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ORIGINAL REPORT

Effect of *BRCA1* Haplotype on Survival of Non–Small-Cell Lung Cancer Patients Treated With Platinum-Based Chemotherapy

Hong-Tae Kim, Jong-Eun Lee, Eun-Soon Shin, Yeon-Kyeong Yoo, Jae-Hwa Cho, Min-Hye Yun, Yeul-Hong Kim, Se-Kyu Kim, Hyun-Jung Kim, Tae-Won Jang, Seung-Min Kwak, Chul-Soo Kim, and Jeong-Seon Ryu

From the Department of Biological Science, Sungkyunkwan University, Suwon; DNA Link Inc; College of Medicine, Korea University; College of Medicine, Yonsei University, Seoul; College of Medicine, Inha University, Incheon; and the College of Medicine, Kosin University, Pusan, South Korea.

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H.-T.K. and J.-E.L. contributed equally to this article.

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Corresponding author: Jeong Seon Ryu, MD, PhD, Pulmonary Division, Department of Internal Medicine, Inha University Hospital, 7-206, 3-Ga, Shinheung Dong, Jung Gu, Inchon, 400-103, Korea; e-mail: jsryu@inha.ac.kr.

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
To determine whether germ-line variations in *BRCA1* affect outcome in non–small-cell lung cancer (NSCLC) patients treated with platinum combination chemotherapy.

Patients and Methods
We evaluated the associations of four tagging *BRCA1* polymorphisms and their haplotypes with treatment outcome in 300 NSCLC patients at stages IIIA (16%), IIIB (31%), and IV (53%).

Results
The median age was 63 years (range, 28 to 89 years). Histologically, 139 (46.3%) of the patients had squamous cell carcinomas and 137 (45.7%) had adenocarcinomas. Patient median survival time (MST) was 13.0 months. We observed no significant association between any of the tagging polymorphisms [S1613G, IVS13-1893 (A>C), IVS12-1207 (C>T), and IVS12+112 (C>A)] and overall survival. Of the five haplotypes evaluated (AACC, AACA, GCTC, GATC, and AATC), the survival of patients with two copies of the AACC (wild-type) haplotype was significantly shorter than that of patients with zero to one copies (MST, 8.47 *v* 14.57 months; log-rank $P = .0066$), even after adjustment for body weight loss, performance status, stage, second-line treatment, and radiation therapy (hazard ratio = 2.097; 95% CI, 1.339 to 3.284). The survival of patients with squamous cell carcinoma and two copies was significantly shorter than that of other patients with squamous cell carcinoma (MST, 6.8 *v* 15.3 months; log-rank $P = 3.6 \times 10^{-5}$), whereas differences in survival between the two adenocarcinoma groups was not significant (log-rank $P = .677$).

Conclusion
These findings suggest that the AACC haplotype of the *BRCA1* gene is an important prognostic marker in NSCLC patients treated with platinum combination chemotherapy.

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INTRODUCTION

Lung cancer is the most common cause of cancer-related death in many countries, but the survival of patients with non–small-cell lung cancer (NSCLC) has slowly been improving; now 15% of NSCLC patients survive for 5 years.[1]

Despite the introduction of new drugs targeting specific molecules,[2-4] cytotoxic chemotherapy is still the mainstay treatment for advanced NSCLC, in which platinum has an essential role as one part of the doublet.[5]

Effective lung cancer pharmacogenomics relies on identifying NSCLC patients who may benefit from DNA-damaging agents, such as platinum. Since individual variations in DNA repair capacity influence the efficiency of DNA-damaging agents, DNA repair pathway genes have generated great interest as potential biomarkers. Accordingly, genes such as *ERCC1*, *RRM1*, *ERCC2*, and *XRCC1*, which are involved in DNA repair, have been focused on potential biomarkers to predict the effects of platinum-based chemotherapy in NSCLC patients.[6-9]

BRCA1 also plays a central role in DNA repair via transcription-coupled nucleotide excision repair.[10] *BRCA1* originally described in terms of its involvement in other repair processes, including homologous recombination repair, nonhomologous end joining, and mismatch repair, as well as in the regulation of mitotic spindle assembly.[11-13] Recent study has identified differential *BRCA1* expression as a reliable predictive/prognostic marker for platinum-based neoadjuvant chemotherapy in

NSCLC patients.[14] However, germ-line variations in *BRCA1* have not been examined in lung cancer patients.

Therefore, we assessed the association between tagging single-nucleotide polymorphisms (tSNPs) and haplotypes of the *BRCA1* gene and outcome in NSCLC patients treated with platinum combination chemotherapy.

METHODS

Selection of Study Population and Acquisition of Clinical Information

The Lung Cancer Cohort of Inha University Hospital (Incheon, South Korea) has constructed a database system that includes clinical information and matched peripheral-blood DNA. The clinical information was prospectively obtained by a well-trained research nurse, who interviewed all lung cancer patients at the time of diagnosis. Information regarding treatment, tumor response, follow-up, and survival, as well as smoking habits, Eastern Cooperative Oncology Group (ECOG) performance status, and weight loss, was collected from the cohort using uniform data sheets. To increase the quality of the information, a physician (J.S.R.) and a research nurse (H.J.K.) conducted biweekly reviews of the electronic charts and the results of radiologic examinations or laboratory studies for each patient. If a patient was transferred to a primary or secondary hospital, or if a patient left our hospital to remain at home for conservative or terminal care, we contacted the patient or relatives by telephone or mail to ascertain his or her current status.

From more than 1,000 NSCLC patients diagnosed between March 2000 and May 2006, we chose 300 patients with advanced-stage disease who were treated with more than two cycles of platinum-based chemotherapy as a first-line treatment, who underwent full follow-up at our hospital, and whose peripheral-blood lymphocytes were available for analysis.

The 300 consecutive NSCLC patients chosen gave their written informed consent and agreed to the purposes of the study. This study was approved by the Institutional Review Board of Inha University Hospital.

Selection of Tagging Polymorphisms

The *BRCA1* gene spans approximately 81.2 kb and contains 24 exons. All polymorphisms were looked for between nucleotide −1,100 upstream from the start codon (ATG) and the 3′ untranslated region of the *BRCA1* gene using two data systems: the international HapMap Project (http://hapmap.org) and Japanese Single-Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp). Thirty-five polymorphisms were selected with minor allele frequencies of more than 10% in Asian ethnic populations. Among these polymorphisms, four tSNPs were selected using a criterion of more than .9 for the value of the correlation coefficient (r^2). Four tSNPs were chosen with the following priorities: (1) nonsynonymous coding polymorphism; (2) synonymous coding polymorphism; (3) polymorphism near the start codon; (4) intronic polymorphism near an exon; and (5) downstream in order. Finally, IVS12+112 (C>A; *rs2070833*), IVS12-1207 (C>T; *rs8067269*), IVS13-1893A/C (*rs8176199*), and S1613G (*rs1799966*) were identified as tSNPs in this study (Table 1; Appendix Fig A1, online only).

Genetic Analysis

Genotyping analyses of IVS12+112 (C>A), IVS12-1207 (C>T), and IVS13-1893 (A>C) were performed using the GenomeLab SNPstream Genotyping System (ultra-high throughput [UHT]; Beckman Coulter, Fullerton, CA), according to the manufacturer's protocol.[15] Polymerase chain reaction (PCR) amplifications were performed in a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham, MA) using a Taq Gold DNA polymerase. The sequences of the PCR and extension primers are listed in Table 1. The S1613G genotype was analyzed using a single-base primer extension assay employing the SNaPShot assay kit, according to the manufacturer's protocol (Applied Biosystems [ABI], Foster City, CA). The PCR product sequences were analyzed using electrophoresis on an ABI Prism 3730 DNA analyzer. The results were analyzed using Gene Mapper software (ABI).

Survival Measurements

The end point of this study was overall survival estimated from the date of the commencement of chemotherapy. Dates of death were obtained principally by contacting the relatives of the patients by phone or mail, or by reviewing the electronic charts. Seven of the patients could not be contacted, because they were lost to follow-up after discharge from the hospital; information on their survival was collected from the National Statistical Office or the National Police Agency of Seoul, South Korea.

Statistical Methods

The χ^2 test for heterogeneity was used to compare the distributions of the clinical variables or genotype frequencies, and the Mann-Whitney *U* test was used for continuous variables. Using the Haploview v. 4.0 software package

Table 1. Primers for Amplification and Allele Frequencies of Tagging Polymorphisms

SNP Name	*rs* No.	Location	Primer Sequence	Strand	Method	Allele Frequency	HWE *P*
S1613G (A>G)	*rs1799966*	Exon 16		Forward	UHT	A:G = 0.692:0.308	.9043
Forward			AACATACCATCTTCAACCTCTGC				
Reverse			AATTCTGGCTTCTCCCTGC				
Extension			RTTGAAAGTTGCAGAATCTGCCCAG				
IVS13-1893 (A>C)	*rs8176199*	Intron 13		Reverse	UHT	A:C = 0.828:0.172	.1697
Forward			TGAATTACAGTCATCAGTGACTTTTT				
Reverse			GCCTGGCCAAGGCGGAAATAT				
Extension			ACGCACGTCCACGGTGATTTTATTTAATAAGT AAAAACAAATAGT				
IVS12-1207 (C>T)	*rs8067269*	Intron 12		Forward	UHT	C:T = 0.599:0.401	.7241
Forward			TTTGGAAAACTCCTTACATACCTAA				
Reverse			CTGTAAGGATAGTTACTGTTTTTAAAATAATG				
Extension			GGATGGCGTTCCGTCCTATTTTATCAAATACT TGGACTTAGCACA				
IVS12+112 (C>A)	*rs2070833*	Intron 12		Reverse	SNaPshot	C:A = 0.724:0.276	.7525
Forward			TCCTTTGCATTCAGTAGTATGTATC				
Reverse			TAAAAGGTAGTATGAGTTCCATCA				
Extension			GGCTATGATTCGCAATGCTTAGGAGACAATG AACCACAAACAATT				

Abbreviations: SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium.

(http://www.broad.mit.edu/mpg/haploview/), we determined whether the allele frequencies of the tSNPs were in Hardy-Weinberg equilibrium, and we estimated the values of Lewontin's coefficient, D′, and r^2. Associations between genotypes or clinical variables and overall survival were estimated using the Kaplan-Meier method and log-rank testing for univariate analysis. The hazard ratio, adjusted for potential confounders, and the 95% CI were determined using the Cox proportional hazards model for multivariate analysis. The association between haplotype copy number and overall survival was

Table 2. Baseline Patient Characteristics and Copy Numbers of the *BRCA1* AACC Haplotype

Variable	All Patients		AACC Haplotype Copy Number				
			0-1		2		
	No.	%	No.	%	No.	%	Pearson χ^2 *P*
Total patients	300		265		27		
Age, years							
Median	63		63		56		.096
Range	28 to 89		28 to 89		41 to 73		
Sex							
Male	238	79.3	208	89.7	24	10.3	.203
Female	62	20.7	57	95.0	3	5.0	
ECOG performance status							
0-1	223	74.3	194	89.4	23	10.6	.122
≥ 2	70	23.3	65	95.6	3	4.4	
Missing	7	2.3	1	50.0	1	50.0	
Histologic cell type							
Squamous cell carcinoma	139	46.3	122	89.1	15	10.9	.548
Adenocarcinoma	137	45.7	122	91.7	11	8.3	
Mixed	24	8.0	21	95.5	1	4.5	
Smoking habit							
Never	59	19.7	54	96.4	2	3.6	.103
Ever	241	80.3	211	89.4	25	10.6	
Current	174						
Former	67						
< 40 pack years	153	51.0	132	88.6	17	11.4	.594
≥ 40 pack years	88	29.3	79	90.8	8	9.2	
Stage							
III	141	47.0	126	91.3	12	8.7	.758
IIIA	48						
IIIB	93						
IV	159	53.0	139	90.3	15	9.7	
Tumor response							
Complete/partial response	112	37.3	98	91.6	9	8.4	.720
Stable/progressive disease	168	56.0	149	90.3	16	9.7	
Missing	20	6.7	18		2		
First-line regimen							
Platinum/gemcitabine	139	46.3	128	93.4	9	6.6	.128
Platinum/taxane	159	53.0	135	88.2	18	11.8	
Paclitaxel	120						
Docetaxel	39						
Platinum/others	2	0.7	2	100.0	—	0.0	
No. of cycles delivered							.375
Mean	3.98		3.97		3.70		
Standard deviation	1.9		1.9		1.4		
Second-line regimen							
Total	163 of 300	54.3	145 of 292	49.7	14 of 292	4.8	
Platinum doublets	71 of 163	43.6	61	89.7	7	10.3	.438
Single cytotoxic agents	77 of 163	47.2	69	90.8	7	9.2	
EGFR TKIs	15 of 163	9.2	15	100.0	0	0.0	
Radiation therapy to primary tumor							
Total	89 of 300	29.7	77 of 292	26.4	11 of 292	3.8	
IIIA	27 of 48	56.3	23	85.2	4	14.8	.369
IIIB	37 of 93	39.8	34	91.9	3	8.1	.99
IV	25 of 159	15.7	20	83.3	4	16.7	.255
Event, deaths	270	90.0	239	90.5	25	9.5	.686

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

determined using a recessive model. All analyses were performed using the SAS software package (version 9.1.3; SAS Institute, Cary, NC).

RESULTS

Patients and Treatment

Table 2 provides the demographic and clinical information for the 300 patients in this study. Their median age was 63 years (range, 28 to 89 years), and 238 of the patients (79%) were men. Most of the patients (223 of 300, 74%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 1 or better. At the time of diagnosis, 16% of patients had stage IIIA disease, 31% had stage IIIB disease, and 53% had stage IV disease. With regard to histologic cell type, 46.3% of the patients had squamous cell carcinomas and 45.7% had adenocarcinomas. As a first-line regimen, 159 patients (53%) received taxane doublets, and 139 patients (46%) received gemcitabine doublets. Among 163 (54%) patients who received second-line chemotherapy, platinum doublets and single-agent chemotherapy with a cytotoxic agent accounted for 91%. Radiation therapy to the primary tumor was administered to 89 patients (30%). We observed events (deaths) in 270 of the 300 patients (90%).

Genotypes and Allele Frequencies of the Tagging Polymorphisms of BRCA1

Three of the tSNPs were located in introns; the other tSNP, in exon 16, was a nonsynonymous polymorphism causing an amino acid change (serine to glycine; Table 1). A Hardy-Weinberg equilibrium was observed for all tSNPs. The frequencies of the variant alleles were as follows: 30% for S1613G, 17% for IVS13-1893 (A>C), 40% for IVS12-1207 (C>T), and 27% for IVS12+112 (C>A). No associations were demonstrated between each tSNP and sex, ECOG performance status (0 to 1 *v* ≥ 2), histologic cell type (squamous cell carcinoma *v* adenocarcinoma), smoking habit (never smoker *v* ever smoker), stage (III *v* IV), tumor response (complete or partial remission *v* stable disease or progression), first-line regimen (gemcitabine *v* taxanes), second-line treatment (yes *v* no), or sequential radiation therapy (yes *v* no; data not shown).

Linkage Disequilibrium and BRCA1 Haplotype

The linkage disequilibrium (LD) values for S1613G, IVS13-1893 (A>C), IVS12-1207 (C>T), and IVS12+112 (C>A) are shown in Appendix Table A1 (online only). High LDs were observed for all four tSNPs with D′ values of 1, but the r^2 values were intermediate (range, .08 to .664). Using the Haploview v. 4.0 software package, we constructed haplotypes of *BRCA1* in the following order: S1613G, IVS13-1893 (A>C), IVS12-1207 (C>T), and IVS12+112 (C>A); we then identified six haplotypes (Appendix Table A2, online only). The five common haplotypes were AACC, AACA, GCTC, GATC, and AATC, which together accounted for 99.9% of all haplotypes. The AACC haplotype was most commonly observed with 32.1% of frequency.

Association of Tagging Polymorphisms With Overall Survival

The median survival time (MST) of the 300 patients was 13.0 months. No associations were demonstrated between the tSNPs and overall survival of the patients. However, patients with the wild-type allele among the four tSNPs tended to survive for shorter periods than patients with one or two variant-type alleles, although this trend was not statistically significant (Table 3).

Association of Clinical Variables or Haplotypes of BRCA1 With Overall Survival

When the association between the clinical variables and overall survival of patients was analyzed, weight loss (≥ 5%), ECOG performance status (≥ 2), stage (IV), second-line treatment (no), or radiation therapy (no) had significant association with shorter survival according to the log-rank tests and Cox proportional hazards model (Table 4). The other clinical variables we examined (sex, smoking habit, histologic cell type, and first-line regimen) were not prognostic factors in the context of this study.

We classified the 292 patients into two groups according to the copy number of the allele in each *BRCA1* haplotype: one group had zero or one copy and the other group had two copies. We analyzed the two groups statistically using a recessive model, and we evaluated the association between copy numbers of the five *BRCA1* haplotypes and

Table 3. Association Between Tagging Polymorphisms of *BRCA1* and Overall Survival

Variable	Patients		MST (months)	95% CI	Hazard Ratio	95% CI	*P**
	No.	%					
S1613G (A>G)							
Ser/Ser	140	47.5	12.7	10.5 to 14.9	1.0	Reference	
Ser/Gly+Gly/Gly	155	52.5	14.4	11.6 to 17.2	0.781	0.604 to 1.011	.061
IVS13-1893 (A>C)							
AA	201	67.2	12.7	10.8 to 14.6	1.0	Reference	
AC+CC	98	32.8	14.6	11.4 to 17.9	0.892	0.632 to 1.087	.174
IVS12-1207 (C>T)							
CC	109	36.5	12.9	10.6 to 15.2	1.0	Reference	
CT+TT	190	63.5	13.0	10.5 to 15.5	0.850	0.652 to 1.108	.230
IVS12+112 (C>A)							
CC	154	51.9	12.8	10.6 to 15.0	1.0	Reference	
CA+AA	143	48.1	13.7	10.8 to 16.5	0.978	0.757 to 1.265	.869

Abbreviations: MST, median survival time; Ser, serine; Gly, glycine.
*Estimated from Cox proportional hazards model, adjusted for body weight loss, Eastern Cooperative Oncology Group performance status, stage, second-line treatment, and radiation therapy.

Table 4. Association Between Copy Number of *BRCA1* AACC Haplotype or Clinical Variables and Overall Survival

Variable	Univariate			Multivariate		
	MST (months)	95% CI	Log-Rank *P*	Hazard Ratio*	95% CI	*P*
AACC						
Copy number, 0-1	14.57	12.40 to 16.73	.0066	1.0	Reference	
Copy number, 2	8.47	6.94 to 9.99		2.097	1.339 to 3.284	.001
Weight loss, %						
< 5	15.47	14.12 to 16.81	.0242	1.0	Reference	
≥ 5	10.83	9.14 to 12.53		1.351	1.050 to 1.739	.020
ECOG performance status						
0-1	14.57	12.40 to 16.73	.0010	1.0	Reference	
≥ 2	10.00	7.68 to 12.32		1.545	1.154 to 2.068	.003
Stage						
IIIA/IIIB	14.67	11.90 to 17.43	.0125	1.0	Reference	
IV	11.60	9.32 to 13.88		1.257	0.958 to 1.649	.098
Second-line treatment						
Yes	15.77	13.64 to 17.89	.0038	1.0	Reference	
No	10.00	8.14 to 11.86		1.502	1.159 to 1.947	.002
Radiation therapy to primary tumor						
Yes	16.63	11.42 to 21.84	.0005	1.0	Reference	
No	11.60	10.18 to 13.02		1.720	1.274 to 2.321	.000
Sex						
Female	15.23	12.21 to 18.26	.4576			
Male	12.23	10.45 to 14.02				
Smoking habit						
Never	15.23	11.86 to 18.60	.2287			
Ever	12.33	10.53 to 14.14				
Histologic cell type						
Squamous cell carcinoma	14.17	11.39 to 16.94	.1847			
Adenocarcinoma	13.30	11.11 to 15.49				
First-line treatment						
Platinum/gemcitabine	11.90	9.59 to 14.21	.1682			
Platinum/taxanes (P,D)	13.67	11.36 to 15.97				

Abbreviations: MST, median survival time; ECOG, Eastern Cooperative Oncology Group; P,D, paclitaxel/docetaxel.
*Estimated from Cox proportional hazards model, adjusted for body weight loss, ECOG performance status, stage, second-line treatment, and radiation therapy.

overall survival. We could not demonstrate an association between any of the four less common haplotypes (AACA, GCTC, GATC, and AATC) and survival time (data not shown), but patients with two copies of AACC, the wild-type haplotype, showed significantly shorter survival than patients with zero or one copy (MST, 8.5 *v* 14.6 months; log-rank $P = .0066$; Table 4; Fig 1). After adjusting for the confounding variables identified by univariate analysis, the Cox model showed that patients with two copies had a two-fold higher risk of death than patients with zero or one copy of the AACC haplotype (hazard ratio [HR] = 2.097; 95% CI, 1.339 to 3.284). No associations were observed between the two groups by AACC haplotype or clinical variables (Table 1).

AACC Haplotype of BRCA1 *and Overall Survival: Subgroup Analysis*

We analyzed the effect of the copy number of the wild-type haplotype AACC on survival by cell type, smoking habit, sex, and chemotherapy regimen (Fig 2). In patients with squamous cell carcinomas, the MST of patients with the two copies was 6.8 months, less than half that of the MST of patients with zero or one copy (log-rank $P = 3.6 \times 10^{-5}$). In the Cox model, patients with two copies showed an increased risk of death (HR = 3.20; 95% CI, 1.74 to 5.97). In contrast, no association was demonstrated in patients with adenocarcinoma (log-rank $P = .6774$; HR = 0.919; 95% CI, 0.429 to 1.968). When the effect of copy number was analyzed according to smoking habit and sex, smokers or males with the two copies displayed significantly shorter survival (log-rank *P* for smokers = .0016; HR = 2.01;

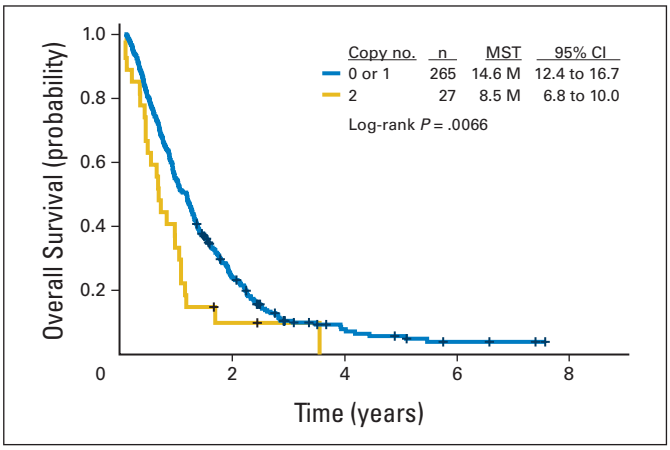

Fig 1. Overall survival according to copy number of AACC haplotype of *BRCA1*. MST, median survival time.

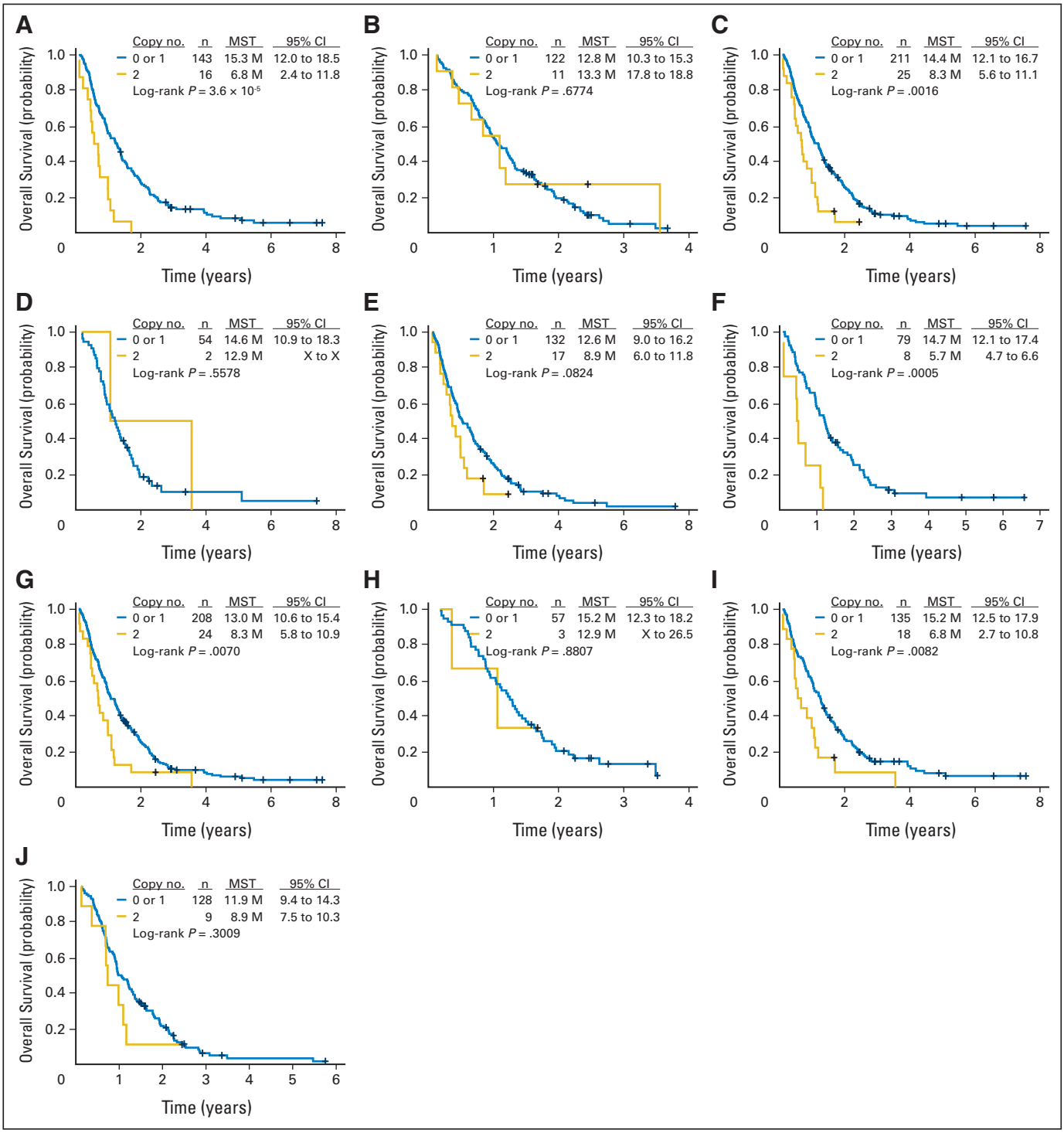

Fig 2. Overall survival of subgroups according to AACC haplotype of *BRCA1*. (A) Squamous cell carcinoma, (B) adenocarcinoma, (C) ever smokers, (D) never smokers, (E) ever-smokers < 40 pack years, (F) ever smokers ≥ 40 pack years, (G) males, (H) females, (I) taxanes/cisplatin, and (J) gemcitabine/cisplatin. MST, median survival time.

95% CI, 1.248 to 3.240; log-rank *P* for males = .0070; HR = 1.96; 95% CI, 1.217 to 3.187). However, no association was observed among never smokers or females (log-rank *P* for never smokers = .5578; HR = 0.994; 95% CI, 0.201 to 4.926; log-rank *P* for females = .8807; HR = 1.586; 95% CI, 0.355 to 7.089). With respect to chemotherapy regimen, the two copies showed significantly shorter survival after taxanes/cisplatin, but this effect was not present in gemcitabine-/cisplatin-treated patients (log-rank *P* for taxanes/cisplatin = .0082; HR = 2.053; 95% CI, 1.123 to 3.752; log-rank *P* for gemcitabine/cisplatin = .3009; HR = 1.606; 95% CI, 0.763 to 3.382).

DISCUSSION

We demonstrated the importance of *BRCA1* haplotype in predicting the prognosis of lung cancer patients treated with platinum-based chemotherapy, especially those with squamous cell carcinomas. *BRCA1* crucially participates in the DNA repair mechanism[10,11] and in regulating mitotic spindle assembly.[12,13] Accordingly, some evidence supports the idea that *BRCA1* mRNA or protein levels could be biomarkers indicating the response to chemotherapeutic agents in cancers of the lung[14] and ovary.[16,17] A lower expression level is predictive of better survival in treatment with DNA-damaging agents, such as platinum, whereas a higher level can indicate a benefit from anticancer drugs that act on tubulin, such as taxanes (paclitaxel or docetaxel).

Most previous studies looking for associations between germline variations in *BRCA1* and survival have investigated cancers in breast,[18] ovary,[19-21] or other tissue,[22] in which the founder mutations or unclassified variants (UVs) of *BRCA1* were evaluated. Chetrit et al[21] recently reported longer survival in ovarian cancers patients with founder mutations, even after adjustment for some confounding factors. In a report by Majdak et al,[20] UVs showed no prognostic significance. However, Garcia et al[23] demonstrated different clinical features among patients with UVs, a finding that warrants a further study with a large number of patients to confirm the relevance of UVs.

In contrast, existing studies have rarely investigated associations between *BRCA1* gene polymorphisms, or their functional significance, with survival of cancer patients. To the best of our knowledge, this study is the first to demonstrate the effects of germ-line polymorphisms on the survival of lung cancer patients.

Regarding possible methodologic issues with this study, only SNPs with a minor allele frequency of more than 10% in the Asian ethnic group were chosen from the two databases to enhance the clinical applicability of the association study. Then, we selected four tSNPs believed to represent corresponding SNPs with 90% probability (Table 1; Fig A1).

In this study, patients with the wild-type alleles of each *BRCA1* tSNP tended to display shorter survival periods than patients harboring one or two variant-type alleles, although the differences were not statistically significant (Table 4). Furthermore, the effect on survival was most evident when the wild-type alleles were combined to construct the AACC wild-type haplotype. This haplotype occurred with the highest frequency in our population, accounting for 32% of the patients. Patients with two copies of the wild-type haplotype AACC showed significantly shorter survival than patients with zero or one copy (Fig 1). To explain these results, we hypothesize that the two copies may convey proficient *BRCA1* function, which hampers the efficacy of DNA-damaging drugs.

In the subgroup analysis, the effect of the two copies depended on cell type, as well as on some clinical variables (sex, smoking habit, and chemotherapy regimen). Squamous cell carcinoma is more common among smokers than adenocarcinoma.[24] Ever smokers have more proficient DNA repair capacity than never smokers;[25-27] therefore, in estimating the effect of a polymorphism, smoking habit is an important determinant in terms of the gene environment interaction.[25-29] So far, no clear evidence has suggested that smoking modulates the effects of *BRCA1* polymorphisms or haplotypes. We assume that the DNA repair capacity can be synergistically enhanced in patients carrying the two copies and with certain phenotypes (squamous cell carcinomas, males, and heavy smokers), in whom the benefit from platinum may decrease. These are common phenotypes among lung cancer patients in South Korea, and in other countries that still have a high prevalence of smoking. This could detract from the benefit of currently available biologic agents.[2-4] Interestingly, we observed significantly shorter survival in patients with the two copies who were treated with taxanes/cisplatin, but not in those treated with gemcitabine. We therefore assume that synergism between taxanes and cisplatin is impaired by efficient *BRCA1* function, whereas gemcitabine is not affected.[14] The results of this study suggest that an alternative option, such as a nonplatinum combination or a nontaxane regimen could be considered in when treating NSCLC patients with the two copies and the phenotypes associated with decreased effectiveness.

There have been publications on genes involving DNA repair. Therefore, gene-gene interactions with *BRCA1* may synergistically potentiate prognostic significance and generate a more robust model for clinicians to utilize as a guide in patient treatment assignment. However, several concerns may hinder developing a useful model. Genetic differences among ethnic groups could affect the benefit from this approach; for example, even though *ERCC2* (Lys751Gln and Asp231Asn) reportedly has a prognostic role in white NSCLC patients,[7] the frequency of Korean patients homozygous for this variant is extremely low.[8,30] Another concern is confounding factors potentially affecting a gene's effect on survival (eg, the effect of DNA repair gene SNPs on survival varies with is effective in stage III, but not in stage IV) or smoking habit.[7,28] Finally, in two genes' interaction analysis between *BRCA1* and *ERCC1* Asn118Asn, a prognostic marker in our cohort,[8,28] only 4.4% (13 of 291) of the patients carried both the two copies of *BRCA1* and *ERCC1* Asn/Asp or Asn/Asn. The death risk was indeed higher for these patients than in patients with the two copies of *BRCA1* alone (log-rank P for combined effect = .0016; HR = 2.611; 95% CI, 1.400 to 4.870 after adjustment for sex, smoking habit, histologic cell type, stage, performance status, and weight loss, unpublished data). However, the size of the stratum holding prognostic significance decreased more than 50%. Although two or more genes' interaction analysis among genes involved in DNA repair may be sound and more informative, how many patients are beneficial from the approach is another important clinical issue or concern.

The results of this study must be interpreted with some caution. First, this study may potentially be limited by its retrospective nature. However, a total of 300 patients were treated in a single institution with platinum-based chemotherapy as the first-line treatment, with clinical information obtained prospectively. We believe that this reduced bias from possible confounding factors. Second, the question of whether the copy number of the wild-type haplotype AACC differentially affects *BRCA1* function remains unanswered, and must be addressed in a future study. Third, the effect of the two copies among subgroups may be limited by small numbers of patients in each stratum, particularly in never smokers.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Hong-Tae Kim, Jong-Eun Lee, Yeon-Kyeong Yoo, Jeong-Seon Ryu
Financial support: Jong-Eun Lee
Provision of study materials or patients: Seung-Min Kwak, Jeong-Seon Ryu
Collection and assembly of data: Eun-Soon Shin, Jae-Hwa Cho, Min-Hye Yun, Hyun-Jung Kim, Seung-Min Kwak, Chul-Soo Kim, Jeong-Seon Ryu
Data analysis and interpretation: Jong-Eun Lee, Eun-Soon Shin, Yeon-Kyeong Yoo, Yeul-Hong Kim, Se-Kyu Kim, Tae-Won Jang, Jeong-Seon Ryu
Manuscript writing: Hong-Tae Kim, Jong-Eun Lee, Jeong-Seon Ryu
Final approval of manuscript: Jeong-Seon Ryu

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