frequency of *ALK* rearrangements in never-smokers remains unknown.[21,25,29-31]

KRAS mutations are oncogenic missense mutations that occur more frequently in adenocarcinomas from smokers.[32,33] However, a more recent study demonstrated that *KRAS* mutations are not rare (approximately 15%) in LCINS; it is noteworthy that *KRAS* mutations in lung cancers from never-smokers are more likely to be transition mutations, unlike those in lung cancers from smokers, which commonly are transversion mutations.[34] Because tumors with *KRAS* mutations display primary resistance to *EGFR* TKIs, molecular evaluation of *KRAS* is important to predict clinical treatment outcomes and to decide on a therapeutic option when treating LCINS.[13,35]

Recently, the frequencies of mutations in oncogenes that repeatedly were mutated in lung cancer patients, including *EGFR*, *KRAS*, neuroblastoma RAS viral oncogene homolog (*NRAS*), v-Ha-ras Harvey rat sarcoma viral oncogene homolog (*HRAS*), human EGFR 2 (*HER2*), v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), *ALK*, phosphoinositide-3-kinase catalytic alpha polypeptide (*PIK3CA*), tumor protein 53 (*TP53*), and serine/threonine kinase 11 (*LKB1*), were examined in never-smokers with lung adenocarcinoma.[36] It is noteworthy that, in the study, approximately 90% of 52 tumor samples harbored well known oncogenic alterations in *EGFR*, *HER2*, *ALK*, and *KRAS*: 78.8% of patients had an *EGFR* mutation, 5.8% had echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion, 3.8% had an *HER2* mutation, and 1.9% had a *KRAS* mutation.[36] However, it is unclear whether the results from that study can be generalized because of the small sample size; furthermore, treatment responses to chemotherapy or specific kinase inhibitors were not evaluated.

Herein, we report a comprehensive and concurrent analysis to date of alterations in 3 major oncogenes associated with lung cancer in the largest ever cohort of East Asian never-smokers with NSCLC. We also evaluated clinicopathologic features and survival outcomes according to genotype. This information has clear therapeutic implications; by identifying clinically relevant molecular subsets of LCINS, patients potentially can be treated successfully with appropriate kinase inhibitors.

MATERIALS AND METHODS

Study Population and Data Collection

Between January 2006 and June 2010, a total of 229 consecutive never-smokers with newly diagnosed and histologically confirmed NSCLC for whom genetic data were available were enrolled at the Yonsei Cancer Center (Seoul, Korea). Tumor histology was classified according to World Health Organization criteria.[37] Never-smokers were defined as individuals who had a lifetime smoking exposure of $<$100 cigarettes.

Medical records of all patients were reviewed to extract data on clinicopathologic characteristics. For patients with metastatic disease, we examined treatment regimens, the overall response rate (ORR), and survival outcomes (progression-free survival [PFS] and overall survival [OS]). Clinical responses were classified using Response Evaluation Criteria in Solid Tumor (version 1.0).[38] PFS was measured from the first day of treatment to tumor progression or death, and OS was measured from the date of diagnosis with metastatic disease until the date of death. In patients with resected NSCLC, recurrence-free survival (RFS) was measured from the date of

surgery for lung cancer until the date of either recurrence or death, and OS was measured from the date of surgery until the date of death. Patients were censored on September 31, 2010 if they were alive and progression-free. Patients without a known date of death were censored at the time of the last follow-up. This study was approved by the Institutional Review Board of Severance Hospital. All patients signed written informed consent for genetic analysis.

EGFR *and* KRAS *Mutation Analysis*

Nucleotide sequencing of the kinase domain of *EGFR* (exons 18 to 21) was performed using nested polymerase chain reaction amplification of the individual exons.[39] Details of sequencing have been described previously. Specific mutations in *KRAS* exon 2 (codons 12/13) were identified as described previously.[34,35]

ALK *Rearrangements*

To identify *ALK* rearrangements, fluorescent in situ hybridization (FISH) studies were performed on formalin-fixed, paraffin-embedded tumors by using a break-apart probe for *ALK* (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbot Park, Ill). A positive FISH result for *ALK* rearrangement was defined as >15% of tumor cells with a split signal and was confirmed by immunohistochemistry. Immunohistochemistry for ALK expression was performed on samples that were identified as positive for *ALK* rearrangement in FISH studies. The formalin-fixed, paraffin-embedded tumors were sectioned at 4 μm thickness and stained using a Ventana automated immunostainer (Ventana Medical Systems, Tucson, Ariz). The antibody against ALK (mouse monoclonal, clone 5A4; Novocastra, Newcastle, United Kingdom) was diluted 1:30, and sections were incubated with this diluted antibody for 2 hours at 42°C. Signals were detected using an iView detection kit (Ventana Medical Systems), which is based on a labeled streptavidin-biotin method.[40]

Statistical Analysis

Significant differences in variables according to each genotype were tested using the chi-square test, the Fisher exact test, or *t* tests, as appropriate. The Kaplan-Meier method was used to estimate PFS, RFS, and OS, and the differences according to genotype were compared using the log-rank test. Adjusted hazard ratios (AHRs) for the risk of progression or death in response to treatment according to genotype were calculated using a Cox regression model that included age, sex, histology, and performance status as independent variables. All *P* values were based on a 2-tailed hypothesis.

RESULTS

Clinicopathologic Characteristics of Distinct Genotypes

We screened 229 never-smokers with NSCLC for genetic analysis. This entire cohort included 30 men and 199 women, and the median patient age was 58 years (range, 30-78 years). Histology revealed that 215 patients had adenocarcinoma (93.9%), 7 patients had squamous cell carcinoma (3.1%), and 3 patients had large cell carcinoma (1.3%) (Table 1). On the basis of their genetic mutations, patients were classified into 4 distinct genotype groups: *EGFR* mutations (n = 110; 48%), *ALK* rearrangements (n = 19; 8.3%), *KRAS* mutations (n = 8; 3.5%), and the wild-type (unmutated) genotype of all 3 genes (*WT/WT/WT*) (n = 92; 40.2%) (Fig. 1). Of the 110 *EGFR* mutations, the majority included deletion mutations in exon 19 (n = 69; 62.7%) and leucine-to-arginine substitution at codon 858 (L858R) point mutations in exon 21 (n = 37; 33.6%). Three patients had double mutations in exon 19 (deletion [del] 2235-2249)/exon 20 (threonine-to-methionine substitution at codon 790 [T790M]), exon 20 (T790M)/exon 21 (L858R), or exon 20 (alanine-to-glycine substitution at codon 871 [A871G])/exon 21 (L858R). In addition, 1 patient had a duplication mutation in exon 20. All *KRAS* mutation-positive tumors had transition mutations in codon 12. The frequency of *ALK* fusion was 17.1% among patients without *EGFR* or *KRAS* mutations.

There were no differences in the baseline characteristics of patients (median age, sex, Eastern Cooperative Oncology Group performance status, histology, the presence of central nervous system metastasis, and stage at diagnosis) according to genotype (Table 2). Consistent with previous studies, *EGFR* mutations, *KRAS* mutations, and *ALK* rearrangements were mutually exclusive. These findings suggested that prospective genotyping for these genetic mutations may lead to the identification of distinct and nonoverlapping molecular subsets of lung cancer patients among never-smokers.

Of 19 *ALK* rearrangement-positive tumors, 18 (94%) were adenocarcinomas, and 1 was a large cell carcinoma. Considering the pathologic subtype of adenocarcinoma according to World Health Organization criteria,[37] *ALK*-positive tumors were significantly more likely to have abundant signet ring cells than the other pathologic tumor types ($P < .001$). When we performed

Table 1. Baseline Characteristics of Genetically Screened Never-Smokers With Nonsmall Cell Lung Cancer (N = 229)

Characteristic	No. of Patients	%
Age, y		
Median	58	
Range	30-78	
Sex		
Men	30	13.1
Women	199	86.9
Histology		
Adenocarcinoma	215	93.9
SCC	7	3.1
Large cell	3	1.3
NSCLC, NOS	4	1.7
Stage[a]		
I	43	18.8
II	31	13.5
IIIA	41	17.9
IIIB	20	8.7
IV	94	41
Genotype		
EGFR	110	48
ALK	19	8.3
KRAS	8	3.5
WT/WT/WT[b]	92	40.2
Type of mutation		
EGFR		
Exon19 deletion	69	62.7
Exon 21 L858R	37	33.6
Others[c]	4	3.7
KRAS		
Gly12Asp (GGT→GAT)	5	62.5
Gly12Ser (GGT→AGT)	3	37.5

Abbreviations: A, adenine; *ALK*, anaplastic lymphoma kinase; Asp, aspartic acid; *EGFR*, epidermal growth factor receptor; G, guanine; Gly, glycine; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; L858R, leucine-to-arginine mutation at codon 858; NOS, not otherwise specified; NSCLC, nonsmall cell lung cancer; SCC, squamous cell carcinoma; Ser, serine; T, thymine; *WT/WT/WT*, wild type for all 3 genes.

[a] Clinical stage at the time of initial diagnosis was determined according to the 6th edition of the American Joint Committee on Cancer *Cancer Staging Manual*.

[b] Three patients had double mutations in exon 19 (deletion 2235-2249)/exon 20 (threonine-to-methionine mutation at codon 790 [T790M]), exon 20 (T790M)/exon 21 (L858R), and exon 20 (alanine-to-glycine mutation at codon 817 [A871G])/exon 21(L858R). In addition, 1 patient had a duplication mutation in exon 20.

[c] *WT/WT/WT* represents the wild-type *EGFR*, *ALK*, and *KRAS* genes.

immunohistochemistry on the FISH-positive tumors, all *ALK* FISH-positive occurrences also were *ALK*-positive in immunohistochemical analyses.

Treatment Outcomes of Metastatic NSCLC According to Genotype

Of 176 patients with metastatic NSCLC, 132 received EGFR TKI treatment (Table 3). In terms of patient genotype, 61 of 80 patients (76.2%) who had *EGFR* mutations, 8 of 13 patients (61.5%) who had *ALK* rearrangements, 5 of 8 patients (71.4%) who had *KRAS* mutations, and 58 of 76 patients (76.3%) who had the *WT/WT/WT* genotype received treatment with EGFR TKIs; the proportion of patients with different genotypes who were treated with EGFR TKIs was not statistically significant (Table 3). Most patients received EGFR TKIs as second-line or third-line treatment (Table 3). None received other TKIs, including *ALK* inhibitors. Among 61 patients who had *EGFR* mutations, 40 patients (65.5%) had a clinical response to EGFR TKIs, including 14 patients (22.9%) who had stable disease (SD) and 7 patients (11.6%) who had had progressive disease (PD) after treatment with EGFR TKIs. None of the patients who had *ALK* rearrangements (n = 8) and received treatment with EGFR TKIs had a clinical response. One patient (12.5%) had SD, and the remaining 7 patients (87.5%) had PD. None of the patients with *KRAS* mutations (n = 5) had a clinical response to EGFR TKIs. One of those patients (20%) had SD, and the remaining 4 patients (80%) had PD. In the group with the *WT/WT/WT* genotype (n = 58), 6 patients (10.3%) had a partial response (PR), 36 patients (62.1%) had SD, and 16 patients (27.6%) had PD after receiving treatment with EGFR TKIs. Patients who had *EGFR* mutations had a significantly higher clinical response rate (65.5%) to EGFR TKIs than patients who had *ALK* rearrangements (0%),

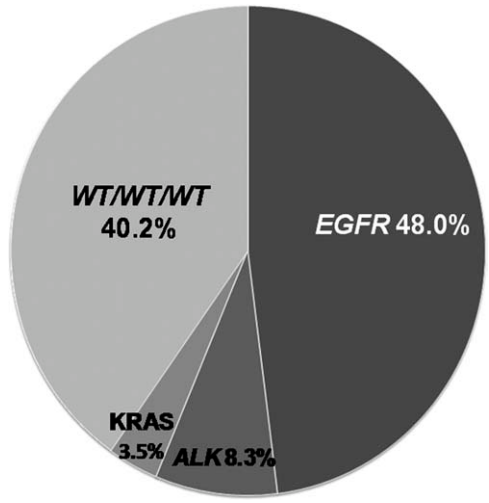

Figure 1. This chart illustrates the proportions of major oncogenic alterations in 229 never-smokers who had nonsmall cell lung cancer. *ALK* indicates anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-*ras*2 Kirsten rat sarcoma viral oncogene homolog; *WT/WT/WT*, wild type (unmutated) of all 3 genes.

Table 2. Clinical Characteristics of Genotype-Specific Subsets of Never-Smokers With Nonsmall Cell Lung Cancer (N = 229)

Characteristic	Genotype: No. of Patients (%)				*P*[a]		
	EGFR, n = 110	*ALK*, n = 19	*KRAS*, n = 8	*WT/WT/WT*, n = 92	*ALK*	*KRAS*	*WT/WT/WT*
Age, y					.372	.582	.954
Median [range]	57 [33-78]	59 [34-78]	61 [46-73]	59 [30-77]			
Sex					.641	.246	.758
Men	16 (14.5)	2 (10.5)	0 (0)	12 (13)			
Women	94 (85.5)	17 (89.5)	8 (100)	80 (87)			
ECOG PS 0-1	110 (1000)	19 (100)	8 (100)	92 (100)			
Histology					.891[b]	.018[b]	.538[b]
Adenocarcinoma	105 (95.5)	18 (94.7)	6 (75)	86 (93.5)			
SCC	2 (1.8)	0 (0)	0 (0)	5 (5.4)			
Large cell	1 (0.9)	1 (5.3)	0 (0)	1 (1.1)			
NSCLC, NOS	2 (1.8)	0 (0)	2 (25)	0 (0)			
CNS metastasis	9 (8.2)	1 (5.3)	1 (12.5)	7 (7.6)			
Stage[c]					.786[d]	.470[d]	.203[d]
I	24(21.9)	4 (21.1)	2 (25)	13 (14.2)			
II	19 (17.3)	3 (15.8)	0 (0)	9 (9.8)			
IIIA	19 (17.3)	4 (21.1)	1 (12.5)	19 (20.7)			
IIIB	6 (5.5)	1 (5.3)	2 (25)	9 (9.8)			
IV	42 (38.2)	7 (36.8)	3 (37.5)	42 (45.7)			

Abbreviations: *ALK*, anaplastic lymphoma kinase; CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NOS, not otherwise specified; NS, nonsignificant; NSCLC, nonsmall cell lung cancer; SCC, squamous cell carcinoma; *WT/WT/WT*, wild type for all 3 genes.

[a] All *P* values were derived by comparing with EGFR mutant genotype.

[b] Adenocarcinoma versus all others.

[c] Clinical stage at the time of initial diagnosis was determined according to the 6th edition of the American Joint Committee on Cancer *Cancer Staging Manual*.

[d] Stages I to IIIA versus stages IIIB/IV.

KRAS mutations (0%), or no mutations in these oncogenes (*WT/WT/WT*; l10.4%; $P < .001$).

We also evaluated the ORR to platinum-based chemotherapy, which was administered as a first-line therapy in the majority of patients. Of 146 patients who received platinum-based chemotherapy, clinical responses were observed in 17 patients (25.4%) with *EGFR* mutations (n = 67), in no patients with *ALK* rearrangements (n = 12), in 1 patient (16.7%) with a *KRAS* mutation (n = 6), and 14 patients (23%) with the *WT/WT/WT* genotype (n = 61) (Table 3). Of the patients who had *EGFR* mutations, 39 had SD, and 10 patients had PD. Of the patients with *ALK* rearrangements, 9 patients had SD, and 3 patients had PD (Table 3). There was no significant difference in the ORR to platinum-based chemotherapy among the genotypes ($P = .352$).

At the time of this analysis, the median follow-up duration of patients with metastatic NSCLC was 22.8 months. Eighty-seven of 176 patients (49.4%) remained alive at the time of analysis. The 4 genotypes differed significantly in terms of median PFS after treatment with EGFR TKIs (12.8 months for patients with *EGFR* mutations vs 6.3 months for patients with the *WT/WT/WT* genotype vs 2.1 months for patients with *KRAS* mutations vs 1.6 months for patients with *ALK* rearrangements) (Fig. 2A). In pair-wise comparisons, we observed that patients with *EGFR* mutations or the *WT/WT/WT* genotype had a significantly longer median PFS than patients with *ALK* rearrangements or *KRAS* mutations (*EGFR* mutation vs *ALK* rearrangement, $P < .001$; *EGFR* mutation vs *KRAS* mutation, $P = .002$; *WT/WT/WT* genotype vs *ALK* rearrangement, $P = .001$; *WT/WT/WT* genotype vs *KRAS* mutation, $P = .029$). Patients with *EGFR* mutations tended to have a longer median PFS after treatment with EGFR TKIs than patients with the *WT/WT/WT* genotype ($P = .093$), and the lack of statistical significance may have been because of the small numbers of patients evaluated.

The median PFS to platinum-based chemotherapy was not significantly different among the genotypes (7.1 months for patients with *EGFR* mutations vs 5.9 months for the *WT/WT/WT* genotype vs 7.2 months for patients with *KRAS* mutations vs 5.0 months for patients with *ALK* rearrangements; $P = .214$) (Fig. 2B). In pair-wise comparisons, we observed that only patients who had *EGFR* mutations had a significantly longer median PFS

Table 3. Summary of Treatment Outcomes by Genotype in Metastatic Nonsmall Cell Lung Cancer

Variable	Genotype: No. of Patients (%)				*P*[a]		
	EGFR, n = 80	*ALK*, n = 13	*KRAS*, n = 7	*WT/WT/WT*, n = 76	*ALK*	*KRAS*	*WT/WT/WT*
Type of treatment							
Chemotherapy	67 (83.3)	12 (84.6)	6 (85.7)	61 (80.3)	NS	.709	.465
EGFR TKI	61 (76.2)	8 (61.5)	5 (71.4)	58 (76.3)	.325	NS	.316
Line of chemotherapy					NS	NS	NS
First line	67 (100)	12 (100)	6 (100)	61 (100)			
Line of EGFR TKI					.104	.851	.733
First line	0 (0)	0 (0)	0 (0)	2 (3.4)			
Second line	41 (67.2)	8 (100)	3 (60)	40 (69)			
Third line	20 (32.8)	0 (0)	2 (40)	16 (27.6)			
Best response to chemotherapy							
PR	17 (25.4)	0 (0)	1 (16.7)	14 (23)			
SD	39 (58.2)	9 (75)	4 (66.7)	33 (54.1)			
PD	10 (14.9)	3 (25)	1 (16.7)	14 (23)			
Unevaluable[b]	1 (1.5)	0 (0)	0 (0)	0 (0)			
ORR	17 (25.4)	0 (0)	1 (16.7)	14 (23)	.060	.635	.837
DCR	56 (83.6)	9 (75)	4 (83.4)	47 (77.1)	.437	NS	.380
Best response to EGFR TKI							
PR	40 (65.5)	0 (0)	0 (0)	6 (10.3)			
SD	14 (22.9)	1 (12.5)	1 (20)	36 (62.1)			
PD	7 (11.6)	7 (87.5)	4 (80)	16 (27.6)			
ORR	40 (65.5)	0 (0)	0 (0)	6 (10.3)	.017	.039	<.001
DCR	54 (88.4)	1 (12.5)	1 (20)	41 (72.4)	<.001	<.001	.008

Abbreviations: *ALK*, anaplastic lymphoma kinase; DCR, disease control rate (complete and partial responses plus stable disease); *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NS, nonsignificant; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor; *WT/WT/WT*, wild type for all 3 genes.

[a] All *P* values were derived from comparisons with the *EGFR* mutant genotype.

[b] One unevaluable patient was lost to follow-up.

compared than patients who had *ALK* rearrangements ($P = .044$). In a Cox regression model adjusted for age, sex, histology, and performance status, the AHR for the risk of disease progression after EGFR TKI treatment was 0.592 (95% confidence interval [CI], 0.401-0.873; $P = .008$) for patients with *EGFR* mutations, 4.583 (95% CI, 2.070-10.148; $P < .001$) for patients with *ALK* rearrangements, and 4.225 (95% CI, 1.653-10.801; $P = .003$) for patients with *KRAS* mutations (Table 4). These results indicate that *EGFR* mutations are a strong, positive predictive factor for a longer median PFS after EGFR TKI treatment, whereas *ALK* rearrangements and *KRAS* mutations have a negative predictive impact. No genotype had a predictive impact on the treatment outcomes in response to platinum-based chemotherapy.

The median OS differed significantly according to genotype and 37.2 months for patients with *EGFR* mutations, 14.3 months for patients with *ALK* rearrangements, 15.6 months for patients with *KRAS* mutations, and 33.3 months for patients with the *WT/WT/WT* genotype ($P = .004$). In pair-wise comparisons, patients with *EGFR* mutations or with the *WT/WT/WT* genotype had a significantly longer median OS than patients with *ALK* rearrangements. In addition, patients with *EGFR* mutations had a significant longer median OS than patients with *KRAS* mutations (*EGFR* mutations vs *ALK* rearrangements, $P = .001$; *WT/WT/WT* vs *ALK* rearrangements, $P = .016$; *EGFR* mutations vs *KRAS* mutations, $P = .026$) (Fig. 2C). In a Cox regression model adjusted for age, sex, histology, and performance status, the AHR for the risk of OS was 0.623 (95% CI, 0.404-0.960; $P = .032$) for patients with *EGFR* mutations, 2.735 (95% CI, 1.329-5.626; $P = .006$) for patients with *ALK* rearrangements, and 2.208 (95% CI, 0.876-5.564; $P = .093$) for patients with *KRAS* mutations (Table 4).

Treatment Outcome of 4 Distinct Genotypes in Resected NSCLC

In addition, we analyzed RFS in 119 patients with resected NSCLC who underwent radical surgery. In our

study, no patients received EGFR TKIs as adjuvant treatment. There was no significant difference in the median RFS among patients according to their genotype (39.7 months for *EGFR* mutations vs 20.0 months for *ALK* rearrangements vs 21.4 months for *KRAS* mutations vs 26.8 months for *WT/WT/WT*; $P = .344$) (Fig. 3A). In a Cox regression model adjusted for age, sex, histology, performance status, and stage, *ALK* rearrangements were associated with a lower OS in patients with resected NSCLC (AHR, 4.162; 95% CI, 1.529-11.341; $P = .005$) (Table 5). This suggests that *ALK* rearrangement may be a negative prognostic factor for early stage NSCLC. However, because the *ALK* rearrangement cohort did not benefit from treatment with EGFR TKIs, it should be noted that the OS is confounded by postrecurrence treatment.

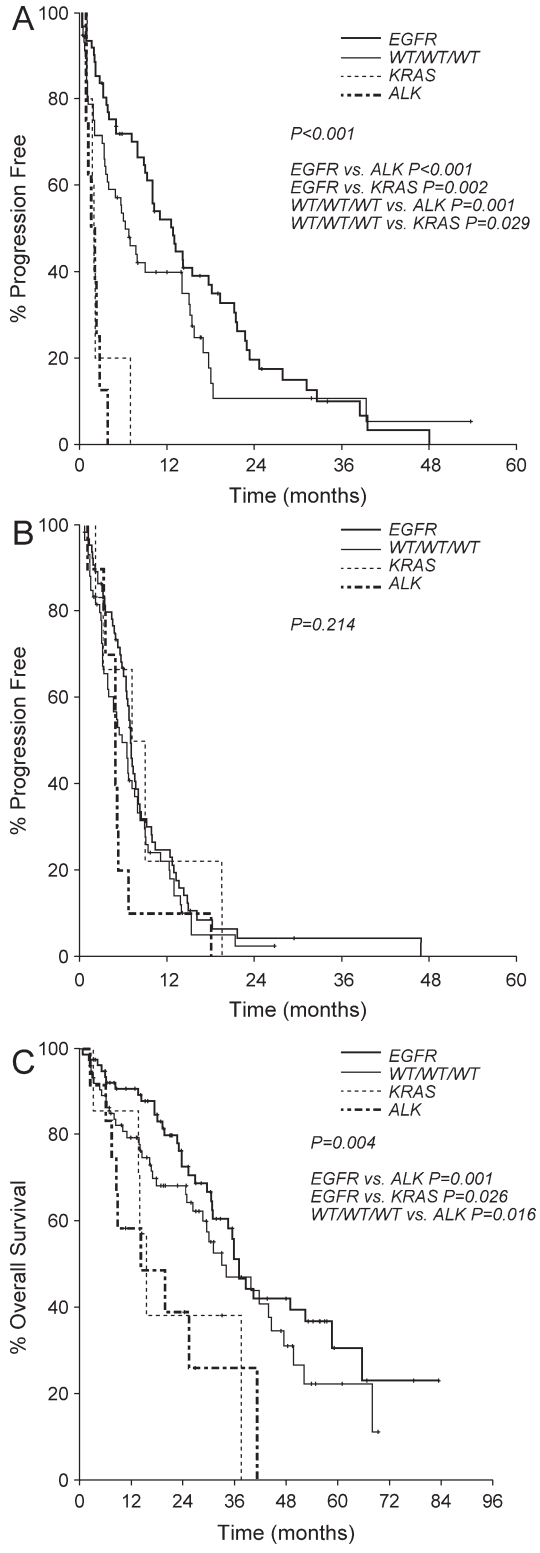

Figure 2. Progression-free survival (PFS) and overall survival (OS) are illustrated in 4 genotypes of metastatic nonsmall cell lung cancer (NSCLC): epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), v-Ki-*ras*2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*), and the unmutated wild type (*WT*) of all 3 genes (*WT/WT/WT*). (A) PFS is illustrated in patients who received EGFR tyrosine kinase inhibitor (TKI) treatment according to genotype: *EGFR* (n = 61), *ALK* (n = 8), *KRAS* (n = 5), and *WT/WT/WT* (n = 58). (B) PFS is illustrated in patients who received platinum-based chemotherapy according to genotype: *EGFR* (n = 67), *ALK* (n = 12), *KRAS* (n = 6), and *WT/WT/WT* (n = 61). (C) This Kaplan-Meier survival plot illustrates the OS of patients with metastatic disease according to genotype: *EGFR* (n = 80), *ALK* (n = 13), *KRAS* (n = 7), and *WT/WT/WT* (n = 76).

DISCUSSION

LCINS is a serious problem that should not be ignored, especially in East Asian countries. Remarkable progress has been made in identifying the genetic and epigenetic alterations that define LCINS as a distinct disease entity.[41-44] Among many alterations, *EGFR* mutations, *ALK* rearrangements, and *KRAS* mutations are the 3 most frequently identified and clinically relevant genetic alterations in LCINS.[2,12-14,20-23,25-29,34] Genetic profiling of alterations in these major oncogenes can help identify genotype-specific subsets with the ultimate goal of oncogene-specific, targeted treatment.

To our knowledge, this is the most comprehensive study to date of alterations in the 3 oncogenes associated with lung cancer in the largest-ever cohort of East Asian never-smokers with NSCLC. Although previous studies have examined the clinical features and treatment outcomes of patients with lung cancer in both never-smokers and smokers,[25] we focused only on LCINS. Other studies have examined LCINS for multiple mutations, including *EGFR* and *KRAS* mutations, but did not include *ALK* rearrangements.[41,45] More recently, Sun et al reported

Table 4. Predictive Impact of Each Gene on Clinical

PFS Outcomes in Patients With Metastatic Nonsmall Cell Lung Cancer

	EGFR TKIs			Chemotherapy			OS[a]		
Genotype	No. of Mutant/WT	AHR (95% CI)[b]	*P*	No. of Mutant/WT	AHR (95% CI)[b]	*P*	No. of Mutant/WT	AHR (95% CI)[b]	*P*
EGFR: Positive vs negative	61/71	0.592 (0.401-0.873)	.008	67/79	0.767 (0.536-1.099)	.148	80/ 96	0.623 (0.404-0.960)	.032
ALK: Positive vs negative	8/124	4.583 (2.070-10.148)	< .001	12/134	1.573 (0.815-3.036)	.177	13/163	2.735 (1.329-5.626)	.006
KRAS: Positive vs negative	5/127	4.225 (1.653-10.801)	.003	6/140	0.665 (0.246-1.798)	.422	7/169	2.208 (0.876-5.564)	.093

Abbreviations: AHR, adjusted hazard ratio; *ALK*, anaplastic lymphoma kinase; CI, confidence interval; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; OS, overall survival; PFS, progression-free survival; TKIs, tyrosine kinase inhibitors; WT, wild type.
[a] OS survival was measured from the date of the diagnosis of metastatic disease until death.
[b] Adjusted for age, sex, histology, and performance status.

multiple genetic alterations in lung adenocarcinoma samples from never-smokers. However, those authors did not examine survival outcomes after chemotherapy or EGFR TKI treatment and only investigated a small patient cohort.[36]

The main finding of our study is that genetic profiling of 3 oncogenes enabled the classification of East Asians with LCINS into 4 distinct genotype groups. Each genotype was associated with a different response and survival after treatment with EGFR TKIs: Patients with *EGFR* mutations had an approximately 40% reduction in disease progression or death, whereas patients with *ALK* rearrangements or *KRAS* mutations had significantly poorer outcomes after treatment with EGFR TKIs. Therefore, before treating LCINS, we highly recommend screening for alterations in these 3 oncogenes.

Clear differences in treatment outcomes among genotypes can be predicted by understanding the molecular basis of the disease. Tumors with *EGFR* mutations become reliant on the constitutive activity of the oncogene, which is also an excellent target for EGFR TKIs.[13] In contrast, patients with *ALK* rearrangements and *KRAS* mutations did not benefit from EGFR TKIs, suggesting that these genotypes are classified as different subsets of LCINS. It is noteworthy that patients who had the *WT/WT/WT* genotype appeared to benefit from EGFR TKI treatment, suggesting that *WT/WT/WT* tumors remain dependent on an active EGFR pathway for their proliferation. In a previous study,[25] patients with a *WT/WT* genotype for *EGFR* and *ALK*, similar to the *ALK* genotype, derived no benefit from EGFR TKIs, which was discordant with our result. This discrepancy may be explained by the finding that direct sequencing may not have sufficient sensitivity to detect *EGFR* mutation.[44] Another possibility is that, in that study, both smokers and those with *KRAS* mutations were included in *WT/WT* genotype, which

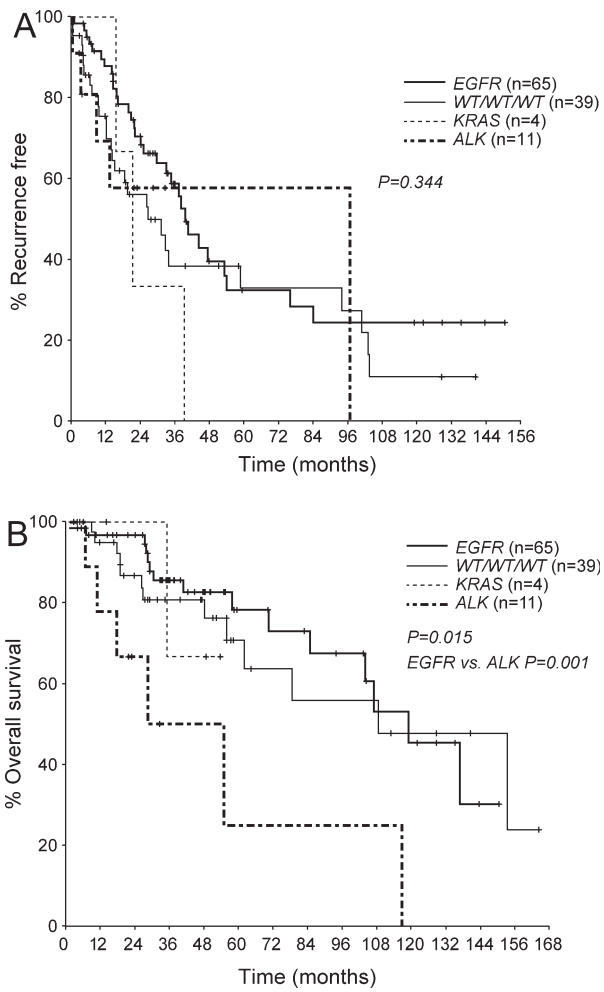

Figure 3. (A) Recurrence-free survival and (B) overall survival are illustrated in patients with resected nonsmall cell lung cancer who underwent lung surgery according to 4 genotypes: epidermal growth factor receptor (*EGFR*) (n = 65), anaplastic lymphoma kinase (*ALK*) (n = 11), v-Ki-*ras*2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (n = 4), and the unmutated wild type (*WT*) of all 3 genes *WT/WT/WT* (n = 39).

Table 5. Prognostic Impact of Each Gene on Clinical Outcomes of Patients With Resected Nonsmall Cell Lung Cancer

		RFS		OS[a]	
Genotype	No. of Mutant/WT	AHR (95%CI)[b]	*P*	AHR (95%CI)[b]	*P*
EGFR: Positive vs negative	65/54	0.838 (0.501-1.400)	.500	0.790 (0.391-1.595)	.511
ALK: Positive vs negative	11/108	1.501 (0.587-3.840)	.397	4.162 (1.529-11.341)	.005
KRAS: Positive vs negative	4/115	1.520 (0.446-5.713)	.503	1.074 (0.137-8.406)	.946

Abbreviations: AHR, adjusted hazard ratio; *ALK*, anaplastic lymphoma kinase; CI, confidence interval; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; OS, overall survival; RFS, recurrence-free survival; WT, wild type.
[a] OS was measured from the date of surgery until death.
[b] Adjusted for age, sex, histology, stage, and performance status.

could have resulted in poor outcomes after EGFR TKI treatment. In addition, the higher response rate (10.3%) to EGFR TKIs among patients with the *WT/WT/WT* genotype that we observed in our study, compared with the response rate documented for individuals with this genotype (1%) in the Iressa Pan-Asian Study (IPASS), may be explained by the aforementioned false-negative *EGFR* mutation testing in our study or by hidden *KRAS* mutations or *ALK* rearrangements in the patients with WT *EGFR* in the IPASS trial.[20] The ORR (25.4%) for patients with *EGFR* mutations to platinum-based chemotherapy in our study was lower than that reported in the IPASS trial (47.3%),[20] including large numbers of *EGFR* mutations. In addition, the ORR (0%) of *ALK*-positive patients in the current study also was lower than what has been reported previously (25%).[25] The small numbers of patients in our study may account for these discrepancies.

Multiple factors, including line of therapy, number of therapies, and type of chemotherapy, may have confounded the survival outcome. It has been reported recently that *ALK*-positive patients may have prolonged responses to pemetrexed-based chemotherapy.[46] Therefore, it is important to know whether each genotype was well balanced in terms of the aforementioned confounding factors. In our study, each genotype was well balanced in terms of the line of therapy and the number of therapies. The proportion of patients exposed to pemetrexed according to genotype did not differ significantly (13 of 80 patients [16.2%] with *EGFR* mutations vs 2 of 13 patients [15.3%] with *ALK* rearrangements vs 1 of 7 patients [14.2%] with *KRAS* mutations vs 11 of 76 patients [14.4%] with the *WT/WT/WT* genotype; $P = .43$). In our study, *EGFR* mutations accounted for the majority of LCINS, whereas a few cases were caused either by *ALK* fusions or *KRAS* mutations. Consistent with previous reports,[25,47] mutations in these 3 genes appear to be mutually exclusive, suggesting that mutations in any 1 of these major oncogenes is sufficient for tumorigenesis and/or the maintenance of a malignant phenotype.

The frequency of *ALK* rearrangements (8.3%) in our study was similar to that reported for never-smoker subgroups in previous reports (range, 4.5%-16.4%).[21,23,25,28-31,36,47] However, the frequency of *ALK* fusions was 17.1% among patients without *EGFR* or *KRAS* mutations, suggesting that this type of genetic alteration is associated with never-smoking status, especially in patients who are negative for *EGFR* mutations. After *EGFR* mutations, *ALK* rearrangements are the genetic alterations that are next most specific for LCINS. Compared with *EGFR* mutations, which have a significant, positive correlation with the papillary subtype,[41] *ALK*-positive tumors are significantly more likely to have abundant signet ring cells, suggesting that these genes may play a role in transformation. Unlike previous reports that indicated an association between *ALK* fusion and younger age or male gender,[14,25,30] we did not observe such associations in our study. This may be explained in part by the finding that the vast majority of patients enrolled in our study were elderly women. The large proportion of elderly women with *ALK* rearrangements in our study also may have confounded the survival outcomes that we documented for the *ALK* rearrangement-positive patients.

Although a previous study reported that *KRAS* mutations occur in 15% of Caucasian never-smokers, the frequency of *KRAS* mutations in Asian populations is consistently lower than this, perhaps reflecting ethnic differences.[34,48] This was confirmed in our study. It is noteworthy that all *KRAS* mutations observed in our study were transition mutations and were not the transversion mutations more commonly observed in lung cancers from smokers.

The molecularly defined subsets of patients in our study may benefit from personalized, targeted therapeutic tools. For example, erlotinib/gefitinib or crizotinib have been proven markedly efficacious in patients with *EGFR* mutations or *ALK* fusions, respectively. Furthermore, based on the encouraging results from the Biomarker Integrated Approached of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial,[49] sorafenib potentially soon may be available to treat lung cancer with *KRAS* mutations. According to current data, a significant proportion of patients with LCINS (approximately 60%) can be assigned successfully to an effective, targeted therapy.

We did not elucidate the driver oncogenic mutations or signaling pathways involved in the remaining 40% of LCINS in our study. A variety of genes, such as v-erb-b2 erythroblastic leukemia viral oncogene (neuroblastoma/glioblastoma-derived) homologs 2 through 4 (*ERBB2-ERBB4*), *BRAF*, *TP53*, and *MET*, may be drivers of tumorigenesis in LCINS.[36,50] Comprehensive, genome-wide studies are expected to reveal many unidentified genes that are mutated or rearranged and, thus, are likely to contribute to the development of LCINS. The identification and characterization of these unknown participants will further our understanding of LCINS and, ultimately, will lead to improved therapies.

In conclusion, East Asian never-smokers with NSCLC could be divided into 4 distinct genotype groups based on genetic profiling of 3 major oncogenes, yielding a unique and nonoverlapping subset of patients with lung cancer who have different therapeutic responses and survival outcomes. Thus, genetic profiling before the initiation of treatment is essential to identify which patients can benefit from specific targeted therapies and should eventually result in personalized therapy options for patients with LCINS.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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