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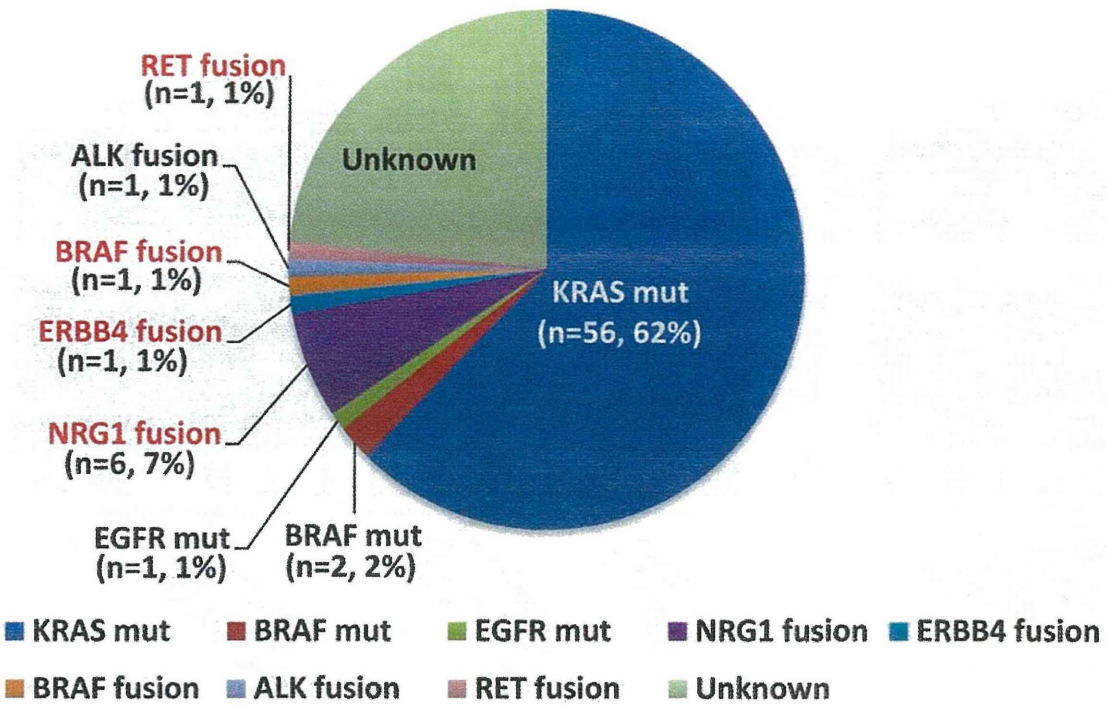
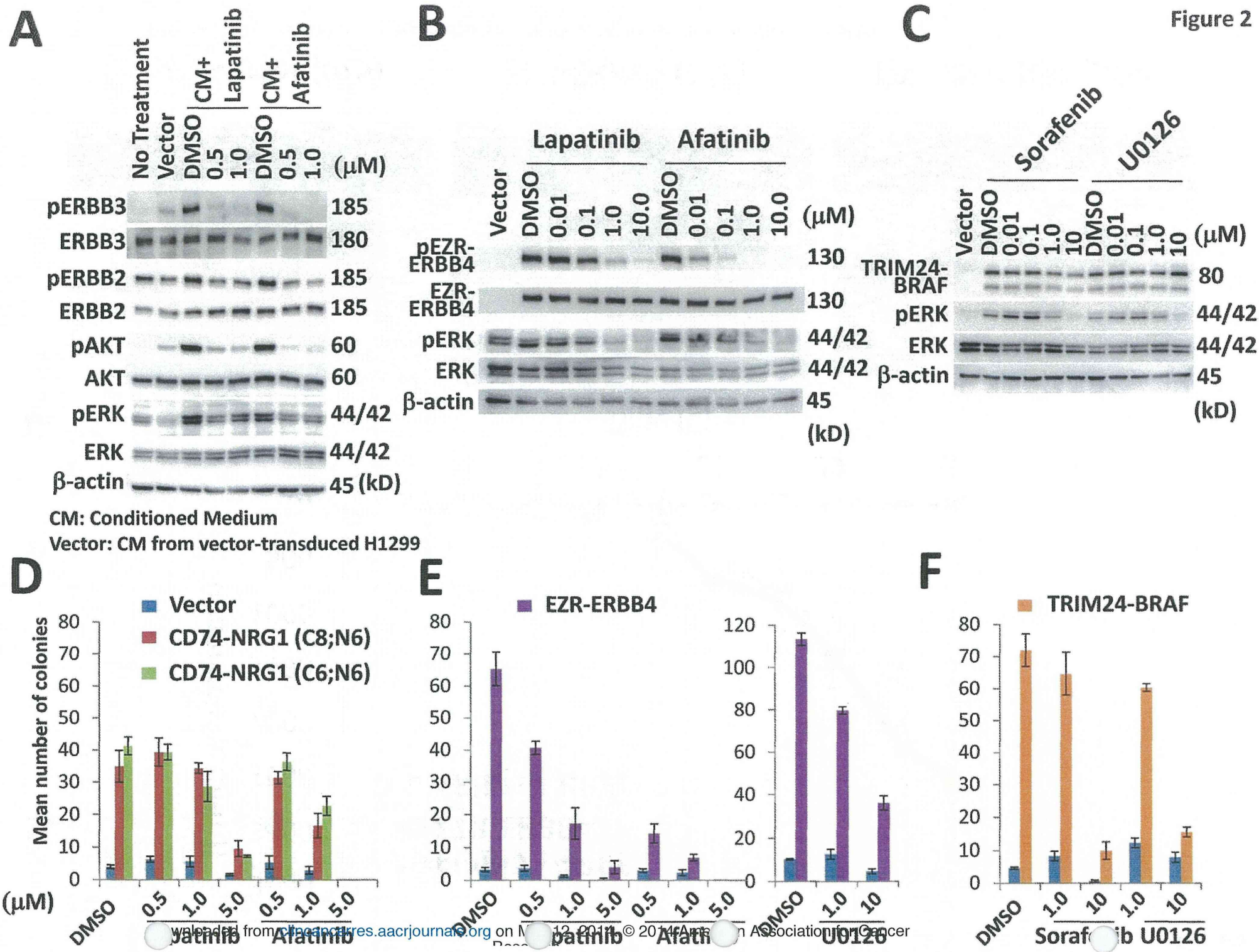


Figure 2



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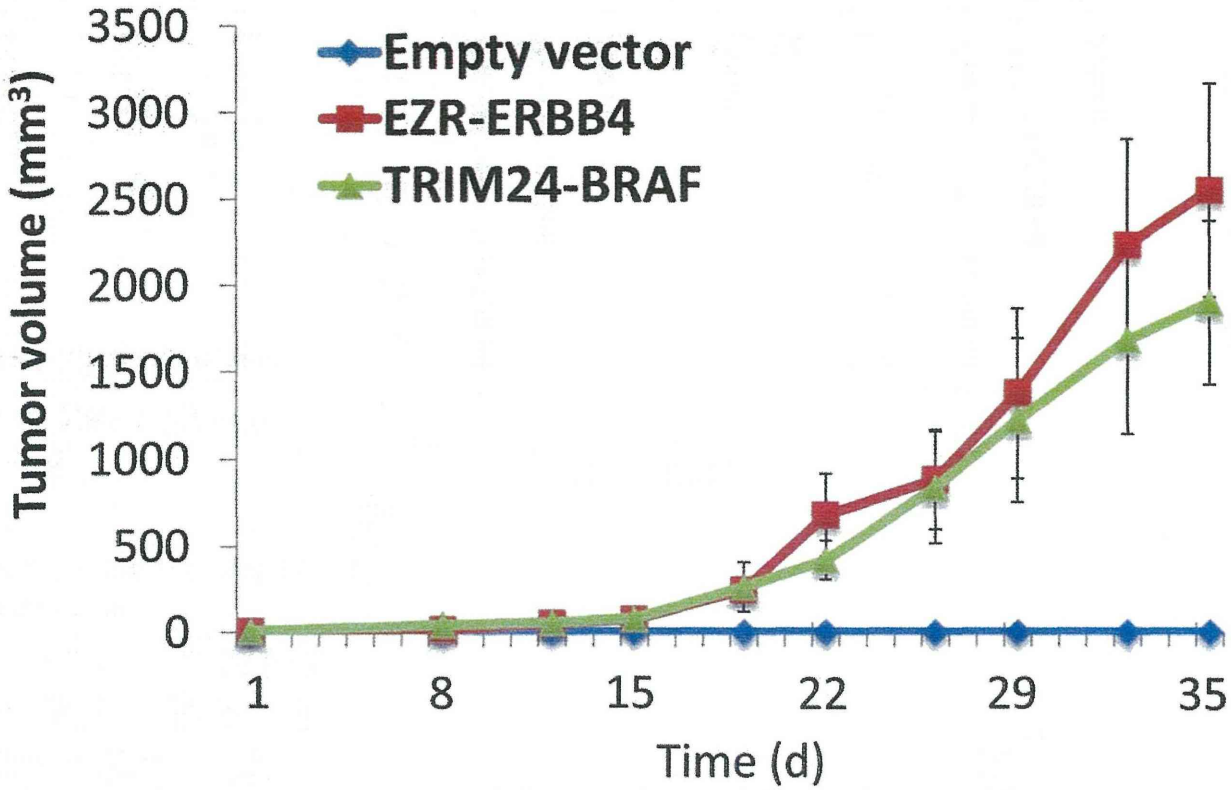

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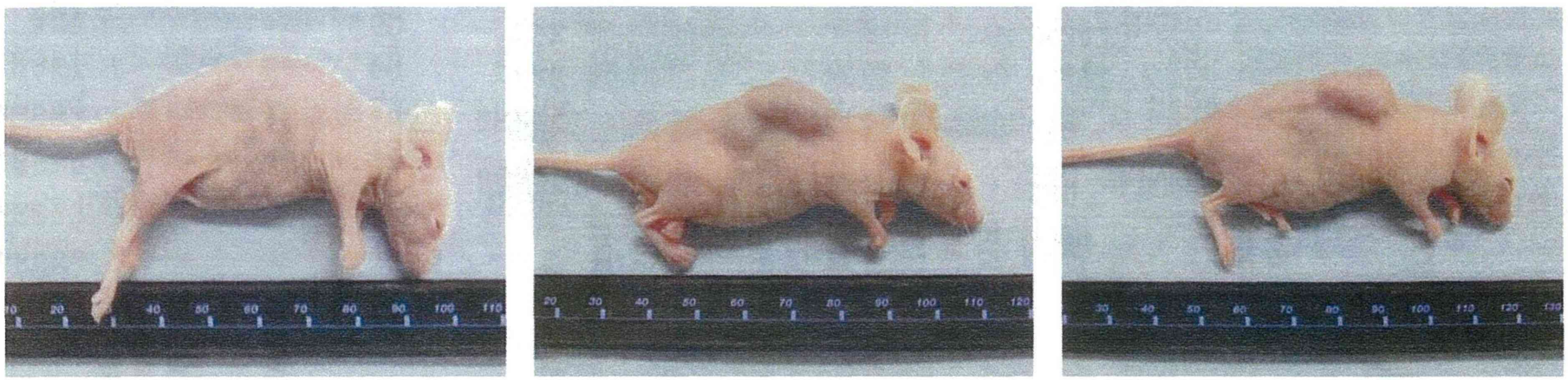

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Association of DNA Repair Gene Polymorphisms With Response to Platinum-Based Doublet Chemotherapy in Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
To identify polymorphisms in DNA repair genes that affect responses to platinum-based doublet chemotherapy in patients with non–small-cell lung cancer (NSCLC).

Patients and Methods
In total, 640 patients with NSCLC who received platinum-based doublet chemotherapy in the National Cancer Center Hospital in Japan from 2000 to 2008 and whose responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) participated in a study of the association between response and genotypes for 30 single nucleotide polymorphisms (SNPs) in 27 DNA repair genes. Candidate SNPs were selected in a discovery set of 201 patients, and their associations were validated in an independent set of 439 patients by prespecified *P* value criteria.

Results
Homozygotes for the minor allele TP53-72Pro of the Arg72Pro SNP in the *TP53* gene showed a better response rate (54.3%) than those for the major allele TP53-72Arg (29.1%; $P = 4.4 \times 10^{-5}$) irrespective of therapeutic regimens, and minor allele homozygotes had significantly longer progression-free and overall survivals than major allele homozygotes (hazard ratio [HR], 0.85; 95% CI, 0.74 to 0.98; $P = .020$; and HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). Minor allele carriers for SNP Lys940Arg in the poly (ADP-ribose) polymerase 1 (*PARP1*) gene showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; *P* for interaction = .019).

Conclusion
Polymorphisms in the *TP53* and *PARP1* genes are involved in inter-individual differences in the response to platinum-based doublet chemotherapy in patients with NSCLC.

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INTRODUCTION

Non–small-cell lung cancer (NSCLC) is a major cause of cancer-related death with 5-year survival rates of < 20%.[1] Cytotoxic chemotherapy is the standard care for patients with advanced NSCLC. The standards of therapeutic regimens are platinum-based doublets (platinum plus another agent).[2] The drugs paired with platinum include microtubule-targeted agents (paclitaxel, docetaxel, or vinorelbine) and DNA-damaging agents (gemcitabine or irinotecan). The efficacy of each combination has been demonstrated to be similar by a series of trials in unselected patients with response rates of 30% to 40%.[3-5] Therefore, predictive factors for the efficacy of these chemotherapy regimens are being investigated for the development of customized therapies.

Considering that agents that damage DNA or disturb chromosomal integrity are used for chemotherapy, activities that repair DNA or chromosome damage possibly influence the outcome of patients with NSCLC after chemotherapy. In fact, expression of *ERCC1*, which is involved in the repair of DNA adducts generated by platinum, has been shown to be a possible predictive factor for the efficacy of the postoperative cisplatin-based adjuvant chemotherapy in resected tumors.[6,7] More recently, a single nucleotide polymorphism (SNP) in the *ERCC1* gene, rs11615, which affects *ERCC1* mRNA levels, was suggested to be associated with response (ie, tumor regression) of patients with advanced NSCLC to platinum-based chemotherapy.[8] Since SNPs can be examined by using blood cells, they will be promising biomarkers in the clinical decision-making process for patients with advanced NSCLC. Reports on the association of SNPs in several other DNA repair genes with prognosis of patients with NSCLC who received chemotherapy also suggested their associations with the outcome of the patients.[7,9-14] However, sample sizes were small (50 to 250 patients), and only four to 15 genomic polymorphisms were investigated in those studies. In addition, the data in each trial were not confirmed by an independent validation set. Therefore, clinical importance of these SNPs still remains unclear.

We previously searched for nonsynonymous (ie, associated with amino acid changes) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome integrity and identified 29 SNPs in 26 DNA repair genes, whose minor allele frequencies were more than 5% in Japanese patients[15] (Table 1). Thus, in this study, we conducted a single-hospital–based retrospective analysis of 640 patients with NSCLC to elucidate associations of these 29 SNPs and the ERCC1 SNP above[8] with the patients' outcome after platinum-based doublet chemotherapy. To minimize type I errors, the significance of candidate SNPs picked up by the first discovery set were validated by using the second independent validation set. We chose the response evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST)[16] as the primary end point of outcome to search for predictive factors for the primary effect of chemotherapy.

Table 1. 30 SNPs in DNA Repair Genes

Pathway	Gene	SNP (rs number)	Amino Acid/ Nucleotide Change	Minor Allele Frequency: Japanese*	Japanese†	Chinese†	European†	African†
29 Nonsynonymous SNPs (associated with amino acid change)								
Base excision repair								
	PARP1	rs1805412	Val762Ala	0.40	0.46	0.48	0.17	0.01
		rs1136471	Lys940Arg	0.05	—	—	—	—
	APEX	rs1130409	Asp148Glu	0.38	0.32	0.46	0.51	0.28
	MBD4	rs140693	Glu346Lys	0.35	0.41	0.27	0.00	0.03
	MTH1	rs4866	Val83Met	0.09	—	—	—	—
	OGG1	rs1052133	Ser326Cys	0.48	0.52	0.50	0.22	0.14
	XRCC1	rs1799782	Arg194Trp	0.33	0.28	0.24	0.09	0.08
		rs25489	Arg280His	0.09	—	—	0.03	0.03
		rs25487	Arg399Gln	0.25	0.28	0.27	—	0.10
Nucleotide excision repair								
	XPG	rs17655	His1104Asp	0.42	0.48	0.56	0.73	0.46
	CSB	rs2228528	Gly399Asp	0.45	0.46	0.40	0.19	0.22
	XPC	rs2228001	Lys939Gln	0.40	0.34	0.38	0.41	0.26
	XPD	rs13181	Lys751Gln	0.05	0.08	0.06	0.33	0.18
Mismatch repair								
	MLH3	rs175080	Pro844Leu	0.18	0.14	0.13	0.43	0.41
	MSH3	rs26279	Thr1045Ala	0.24	0.22	0.37	0.22	0.40
	MSH6	rs1042821	Gly39Glu	0.32	—	—	—	—
DNA double-strand break repair								
	BRCA2	rs144848	Asn372His	0.22	0.31	0.21	0.29	0.13
	SNM1	rs3750898	His317Asp	0.26	0.26	0.10	0.27	0.74
	NBS1	rs1805794	Gln185Glu	0.50	0.46	0.49	0.28	0.16
	XRCC3	rs861539	Thr241Met	0.09	0.15	0.07	0.42	0.24
DNA damage response								
	TP53	rs1042522	Arg72Pro	0.33	0.23	0.49	0.41	0.67
DNA polymerase								
	POLD1	rs1726801	Arg119His	0.20	0.22	0.18	0.06	0.35
	POLI	rs8305	Thr731Ala	0.25	0.28	0.29	0.26	0.00
	REV1	rs3087386	Phe257Ser	0.33	0.30	0.37	0.50	0.30
	POLZ	rs462779	Thr1224Ile	0.35	0.43	0.49	0.82	0.38
Other pathways								
	BLM	rs28384991	Thr298Met	0.09	—	—	—	—
	FANCA	rs2239359	Ser501Gly	0.17	0.16	0.21	0.62	0.33
	FANCG	rs2237857	Thr297Ile	0.12	0.13	0.01	0.00	0.14
	WRN	rs1346044	Cys1367Arg	0.09	0.07	0.08	0.23	0.15
One synonymous SNP (not associated with amino acid change)								
Nucleotide excision repair								
	ERCC1	rs11615	C118T	—	0.29‡	0.22	0.65	0.02

Abbreviation: SNP, single nucleotide polymorphism.
*Frequency in Japanese determined by Sakiyama et al.[15]
†Frequency determined by the HapMap project.
‡Frequency in Japanese (T. Kohno, unpublished data).

PATIENTS AND METHODS

Selection of Study Population and Acquisition of Clinical Information

In total, 987 patients with NSCLC with clinical stages IIIA, IIIB, and IV tumors, who had not received prior platinum-based chemotherapy, were given platinum-based chemotherapy at the National Cancer Center Hospital in Tokyo, Japan, from 2000 to 2008 (Fig 1A). Clinical information was obtained by attending physicians and nurses. Of the 987 patients, 640 were eligible for the study according to the following criteria: they were not indicated for definitive chemoradiotherapy; they received a platinum-based doublet but not single or triplet chemotherapy; and their tumor response was evaluable according to RECIST[16] on the basis of data from computed tomography scans. However, those with clinical or radiologic evidence of early progression, such as emergence of new lesions, were included as patients with progressive disease (PD) in the analysis, even when unaccompanied by corresponding computed tomography scans, according to the definition in RECIST.[16] All patients were Japanese and were diagnosed with adenocarcinoma (ADC), squamous cell carcinoma (SQC), or other histologic types of NSCLC according to WHO classification[17,18] (Table 2).

Written informed consent was obtained from all patients for the use of blood cells for the analysis of genetic polymorphisms in association with clinical findings, including response to chemotherapy. Thus, 201 patients in the discovery set received therapy from 2000 to 2004, and 439 patients in the validation set received therapy from 2004 to 2008. Information on response in a subset of patients was obtained from the data in clinical trials conducted at the National Cancer Center Hospital.[3,19,20] This study was approved by the institutional review boards of the National Cancer Center. Smoking habit was recorded by pack-years. Patients with pack-years > 0 were defined as smokers, including both former and current smokers. Patients who report no smoking history (ie, pack-years = 0) were defined as never-smokers.

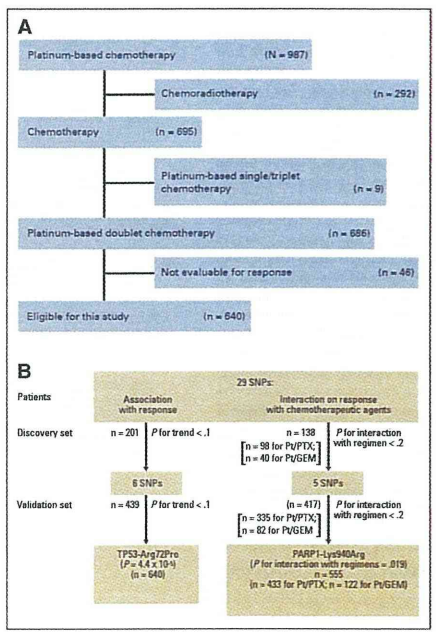

Fig 1. Patients and strategy. (A) Selection of eligible cases. (B) A two-phase screening of single nucleotide polymorphisms (SNPs) associated with responses to platinum-based doublet chemotherapy. Pt, platinum; PTX, paclitaxel; GEM, gemcitabine.

Chemotherapy

Patients were treated with one of the following regimens: (1) paclitaxel 200 mg/m^2 followed by cisplatin 80 mg/m^2, carboplatin at a dose calculated to produce an area under the serum concentration-time curve of 6.0 min · mg/mL, or nedaplatin 100 mg/m^2 on day 1, repeated every 3 weeks; (2) docetaxel 60 mg/m^2 followed by cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (3) vinorelbine 25 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (4) gemcitabine 1,000 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 or carboplatin to area under the serum concentration-time curve of 5.0 min · mg/mL on day 1, repeated every 3 weeks; or (5) irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 80 mg/m^2 on day 1, repeated every 4 weeks. Each treatment was repeated for two or more cycles unless the patient met the criteria for PD or experienced unacceptable toxicity. Chemotherapy dosage was modified by toxicities in subsequent courses.

Genetic Analysis

A 20 mL whole-blood sample was obtained from each patient, and genomic DNA was extracted from whole-blood cells.[15] Genotyping for 30 SNPs in 27 genes was performed by pyrosequencing or TaqMan methods as previously described.[15,21]

Statistical Analysis

Patients were divided into two categories: responders were those with complete response and partial response, and nonresponders were those with stable disease and PD. Odds ratios (ORs) and 95% CIs for the response (ie, responder *v* nonresponder) according to genotypes were calculated as a measure of difference in the response rate against therapy. ORs were calculated by adjusting sex (male *v* female), age (increase by 10 years), performance status (0 *v* 1 to 2), smoking status (never-smoker *v* smoker), stage (III *v* IV), and chemotherapy (platinum plus a DNA-damaging agent *v* platinum plus a microtubule-targeting agent) by using an unconditional logistic regression analysis.[22] *P* value by the trend test was also calculated by using an unconditional logistic regression analysis under the same adjustments as above. Differences in the response between two chemotherapeutic regimens according to genotypes were examined by calculating *P* values for interaction with the regimens on the trend of OR for response.

A two-phase screening was used to search for SNPs associated with the response to chemotherapy (Fig 1B). In the first phase, 29 SNPs were examined for associations with the response and differences in the association according to regimens in 201 and 138 patients (for whom paclitaxel or gemcitabine therapy was used, respectively) in the discovery set. In the second phase, SNPs that showed *P* values < .1 by the trend test for association with the response and *P* values < .2 for interaction with the regimen were subjected to genotyping of 439 and 417 patients (for whom paclitaxel or gemcitabine was used, respectively) in the validation set. SNPs that showed *P* values < .1 for association with the response and *P* values < .2 for interaction with the regimen in patients in the validation set were further subjected to analysis in all 640 and 555 patients, respectively. Progression-free survival (PFS) was defined as the period from the first day of chemotherapy to the date of documentation of disease progression by RECIST and overall survival (OS) was defined as the period from the first day of chemotherapy to death. Hazard ratios (HRs) for PFS and OS and 95% CIs were calculated by using multivariate Cox proportional hazards models with adjustment for sex, age, histology, performance status, smoking status, clinical stage, and treatment as above. Statistical analyses were performed using JMP version 8.0 software (SAS Institute, Cary, NC). A level of $P < .05$ was considered significant, whereas a level of $P < .10$ was considered marginal.

Table 2. Patient Characteristics

Variant	All No.	All %	All 95% CI	Discovery Set* No.	Discovery Set* %	Validation Set No.	Validation Set %
Total patients	640			201		439	
Age, years							
Mean		57.9			57.2		58.2
Range		22-78			22-78		26-74
± Standard deviation		9.2			10.0		9.1
Sex							
Male	402	62.8		136	67.7	266	60.6
Female	238	37.2		65	32.3	173	39.4
ECOG performance status							
0	218	34.1		46	22.9	172	39.2
1	402	62.8		153	76.1	249	56.7
2	20	3.1		2	1.0	18	4.1
Histologic cell type							
Adenocarcinoma	549	85.8		167	83.1	382	87.0
Squamous cell carcinoma	84	13.1		34	16.9	50	11.4
Others	7	1.1		0	0.0	7	1.6
Smoking habit							
Never-smoker	233	36.4		74	36.8	159	36.2
Smoker	407	63.6		127	63.2	280	63.8
Pack-years of smokers							
Mean		46.3			45.9		46.5
± Standard deviation		29.6			29.4		29.7
Stage							
III	172	26.9		60	29.9	112	25.5
IIIA	24	3.8		12	6.0	12	2.7
IIIB	148	23.1		48	23.9	100	22.8
IV	468	73.1		141	70.1	327	74.5
Tumor response							
Responder	231	36.1		74	36.8	157	35.8
CR	4	0.6		0	0.0	4	0.9
PR	227	35.5		74	36.8	153	34.9
Non-responder	409	63.9		127	63.2	282	64.2
SD	232	36.3		70	34.8	162	36.9
PD	177	27.7		57	28.4	120	27.3
Platinum-based regimens							
Platinum + a microtubule-targeted agent	476	74.4		129	64.2	347	79.0
Paclitaxel†	433	67.7		98	48.8	335	76.3
Docetaxel‡	8	1.3		2	1.0	6	1.4
Vinorelbine§	35	5.5		29	14.4	6	1.4
Platinum + a DNA-damaging agent	164	25.6		72	35.8	92	21.0
Gemcitabine¶	122	19.1		40	19.9	82	18.7
Irinotecan‖	42	6.6		32	15.9	10	2.3
PFS, median month							
Platinum + Paclitaxel		4.7	4.2 to 5.3				
Platinum + Gemcitabine		4.6	3.8 to 5.4				
Responder		6.1	5.7 to 6.4				
Nonresponder		3.0	2.7 to 3.3				

Abbreviations: ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival.
*Genotype for 29 nonsynonymous DNA repair gene single nucleotide polymorphisms were determined by Sakiyama et al.[15]
†Cisplatin or carboplatin or nedaplatin + paclitaxel.
‡Cisplatin + docetaxel.
§Cisplatin + vinorelbine.
¶Cisplatin or carboplatin + gemcitabine.
‖Cisplatin + irinotecan.

RESULTS

Association of a TP53-Arg72Pro SNP With Response to Platinum-Based Doublet Chemotherapy

Among 987 patients with NSCLC who were treated with platinum-based chemotherapy, 640 were eligible for this study (Fig 1A). Characteristics of these patients are summarized in Table 2. Genotypes for the 29 nonsynonymous SNPs in 26 DNA repair genes had been determined in 201 of the 640 patients in our previous study[15] (the discovery set in Table 2). Therefore, associations of these 29 SNPs with responses to chemotherapy were first investigated in these patients (Fig 1B). Six of the 29 SNPs fulfilled the criteria described above ($P < .1$ by the trend test; Appendix Table A1, online only); thus, they were further genotyped in the remaining 439 patients (the validation set in Table 2). Only one SNP, TP53-Arg72Pro, reproducibly showed an association that met the criteria ($P < .1$; Fig 1B and Appendix Table A1). In the analysis of all 640 patients, TP53-72Pro, the minor allele, was associated with a better response ($P = 9.5 \times 10^{-5}$ by the trend test; Table 3), and response rates increased according to the increase in the number of minor alleles (Fig 2A). Minor allele homozygotes showed a better response rate (54.3%) than major allele homozygotes (29.1%; $P = 4.4 \times 10^{-5}$). The association remained significant after Bonferroni correction (ie, $< 0.05/29 = 1.7 \times 10^{-3}$). Response rates of heterozygotes and homozygotes for the TP53-72Pro allele were higher in SQC than in ADC (Fig 2A and Table 3).

In the Cox proportional hazard model, minor allele homozygotes showed a significantly longer PFS than major allele homozygotes (HR, 0.85; 95% CI, 0.74 to 0.98; $P = .020$). The HR for progression of these homozygotes in SQC (HR, 0.67; 95% CI, 0.45 to 0.98; $P = .041$) was lower than that in ADC (HR, 0.89; 95% CI, 0.76 to 1.03; $P = .13$). Minor allele homozygotes showed a significantly longer OS than major allele homozygotes (HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). The HR for death of these homozygotes in SQC (HR, 0.66; 95% CI, 0.43 to 0.98; $P = .037$) was lower than that in ADC (HR, 0.87; 95% CI, 0.74 to 1.02; $P = .13$).

SNP rs11615 (C118T) in the *ERCC1* gene was reported to be associated with response to platinum-based chemotherapy of NSCLC[8]; thus, it was also examined for association with response in all 640 patients. Minor allele homozygotes for the *ERCC1* SNP showed a higher response rate than others, consistent with a recent report[8]; however, the association was not statistically significant (Appendix Table A2, online only).

Differential Response According to Chemotherapeutic Regimens by PARP1 Genotypes

We next investigated whether or not SNPs in DNA repair genes affect responses differentially according to chemotherapeutic agents. Paclitaxel (433 patients; 68%) and gemcitabine (122 patients; 19%) were the most and second-most commonly used drugs in the platinum-based regimens (other drugs were also used but less frequently [< 10%; Table 2]). Therefore, differences in the response among the agents according to genotypes were investigated in 555 patients who received chemotherapy with either of these two regimens.

Among 201 patients in the discovery set, 138 received chemotherapy with regimens using paclitaxel (98 patients) or gemcitabine (40 patients; Fig 1B). Five of the 29 SNPs met the criteria in these 138 patients ($P < .2$ for interaction). Therefore, these five SNPs were further genotyped for 417 patients who received chemotherapy with regimens using paclitaxel (335 patients) or gemcitabine (82 patients) among 439 patients in the validation set. Only one SNP, poly (ADP-ribose) polymerase 1 (PARP1) -Lys940Arg, reproducibly showed $P < .2$ for interaction (Appendix Table A3, online only). This SNP showed a statistically significant interaction with the regimens on the response when analyzed in all 555 patients ($P = .019$ for interaction; Fig 1B, Appendix Table A4, online only), although the association did not remain significant after Bonferroni correction (ie, > 0.05 of 29 SNPs tested $= 1.7 \times 10^{-3}$). Heterozygotes for this SNP showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; Fig 2B). There were no minor allele homozygotes for this SNP in this population.

PFS according to the PARP1-Lys940Arg genotype was compared between the two regimens. In the Cox proportional hazard model, the risk for progression of major allele homozygotes with the platinum/paclitaxel treatment was similar to that with the platinum/gemcitabin treatment (HR, 0.97; 95% CI, 0.86 to 1.09; $P = .60$). Conversely, the risk of heterozygotes with the platinum/paclitaxel treatment was smaller than that with the platinum/gemcitabine treatment, although it was not statistically significant (HR, 0.82; 95% CI, 0.59 to 1.17; $P = .27$). SNPs in *TP53* and *ERCC1* did not show differential associations according to regimens (Appendix Table A4).

Table 3. Association of *TP53* Genotypes With Response to Chemotherapy in 640 Patients With NSCLC

NSCLC	Genotype	Nonresponders No.	Nonresponders %	Responders No.	Responders %	Response Rate (%)*	OR	95% CI	P	P by Trend Test
All	Arg/Arg	175	42.8	72	31.2	29.1	Reference			9.5×10^{-5}
	Arg/Pro	197	48.2	115	49.8	36.9	1.38	0.96 to 1.99	.082†	
	Pro/Pro	37	9.0	44	19.0	54.3	3.02	1.77 to 5.18	4.4×10^{-5}†	
	Dominant						1.63	1.15 to 2.30	.0053†	
	Recessive						2.48	1.54 to 4.04	2.1×10^{-4}†	
Adenocarcinoma	Arg/Arg	152	42.2	64	33.9	29.6	Reference			.0024
	Arg/Pro	176	48.9	90	47.6	33.8	1.19	0.81 to 1.77	.38‡	
	Pro/Pro	32	8.9	35	18.5	52.2	2.67	1.50 to 4.81	8.7×10^{-4}‡	
	Dominant						1.42	0.98 to 2.07	.062‡	
	Recessive						2.44	1.44 to 4.15	9.2×10^{-4}‡	
Squamous cell carcinoma	Arg/Arg	21	46.7	7	17.9	25.0	Reference			.0032
	Arg/Pro	19	42.2	23	59.0	54.8	3.63	1.10 to 13.5	.033‡	
	Pro/Pro	5	11.1	9	23.1	64.3	8.71	1.64 to 62.5	.010‡	
	Dominant						4.62	1.52 to 16.3	.0062‡	
	Recessive						3.85	1.02 to 17.6	.047‡	
Smoker	Arg/Arg	98	39.5	44	27.7	31.0	Reference			.0084
	Arg/Pro	124	50.0	88	55.3	41.5	1.52	0.97 to 2.41	.069§	
	Pro/Pro	26	10.5	27	17.0	50.9	2.31	1.19 to 4.50	.013§	
	Dominant						1.65	1.07 to 2.57	.023§	
	Recessive						1.78	0.99 to 3.23	.056§	
Never-smoker	Arg/Arg	77	47.0	28	38.9	26.7	Reference			.0052
	Arg/Pro	73	44.5	27	37.5	27.0	1.06	0.55 to 2.02	.875	
	Pro/Pro	11	6.7	17	23.6	60.7	5.31	2.00 to 15.3	6.8×10^{-4}§	
	Dominant	161					1.56	0.86 to 2.86	.145	
	Recessive						4.76	2.02 to 11.8	3.6×10^{-4}§	

Abbreviations: NSCLC, non–small-cell lung cancer; OR, odds ratio.
*Fraction of responder.
†OR for responder against nonresponder adjusted for sex, age, histology, smoking status, clinical stage, performance status, and treatment.
‡OR for responder against nonresponder adjusted for sex, age, smoking status, clinical stage, performance status, and treatment.
§OR for responder against nonresponder adjusted for sex, age, histology, clinical stage, performance status, and treatment.

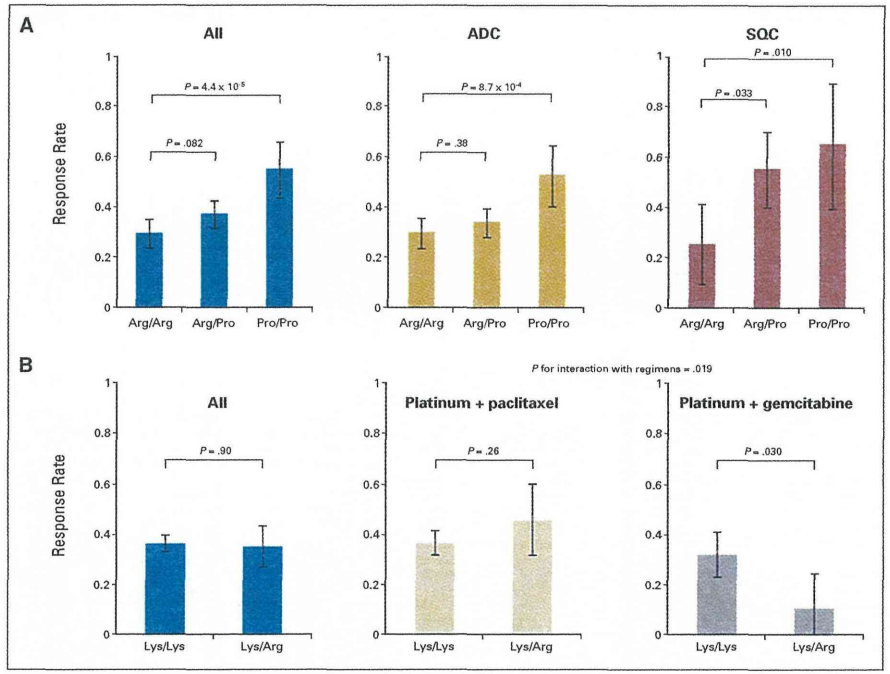

Fig 2. (A) Response rates according to *TP53* genotypes in (left) all patients and those with (middle) aenocarcinoma (ADC) and (right) squamous cell carcinoma (SQC). (B) Response rates according to *PARP1* genotypes in (left) all patients and those treated with (middle) platinum plus pacliteaxel or (right) platinum plus gemcitabine. Response rate is shown with its sampling variations estimated by 95% CI.

DISCUSSION

An SNP in the *TP53* genes was shown to be associated with the response to platinum-based doublet chemotherapy. In this study, association results obtained by the discovery set were confirmed by using an independent validation set. The association of the p53-72Pro allele with a better response to platinum-based doublet chemotherapy

retained statistical significance after Bonferroni correction. Therefore, the results strongly indicate the importance of p53-Arg72Pro SNP as a determinant for the response to platinum-based chemotherapy.

TP53 is a tumor suppressor gene somatically mutated in 40% to 70% of NSCLCs.[23] p53-72Arg protein has a greater activity to induce apoptosis than p53-72Pro protein[24]; however, the relationship was reported as being the reverse in mutant p53 proteins.[25,26] p73, a p53-related protein, plays a role in apoptosis in anticancer agents for cancer cells carrying *TP53* mutations; however, its function is abrogated by mutant p53 proteins. The abrogating activity is greater in mutant p53 proteins with the Arg residue at codon 72 than in those with the Pro residue.[25,26] In an analysis of 25 patients with head and neck cancer, those with a *TP53* mutation on the 72Pro allele showed a better response than those with a mutation on the 72Arg allele with cisplatin-based chemoradiotherapy.[25] Similarly, in this study, the TP53-72Pro allele appeared to confer a better response to platinum-based doublet chemotherapy in patients with NSCLC (Fig 2A). In a previous study,[11] patients with NSCLC who carry the TP53-72Pro allele also showed a better OS after cisplatin-gemcitabine treatment, although the association did not reach statistical significance. These results indicate that p53 mutants with the Pro residue at codon 72 only weakly inhibit the function of p73 protein in NSCLC cells and therefore efficiently induce apoptosis of NSCLC cells treated with platinum and other anticancer agents. In fact, the effect of this SNP was more apparent in patients with SQC than in patients with ADC (Fig 1A), consistent with the fact that *TP53* mutations are more frequent in SQC than in ADC.[27] Since tumor specimens for examination of somatic *TP53* mutations were not available for these patients, *TP53* status in their tumor cells could not be determined. Therefore, we could not conclude whether this differential association was really due to differences in *TP53* mutations. An association study of patients with NSCLC informative for somatic *TP53* mutation will provide a more complete picture of the role of *TP53* SNP in chemotherapeutic responses.

The PARP1-Lys940Arg genotype was suggested to differentially affect the response according to chemotherapeutic agents (Fig 2B), although the association was not significant after Bonferroni correction and needs validation. The *PARP1* gene encodes poly (ADP-ribose) polymerase 1, which regulates multiple processes for DNA repair, such as DNA strand break repair.[28] It is noted that suppression of PARP activity has been recognized as a method of tumor suppression in breast and other cancers[29] and that a PARP inhibitor enhanced the cytotoxic activity of gemcitabine.[30] The biologic significance of the PARP1-Lys940Arg SNP is unknown at present; however, the lysine-arginine residue at codon 940 is located in the catalytic domain of the PARP1 protein.[31] Therefore, this polymorphism may cause differences in the activity of PARP1 protein that affect the response to some chemotherapeutic agents, in particular to DNA-damaging agents.

Interestingly, the frequencies of the TP53-72Pro allele are known to be different among ethnic populations, although those of the PARP1-940Arg allele in other ethnic populations are unknown at present (Table 1). Therefore, examination of these two SNPs in NSCLC patient populations other than Japanese will also help elucidate the mechanism of interethnic differences in the outcome of patients after chemotherapy, as recently discussed.[32]

Identification of polymorphisms associated with drug toxicities is also important to develop customized chemotherapies. For instance, the *UGT1A1* gene polymorphisms are known to be associated with the toxicity of irinotecan, such as neutropenia.[33] In this study, the *TP53* and *PARP1* SNPs were not associated with grade 4 hematologic toxicities, including neutropenia (data not shown). Therefore, genetic factors responsible for response are likely to be different from those for toxicity. In addition, associations of these two SNPs with responses were not significantly different according to smoking habit ($P > .05$ for interaction with smoking; for *TP53*, see Table 3); therefore, these SNPs are likely to contribute to the response irrespective of smoking.

Our study has several limitations. This is a single-institution retrospective study with various therapeutic regimens. Therefore, the effects of SNPs on differential responses according to chemotherapeutic agents were only preliminarily investigated. The results should be confirmed by a larger, preferably prospective, cohort using a defined set of agents. More extensive analyses of interaction between SNPs and responses to chemotherapeutic agents will also be worth performing. Another limitation of this study is that, although the *TP53* polymorphism was significantly associated with response to chemotherapy, differences in PFS and OS were only modest. We chose the response as the primary end point of efficacy to pick up subgroups for which chemotherapy does work. Although this information would be potentially valuable, clinical response alone would be inadequate to improve the outcome of patients with advanced NSCLC. Therefore, investigation of polymorphisms in other genes might provide more information for individually optimized chemotherapy. Indeed, a few other SNPs in DNA repair genes have been reported to be associated with prognosis of patients with NSCLC.[7,9-14] In addition to ERCC1-118T, the APEX-148Asp, XRCC1-399Arg, and XPD-751Gln alleles, which had been reported to be associated with favorable prognosis of patients,[9,13,14] were consistently more frequent in responders than in nonresponders in our study population (Appendix Table A2), although these SNPs did not fulfill the criteria as validated predictive factors in this study.

In conclusion, our extensive analysis of 30 SNPs in 27 DNA repair genes identified the *TP53* and *PARP1* SNPs as strong candidates for defining inter-individual differences in the response to platinum-based chemotherapy of NSCLC. Our results indicate the significance of SNPs in DNA repair genes in the outcome of patients with NSCLC and also imply the utility of these SNPs as predictive markers for responses to chemotherapy. Further investigation is warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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Appendix

Table A1. Association of Six DNA Repair Gene SNPs With Response to Platinum-Doublet Chemotherapy

			Discovery Set (n = 201)									Validation Set (n = 439)								
			Non-responders		Responders							Non-responders		Responders						
Gene	SNP	Genotype	No.	%	No.	%	Response Rate (%)*	OR†	95% CI	*P*	Trend *P*	No.	%	No.	%	Response Rate %*	OR†	95% CI	*P*	Trend *P*
TP53	Arg72Pro	Arg/Arg	54	42.5	23	31.1	29.9	Reference			.084	121	42.9	49	31.2	28.8	Reference			8.2×10^{-4}
		Arg/Pro	59	46.5	36	48.6	37.9	1.22	0.61 to 2.44	.58		138	48.9	79	50.3	36.4	1.42	0.92 to 2.20	.12	
		Pro/Pro	14	11.0	15	20.3	51.7	2.31	0.90 to 6.06	.083		23	8.2	29	18.5	55.8	3.16	1.64 to 6.21	6.1×10^{-4}	
		Dominant						1.48	0.79 to 2.83	.22							1.66	1.10 to 2.53	.016	
		Recessive						2.10	0.88 to 5.02	.094							2.59	1.43 to 4.75	.0017	
ERCC6	Gly399Asp	Gly/Gly	26	20.5	22	29.7	45.8	Reference			.048	85	30.1	60	38.2	41.4	Reference			.17
		Gly/Asp	77	60.6	43	58.1	35.8	0.54	0.26 to 1.11	.094		141	50.0	68	43.3	32.5	0.68	0.43 to 1.06	.087	
		Asp/Asp	24	18.9	9	12.2	27.3	0.32	0.095 to 0.94	.038		56	19.9	29	18.5	34.1	0.73	0.41 to 1.28	.27	
		Dominant						0.51	0.25 to 1.03	.062							0.69	0.45 to 1.05	.081	
		Recessive						0.59	0.24 to 1.38	.23							0.91	0.56 to 1.50	.72	
POLD1	Arg119His	Arg/Arg	94	74.0	38	51.4	28.8	Reference			.0026	179	63.5	91	58.0	33.7	Reference			.20
		Arg/His	29	22.8	34	45.9	54.0	3.55	1.81 to 7.15	2.1×10^{-4}		90	31.9	56	35.7	38.4	1.24	0.81 to 1.90	.32	
		His/His	4	3.1	2	2.7	33.3	1.26	0.15 to 7.51	.81		13	4.6	10	6.4	43.5	1.56	0.63 to 3.75	.33	
		Dominant						3.21	1.68 to 6.29	4.1×10^{-4}							1.26	0.85 to 1.92	.23	
		Recessive						0.78	0.099 to 4.57	.79							1.43	0.59 to 3.39	.43	
APEX1	Asp148Glu	Asp/Asp	51	40.2	24	32.4	32.0	Reference			.075	113	40.1	65	41.4	36.5	Reference			.85
		Asp/Glu	62	48.8	37	50.0	37.4	1.38	0.71 to 2.75	.35		129	45.7	68	43.3	34.5	0.87	0.56 to 1.34	.52	
		Glu/Glu	14	11.0	13	17.6	48.1	2.34	0.89 to 6.31	.086		40	14.2	24	15.3	37.5	0.98	0.52 to 1.83	.95	
		Dominant						1.55	0.82 to 2.99	.17							0.91	0.61 to 1.37	.66	
		Recessive						2.00	0.84 to 4.83	.12							1.07	0.60 to 1.87	.81	
MSH3	Thr1045Ala	Thr/Thr	67	52.8	47	63.5	41.2	Reference			.041	158	56.0	94	59.9	37.3	Reference			.79
		Thr/Ala	48	37.8	25	33.8	34.2	0.70	0.36 to 1.34	.28		110	39.0	53	33.8	32.5	0.87	0.57 to 1.33	.53	
		Ala/Ala	12	9.4	2	2.7	14.3	0.25	0.036 to 1.11	.071		14	5.0	10	6.4	41.7	1.14	0.46 to 2.72	.77	
		Dominant						0.60	0.32 to 1.13	.11							0.89	0.59 to 1.33	.57	
		Recessive						0.25	0.037 to 1.01	.052							1.26	0.53 to 2.93	.59	
FANCA	Ser501Gly	Ser/Ser	94	74.0	48	64.9	33.8	Reference			.071	197	69.9	108	68.8	35.4	Reference			.96
		Ser/Gly	33	26.0	25	33.8	43.1	1.64	0.85 to 3.19	.14		76	27.0	44	28.0	36.7	1.01	0.65 to 1.58	.95	
		Gly/Gly	0	0	1	1.4	100	—		.088		9	3.2	5	3.2	35.7	0.95	0.28 to 2.86	.92	
		Dominant						1.72	0.89 to 3.32	.10							1.02	0.66 to 1.56	.94	
		Recessive						—		.11							0.98	0.29 to 2.93	.97	

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio.
*Fraction of responders.
†OR for responders against nonresponders adjusted for sex, age, histology, smoking status, clinical stage, performance status, and treatment.

Table A2. Association of Genotypes for DNA Repair Genes With Response to Chemotherapy of 640 Patients With NSCLC

				Nonresponders		Responders						
Gene	SNP	SNP ID	Genotype	No.	%	No.	%	Response Rate (%)*	OR†	95% CI	*P*	*P* by Trend Test
ERCC1‡	C118T (Asn3Asn)	rs11615	C/C	212	51.8	127	55.0	37.5	Reference			.87
			C/T	174	42.5	86	37.2	33.1	0.83	0.58 to 1.16	.27	
			T/T	23	5.6	18	7.8	43.9	1.36	0.69 to 2.64	.37	
			Dominant						0.89	0.64 to 1.23	.47	
			Recessive						1.45	0.74 to 2.72	.29	
APEX1‡	Asp148Glu	rs1130409	Asp/Asp	164	40.1	89	38.5	35.2	Reference			.36
			Asp/Glu	191	46.7	105	45.5	35.5	1.01	0.71 to 1.45	.95	
			Glu/Glu	54	13.2	37	16.0	40.7	1.29	0.77 to 2.14	.33	
			Dominant						1.08	0.77 to 1.51	.66	
			Recessive						1.32	0.82 to 2.09	.25	
XRCC1§	Arg399Gln	rs25487	Arg/Arg	69	54.3	44	59.5	38.9	Reference			.22
			Arg/Gln	45	35.4	25	33.8	35.7	0.89	0.45 to 1.74	.74	
			Gln/Gln	13	10.2	5	6.8	27.8	0.38	0.10 to 1.22	.11	
			Dominant						0.78	0.41 to 1.45	.43	
			Recessive						0.43	0.12 to 1.32	.14	
XPD§	Lys751Gln	rs13181	Lys/Lys	116	91.3	65	87.8	35.9	Reference			.32
			Lys/Gln	11	8.7	9	12.2	45.0	1.68	0.60 to 4.60	.32	
			Gln/Gln	0	0	0	0	—	—			
			Dominant						1.68	0.60 to 4.60	.32	
			Recessive						—			

Abbreviations: NSCLC, non–small-cell lung cancer; SNP, single nucleotide polymorphism; ID, identification; OR, odds ratio.
*Fraction of responder.
†OR for responder against nonresponder adjusted for sex, age, histology, smoking status, clinical stage, performance status, and treatment.
‡Result in the discovery and validation set patients.
§Result in the discovery set patients.

Table A3. Differences in Responses to Chemotherapeutic Agents According to Genotypes for Five DNA Repair Genes

			Discovery Set (n = 138)									
			Platinum + Paclitaxel					Platinum + Gemcitabine				
			Nonresponders		Responders			Nonresponders		Responders		
Gene	SNP	Genotype	No.	%	No.	%	Response Rate (%)†	No.	%	No.	%	Response Rate (%)†	*P* for Interaction†
PARP1	Lys940Arg	Lys/Lys	55	91.7	29	76.3	34.5	26	86.7	10	100	27.8	.021
		Lys/Arg	5	8.3	9	23.7	64.3	4	13.3	0	0	0	
		Arg/Arg	0	0	0	0	—	0	0	0	0	—	
ERCC2	Lys751Gln	Lys/Lys	57	95.0	32	84.2	36.0	26	86.7	10	100	27.8	.021
		Lys/Gln	3	5.0	6	15.8	66.7	4	13.3	0	0	0	
		Gln/Gln	0	0	0	0	—	0	0	0	0	—	
BRCA2	Asn372His	Asn/Asn	38	63.3	20	52.6	34.5	22	73.3	8	80.0	26.7	.14
		Asn/His	21	35.0	13	34.2	38.2	7	23.3	2	20.0	22.2	
		His/His	1	1.7	5	13.2	83.3	1	3.3	0	0	0	
REV1	Phe257Ser	Phe/Phe	23	38.3	17	44.7	42.5	12	40.0	3	30.0	20.0	.18
		Phe/Ser	31	51.7	18	47.4	36.7	16	53.3	5	50.0	23.8	
		Ser/Ser	6	10.0	3	7.9	33.3	2	6.7	2	20.0	50.0	
REV3L	Thr1224Ile	Thr/Thr	25	41.7	16	42.1	39.0	16	53.3	3	30.0	15.8	.080
		Thr/Ile	28	46.7	19	50.0	40.4	13	43.3	5	50.0	27.8	
		Ile/Ile	7	11.7	3	7.9	30.0	1	3.3	2	20.0	66.7	

	Validation Set (n = 417)									
	Platinum + Paclitaxel					Platinum + Gemcitabine				
	Nonresponders		Responders			Nonresponders		Responders		
Gene	No.	%	No.	%	Response Rate (%)†	No.	%	No.	%	Response Rate (%)†	*P* for Interaction*
PARP1	189	90.0	112	89.6	37.2	44	77.2	23	92.0	34.3	.16
	21	10.0	13	10.4	38.2	13	22.8	2	8.0	13.3	
	0	0	0	0	—	0	0	0	0	—	
ERCC2	191	91.0	115	92.0	37.6	54	94.7	24	96.0	30.8	.98
	19	9.0	10	8.0	34.5	3	5.3	1	4.0	25.0	
	0	0	0	0	—	0	0	0	0	—	
BRCA2	127	60.5	78	62.4	38.0	34	59.6	16	64.0	32.0	.81
	74	35.2	42	33.6	36.2	22	38.6	7	28.0	24.1	
	9	4.3	5	4.0	35.7	1	1.8	2	8.0	66.7	
REV1	107	51.0	52	41.6	32.7	29	50.9	13	52.0	31.0	.56
	87	41.4	60	48.0	40.8	25	43.9	10	40.0	28.6	
	16	7.6	13	10.4	44.8	3	5.3	2	8.0	40.0	
REV3L	86	41.0	62	49.6	41.9	21	36.8	9	36.0	30.0	.64
	97	46.2	53	42.4	35.3	28	49.1	14	56.0	33.3	
	27	12.9	10	8.0	27.0	8	14.0	2	8.0	20.0	

Abbreviation: SNP, single nucleotide polymorphism.
*Interaction with chemotherapeutic regimens on response.
†Fraction of responders.

Table A4. Differences in Responses to Chemotherapeutic Agents According to Genotypes for DNA Repair Genes

	Platinum + Paclitaxel (n = 433)								Platinum + Gemcitabine (n = 122)							
	Nonresponders		Responders						Nonresponders		Responders					
Genotype	No.	%	No.	%	Response Rate (%)†	OR‡	95% CI	*P*	No.	%	No.	%	Response Rate (%)†	OR‡	95% CI	*P*	*P* for Interaction*
TP53-Arg72Pro																	.35
Arg/Arg	118	43.7	54	33.1	31.4	Reference			39	44.8	9	25.7	18.8	Reference			
Arg/Pro	130	48.1	83	50.9	39.0	1.33	0.87 to 2.05	.19	39	44.8	17	48.6	30.4	1.80	0.68 to 4.96	.24	
Pro/Pro	22	8.1	26	16.0	54.2	2.51	1.29 to 4.95	.0068	9	10.3	9	25.7	50.0	4.56	1.29 to 17.6	.019	
PARP1-Lys940Arg																	.019
Lys/Lys	244	90.4	141	86.5	36.6	Reference			70	80.5	33	94.3	32.0	Reference			
Lys/Arg	26	9.6	22	13.5	45.8	1.43	0.77 to 2.63	.26	17	19.5	2	5.7	10.5	0.22	0.033 to 0.88	.030	
Arg/Arg	0	0.0	0	0	—	—			0	0	0	0	—	—			
ERCC1-C118T																	.58
C/C	136	50.4	89	54.6	39.6	Reference			49	56.3	21	60.0	30.0	Reference			
C/T	118	43.7	62	38.0	34.4	0.83	0.55 to 1.25	.37	36	41.4	10	28.6	21.7	0.68	0.27 to 1.65	.40	
T/T	16	5.9	12	7.4	42.9	1.30	0.57 to 2.94	.53	2	2.3	4	11.4	66.7	5.78	0.99 to 48.3	.066	

Abbreviation: OR, odds ratio.
*Interaction with chemotherapeutic regimens on response.
†Fraction of responders.
‡OR for responders against nonresponders adjusted for sex, age, histology, smoking status, clinical stage, and performance status.

Contribution of the *TP53*, *OGG1*, *CHRNA3*, and *HLA-DQA1* Genes to the Risk for Lung Squamous Cell Carcinoma

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Introduction: Recent genome-wide association studies (GWASs) have identified polymorphisms in several genes associated with lung cancer risk. Nevertheless, functional polymorphisms in DNA repair and metabolic genes that had been reported as being associated with risk for lung cancer, particularly for lung squamous cell carcinoma (SQC), were not examined in those studies. Therefore, significance of these functional polymorphisms was evaluated in a population, in which polymorphisms in the GWAS genes showed associations with lung SQC risk.

Methods: Polymorphisms in three DNA repair genes, *TP53*, *MDM2*, and *OGG1*, and two metabolic genes, *CYP1A1* and *GSTM1*, were examined for associations with lung SQC risk in a hospital-based case-control study consisting of 377 cases and 325 controls, which had been previously subjected to association studies on GWAS genes, *CHRNA3*, *TERT*, and *HLA-DQA1*.

Results: Genotypes for two DNA repair genes, *TP53* and *OGG1*, showed significant associations with SQC risk ($p < 0.05$), and those for two GWAS genes, *CHRNA3* and *HLA-DQA1*, showed significant associations with SQC risk ($P < 0.05$) with odds ratios between 1.65 (95% confidence interval = 1.06–2.57 for *OGG1*) and 2.57 (95% confidence interval = 1.03–6.87 for *CHRNA3*). Marginally significant associations were also observed for *MDM2* and *CYP1A1* genes. Interactions among these polymorphisms on SQC risk were not observed.

Conclusions: Association of functional polymorphisms in DNA repair and metabolic genes with lung SQC risk was appreciated. This result indicates the necessity of reevaluation for the significance of functional polymorphisms in DNA repair and metabolic genes on lung cancer risk in other populations subjected to GWASs.

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Several genome-wide association studies (GWASs) on single-nucleotide polymorphisms (SNPs) have led to the identification of three loci, chromosomes 15q25, 5p13, and 6p21, containing SNPs associated with lung cancer risk in Europeans and Americans.[1–3] Associations with lung cancer risk of a SNP in the *CHRNA3* gene at 15q25 encoding a nicotinic acetylcholine receptor subunit and a SNP in the *TERT* gene at 5p15 encoding a telomerase reverse transcription were also validated in Asians.[4,5] Significance of SNPs at 6p21 on lung cancer risk of Asians have not been investigated fully; however, our GWAS on Japanese indicated *HLA-DQA1*, encoding a human leukocyte antigen class II protein, as a responsible locus at 6p21.[5] A recent combined study on multiple GWASs in Europeans and Americans indicated that major common genes conferring lung cancer risk have been identified already.[6]

Studies on DNA adducts/damages, including those produced by tobacco carcinogens and their repair processes led to identification of various metabolic and DNA repair genes carrying functional polymorphisms, which possibly cause interindividual differences in the rate of somatic mutation and lung cancer susceptibility.[7] *CYP1A1* and *GSTM1* are representative, because their polymorphisms have been reported to be associated with risk for lung cancer of Asians, particularly for squamous cell carcinoma (SQC), a major histological type of lung cancer mostly developed in cigarette smokers.[8] The risk (462Val) allele for the Ile462Val SNP in the *CYP1A1* gene encodes a protein with a higher activity to bioactivate polycyclic aromatic hydrocarbons, major tobacco procarcinogens, than the 462Ile allele.[7] The risk (absence) allele for the presence or absence polymorphism in the *GSTM1* gene does not encode GSTM1 protein to detoxify activated polycyclic aromatic hydrocarbon-intermediates.[7] *TP53*, *MDM2*, and *OGG1* are representative DNA repair genes associated with lung cancer risk in Asians.[9–11] The risk (72Pro) allele for the TP53-Arg72Pro SNP in the *TP53* gene encodes a protein with a weaker apoptosis activity allowing survival of DNA-damaged cells than the 72Arg allele.[7] The risk (G) allele for a T/G SNP in the promoter region of the *MDM2* gene (called