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BRIEF COMMUNICATION

Replication of results of a genome-wide association study on lung cancer survival in a Korean population

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Recently, a genome-wide association study (GWAS) identified single nucleotide polymorphisms (SNPs) that may influence the prognosis of early-stage non-small cell lung cancer (NSCLC) in Caucasians. We attempted to replicate the impact of genetic variants identified in the GWAS on lung cancer survival in a Korean population. A total of 363 patients with surgically resected NSCLCs were enrolled, and 12 SNPs were genotyped using the SEQUENOM MassARRAY iPLEX assay, TaqMan assay, or a polymerase chain reaction–restriction fragment length polymorphism analysis. The association between genotypes and overall survival (OS) was analyzed. Among the 12 SNPs, the rs6034368T>C was associated with OS. Patients with the rs6034368C allele showed a better OS than the patients with the rs6034368T allele (adjusted hazard ratio = 0.72, confidence interval = 0.56–0.93, $P = 0.01$). The rs12446308A>G had an effect on OS, but it was marginally significant (under a codominant model, adjusted hazard ratio = 1.85, confidence interval = 0.98–3.47, $P = 0.06$). We identified that the rs6034368T>C was associated with survival in early-stage NSCLC in a Korean population.

Keywords Genome-wide association study, non-small cell lung cancer, polymorphism, survival

Lung cancer is the most common cause of cancer-related deaths worldwide. Surgical resection is the treatment of choice for early-stage non-small cell lung cancer (NSCLC) (1). Although the TNM staging system is the best prognostic index for NSCLC, a considerable difference exists in the survival outcome during the same stage of the disease (2). The 5-year survival rate of surgically resected NSCLC ranges from 58–73% in stage I and from 36–46% in stage II (2). The wide variation in prognosis may indicate the inadequacy of the TNM staging system in fully accounting for heterogeneity. Therefore, numerous investigators have tried to identify additional prognostic markers for NSCLC.

A number of single nucleotide polymorphisms (SNPs) are reportedly associated with susceptibility to NSCLC (3–5). Furthermore, it has been reported that some SNPs also influence the progression and prognosis of NSCLC (6–8). Recently, a genome-wide association study (GWAS) identified SNPs that may be associated with overall survival (OS) in early-stage NSCLC in Caucasians (9). The effect of genetic polymorphisms on survival may be different according to the ethnic group. Therefore, the SNPs identified by the GWAS in Caucasians need to be verified in diverse ethnic groups. In the present study, we tried to replicate the impact of the genetic variants identified in the GWAS on lung cancer prognosis in a Korean population.

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Materials and methods

A total of 363 NSCLC patients who underwent curative surgical resection at the Kyungpook National University Hospital (Daegu, Korea) between September 1998 and December 2007 were included in the study. The enrolled patients were the same as those in our previous study (10). To avoid confounding effects on the DNA, the patients who received chemotherapy or radiotherapy before surgery were excluded. All of the patients included in this study were ethnic Koreans. Tumor samples were provided by the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs. This study was approved by the Institutional Review Board of the Kyungpook National University Hospital.

We selected 13 SNPs from the GWAS in Caucasians (9). The GWAS included 100 patients from Massachusetts General Hospital (MGH) (Boston, MA), as a discovery set, and 89 patients from the National Institute of Occupational Health (Oslo, Norway), as a validation set. A total of 13 SNPs were validated in the Norwegian cohort with a *P*-value <0.1: 10 SNPs were associated with OS in the same direction and 3 SNPs showed opposite directions in the MGH and Norwegian cohorts (9). According to the HapMap data, the minor allele frequency (MAF) for the rs7926262T>C was zero in Asians. Therefore, we analyzed the remaining 12 SNPs: 7 SNPs (rs4438452, rs9290781, rs11718245, rs10517215, rs1374653, rs12446308, and rs13041757) were genotyped with the SEQUENOM MassARRAY iPLEX assay (SEQUENOM Inc., San Diego, CA); 3 SNPs (rs6034368, rs1893784, and rs9307270) were genotyped with the TaqMan assay (Applied Biosystems, Foster City, CA);and 2 SNPs (rs10176669 and rs7078980) were genotyped with a polymerase chain reaction−restriction fragment length polymorphism analysis. The Taqman probes were predesigned and synthesized by Applied Biosystems. The distribution of genotypes was tested for the Hardy-Weinberg equilibrium. Differences in the distribution of genotypes according to the clinicopathological factors of the patients were compared using the chi-square test for categorical variables. The primary outcome for this study was OS, which was measured from the day of surgery until death or the date of the last follow-up. The survival estimates were calculated using the Kaplan-Meier method. The difference in OS across different genotypes was compared using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using multivariate Cox proportional hazards models, with adjustment for age (≤63 years vs. >63 years), gender (male vs. female), smoking status (never vs. ever), pathological stage (I vs. II or IIIA), and adjuvant therapy (yes vs. no). All of the analyses were performed using the Statistical Analysis System for Windows, version 9.1 (SAS Institute, Cary, NC).

Results

The clinical and pathological characteristics of the study population are shown in Table 1. There were 141 deaths (38.8%) among 363 patients and the estimated 5-year OS was 54% (95% CI, 48−60%). By univariate analysis, the pathological stage was significantly associated with OS (log-rank $P = 4.9 \times 10^{-8}$, Table 1). The genotype frequencies of the 12 SNPs were in Hardy-Weinberg equilibrium. None of the 12 SNPs were significantly associated with patient- or tumor-related factors, such as age, gender, smoking status, pathological stage, or adjuvant therapy (data not shown).

Table 1 Univariate analysis for overall survival by age, gender, smoking status, histological type, pathological stage, and adjuvant therapy

Variables	No. of cases	No. of deaths (%)[a]	5Y-OSR (%)	Log-rank *P*[b]
Overall	363	141 (38.8)	54	
Age (y)				
≤63	185	66 (35.7)	59	0.07
>63	178	75 (42.1)	49	
Gender				
Female	85	24 (28.2)	61	0.14
Male	278	117 (42.1)	53	
Smoking status				
Never	82	25 (30.5)	62	0.27
Ever	281	116 (41.3)	52	
Pack-years[c]				
≤39	127	49 (38.6)	55	0.25
>39	154	67 (43.5)	51	
Histological type				
Squamous cell carcinoma	202	75 (37.1)	57	0.65
Adenocarcioma	155	63 (40.7)	49	
Large cell carcinoma	6	3 (50.0)	67	
Pathological stage				
I	220	58 (26.4)	65	4.9×10^{-8}
II or IIIA	143	83 (58.0)	40	
Adjuvant therapy[d]				
No	79	42 (53.2)	41	0.81
Yes	64	41 (64.1)	39	

Abbreviations: No, number; 5Y-OSR, five-year overall survival rate.
[a] Row percentage.
[b] *P* value was derived from Kaplan-Meier analysis.
[c] In ever-smokers.
[d] In pathological stage II or IIIA: 59 cases received chemotherapy, 2 cases received radiotherapy, and 3 cases received chemotherapy and radiotherapy.

By multivariate analysis, the rs6034368T>C was significantly associated with OS. Patients with the rs6034368C allele exhibited a better survival outcome than patients with the rs6034368T allele (adjusted HR = 0.72, CI = 0.56−0.93, $P = 0.01$, Table 2). The rs12446308A>G was associated with OS, but it was marginally significant (under a codominant model, adjusted HR = 1.85, CI = 0.98−3.47, $P = 0.06$, Table 2).

The association of the rs6034368T>C with survival outcome was further examined after categorizing the patients according to the clinicopathological factors such as age, gender, smoking status, pack-years, histologic type, pathologic stage, and adjuvant therapy. The effect of the rs6034368T>C on OS did not differ according to these factors (*P*-values for homogeneity test >0.05, Supplementary Table 1).