Table 3. Toxicity analysis

	FACS ($N = 145$)	LC00-03 ($N = 197$)	S0003 ($N = 186$)	*P*-value
Neutropenia (group 4), *N* (%)	102 (69)	106 (69)	48 (26)	<0.0001
Febrile neutropenia (groups 3–4), *N* (%)	26 (18)	38 (19)	6 (3%)	<0.0001

Gandara: ASCO 2004; Crowley: ASCO 2006; Gandara JCO 2009 (27).

Table 4. Efficacy

	FACS ($N = 145$)	LC00-03 ($N = 197$)	S0003 ($N = 182$)	*P*-value
Complete + partial response, *N* (%)	47 (32)	71 (36)	61 (34)	0.61
PFS (months)	4.5	6	4	NA
MST (months)	12	14	9	NA
1-year survival rate (%)	51	57	37	0.001

NA, statistical comparison not applicable.
Gandara: ASCO 2004; Crowley: ASCO 2006; Gandara JCO 2009 (27).

Table 5. Solution to drug lag in East Asia

Pharmaceutical companies
Simultaneous clinical development
Multinational clinical trial
Asian clinical trial
Investigations
Quick accrual of patients
Regulatories
Established high quality and speedy approval process
Regulatory harmonization and more collaborations among regulatory agencies

population: 51 and 57% versus 37%. Korean and Chinese trials have shown the same tendency.

Another very important factor is the lag time until drug approval. Comparison of Japan with the EU and the US shows that the average time from the first approval anywhere in the world until approval in each other country was about 500 days in the US and the UK, but over 1400 days in Japan. Looking at drug lag in East Asia shows that Taiwan and Korea were a little bit quicker than Japan and China for approval of some drugs. To solve this problem of drug lag in East Asia, it will be necessary for pharmaceutical companies to perform simultaneous clinical development in multiple countries, multinational clinical trials and Asian clinical trials. Also, investigators need to achieve quick accrual of patients, while the regulatory authorities need to establish high quality and speedy approval processes, and achieve regulatory harmonization and better collaboration among agencies (Table 5).

The National Comprehensive Cancer Network (NCCN) is an alliance of 21 of the world's leading cancer centers that is based in the USA. The NCCN promotes the importance of continuous quality improvement and creation of international and national clinical practice guidelines (10). The NCCN has international initiatives in Asia, including adaptation of NCCN Clinical Practice Guidelines in Oncology to create NCCN approved, translated and/or regionally adapted materials for national use. The process for such adaptation is that the NCCN authorizes selected groups to adapt its Practice Guidelines for national use. The participating countries select disease-specific representatives to review and suggest modifications to specific guidelines. Then the NCCN guidelines are circulated to multidisciplinary physicians in that country to determine where local practice is not concordant with the NCCN version. Regional meetings are held to agree on proposals, supported by data, for adaptation of the guidelines. A consensus for adaptation is approved by the NCCN, and the changes from the NCCN version are identified in the adaptation.

Asian consensus statements are intended as a reference and stepping stone for individual countries in Asia that do not yet have local editions of the NCCN guidelines so that they can develop their own guidelines. There have still been no pan-Asian guidelines developed for NSCLC. In general, the NCCN guidelines or national adaptations, or other recognized guidelines (e.g. ASCO, ACCP), are followed. Asian consensus statements are developed through the NCCN to help individual countries establish their own guidelines. As national NSCLC guidelines, Korea, China and Thailand adapted the NCCN guidelines. In Japan, the Japanese Society of Lung Cancer developed a Lung Cancer Practice Guideline in 2003 (13); this is different from the NCCN guidelines. China also has a Chinese Lung Cancer Management Guideline that is based on Chinese clinical practice and is used by most Chinese doctors. It was issued by the Chinese Society of Lung Cancer and is revised every 2 years. Hong Kong, India, Malaysia, Taiwan and Singapore have no NSCLC guideline (Table 6).

There are several differences between the NCCN version 2/2009 and the Korean NCCN 2008. For Stage IIIB resectable satellite lesions, the Korean NCCN guidelines specify the strategies for pN 0-1 and pN0. The therapy for recurrent and metastatic disease, chemotherapy for progressive disease and adjuvant chemotherapy regimens also differ between these guidelines. Comparison of the Korean NCCN guidelines and the ASCO guidelines shows that key differences exist in relation to Stage I disease and resected Stages I–IIIA. For Stage I, the Korean NCCN guidelines suggest adjuvant chemotherapy as an option, whereas it is not recommended in the ASCO guidelines (29). For resected Stages I–IIIA, the Korean NCCN guidelines suggest adjuvant radiotherapy when margins are positive, but it is not routinely recommended in the ASCO guidelines. The ASCO

Table 6. Current NSCLC guidelines in Asia

Pan-Asian guidelines
There are no pan-Asian guidelines developed for NSCLC
NCCN guidelines (or national adaptations of these) or other recognised guidelines (e.g. ASCO, ACCP) are generally followed
Asia Consensus Statements are developed through NCCN to help countries develop their own guidelines
National guidelines
Korea, Thailand: adaptation of NCCN guidelines
Japan: Japanese Society Lung Cancer developed Lung Cancer Practice guideline (2003)
China: adaptation of NCCN guidelines, Chinese LC Management Guideline
The following countries do not appear to have individual national guidelines
Hong Kong, India, Malaysia, Taiwan, Singapore

guidelines are very conservative and revised every 5 years, whereas the Korean NCCN guidelines are revised very frequently. Major institutions generally apply the Korean KCCN guidelines (11).

Regarding the current guideline for NSCLC in Japan, the background of its preparation includes such factors as that lung cancer is the number-one cause of death in Japan, the death rate due to lung cancer is increasing rapid, the cure rate is low at about 10–15%, there has been development of diverse diagnostic and treatment methods, and there is a need for a guideline that indicates standard medical care for lung cancer. The guideline should be evidence based, with scientific evidence obtained from clinical trials, should take into account the patients' requirements and preferences, and should also take into account physicians' professional experience and knowledge. As the method for development of a guideline, a systematic search of the published literature during the last 10–20 years should encompass PubMed, the Cochrane Review, Japanese medical journals, etc., critical and quantitative/qualitative evaluation of evidence, and scientific recommendations. Various key words are used to search the literature.

With regard to the history of development of a guideline for medical care of lung cancer in Japan, a study group was formed in 2001, with support from the Japanese Ministry of Health, Labour and Welfare (MHLW). The study group consisted of representatives from various Japanese medical societies, including the Japanese Society of Lung Cancer and the Japanese Society of Respiratory Disease. In 2003, the first 'Guideline for Medical Care in Lung Cancer (13),' also supported by grants from the MHLW, was developed. In 2005, the Guideline was revised by the Japanese Society of Lung Cancer. The contents of the guideline consisted of medical care (diagnosis and treatment modalities) and staging. The classification of the evidence level was similar to that for other guidelines. The highest level of evidence was (i) systematic review and meta-analysis of multiple randomized clinical trials. Subsequent levels consisted of (ii) more than one RCT, (iii) a non-RCT such as a Phase II study, (iv) an analytical-epidemiological study such as a cohort study or case-controlled study, (v) case reports and/or case series, and (vi) personal opinions of specialists or committee members. The recommendation levels consisted of (A) strongly recommended, (B) recommended, (C) not enough data for recommendation and (D) recommended not to do. Decision-making regarding the recommendation was based on the (A) evidence level, (B) amount of evidence and consistency, (C) hazard ratio (difference in efficacy), (D) clinical applicability and (E) evidence of toxicity and cost.

In the EBM guideline to chemotherapy for lung cancer, the recommendations regarding the roles of chemotherapy for advanced NSCLC are (i) chemotherapy in unresectable advanced NSCLC patients prolongs survival, improves QOL and is strongly recommended in this group of patients (Grade A recommendation) and (ii) chemotherapy in elderly, unresectable advanced NSCLC patients prolongs survival, improves QOL and is strongly recommended in this group of patients (Grade B recommendation). The recommendations regarding the target population for chemotherapy are (i) chemotherapy is recommended in patients less than 75 years old with a good performance status (PS 0, 1) (Grade A), (ii) chemotherapy is also recommended in patients more than 75 years old with a good PS (0, 1) (Grade B) and (iii) possibility of chemotherapy in PS 2 patients, but there is no evidence (Grade C). (underlining indicates a difference from Western guidelines.) There is the issue of use of gefitinib in patients with EGFR mutation, and the guideline thus needs to be revised.

The recommendations regarding the selection of anti-cancer drugs are (i) cisplatin-containing doublets are strongly recommended in patients less than 75 years old with a good PS (0, 1) (Grade A), (ii) drugs to be combined with cisplatin are irinotecan, vinorelbine, gemcitabine, paclitaxel and docetaxel (Grade A), and (iii) non-platinum doublets are recommended in patients who might be suffering from cisplatin-induced toxicity (Grade A). Questions remain regarding the use of gefitinib in patients with EGFR mutation and whether pemetrexed should be used, and the guideline thus needs to be revised.

The recommendation regarding the duration of chemotherapy is that first-line chemotherapy should consist of three to six courses (Grade B). But recently there has been development of the concepts of consolidation and maintenance therapy, so this recommendation also needs to be revised. For second-line chemotherapy (defined as chemotherapy for refractory or recurrent NSCLC after first-line chemotherapy), it is recommended that docetaxel be administered for refractory or recurrent NSCLC after first-line chemotherapy (Grade B). However, pemetrexed, erlotinib and gefitinib are now available, and this recommendation thus needs to be revised. With regard to molecular-target-based therapy, there is insufficient evidence for recommendation of EGFR/TKI in NSCLC (Grade C). However, positive results have since been obtained in EGFR-mutated NSCLC, and this description in the guideline thus also needs to be revised.

With regard to chemoradiotherapy (CRT) for locally advanced NSCLC, the recommendations are as follows: (i) CRT containing cisplatin is strongly recommended for inoperable, locally advanced NSCLC (Grade A); (ii) CRT is strongly recommended for patients with a good PS (0, 1) (Grade A); (iii) Chemotherapy should be given concurrently (Grade A); (iv) The dose of radiotherapy should be 60 Gy by usual fractionation (1.8–2.0 Gy/day) (Grade A); (v) there is no evidence for an effect of split-course radiotherapy on survival benefit, while there is not enough data for recommending not to split radiotherapy (Grade C); (vi) the chemotherapy regimen for concurrent CRT should be a platinum-containing doublet or triplet (Grade B). There is not enough data from large clinical trials regarding CRT-containing irinotecan, paclitaxel, docetaxel, vinorelbine and gemcitabine, and these drugs should be used only in clinical trials (Grade C). However, positive results have recently been obtained with paclitaxel and vinorelbine, and this description in the guideline thus also needs to be revised.

The recommendation with regard to adjuvant immunotherapy (postoperative) is that there is not enough evidence for an improved prognosis by using an immunostimulant. There is also no clear evidence for recommending use of an immunostimulant after surgery (Grade C). The recommendation with regard to preoperative chemotherapy in Stage I/II NSCLC is that there is not enough data to recommend preoperative chemotherapy (Grade C).

In addition to the guideline, since 2005 Japan has had a guidance for gefitinib prescription. The indication for gefitinib is inoperable or recurrent NSCLC. Gefitinib is not indicated for patients without prior chemotherapy, as adjuvant therapy, as maintenance therapy after CRT or in combination with anti-cancer drugs or radiotherapy. Gefitinib is recommended for the following patients: females, adenocarcinoma, non-smokers, Japanese (Asians) and patients with EGFR mutation.

Thus, Japan has an NSCLC guideline and a gefitinib guidance, but the reality is somewhat different. With regard to the market share of the first-line regimens for NSCLC in Japan, carboplatin/paclitaxel is number one, followed by gefitinib, which is surprising. As the second-line regimen, gefitinib is number one, followed by docetaxel. There is thus a discrepancy between the guidelines and actual clinical practice.

Based on the discussions among the study group members from various Asian countries, it seems difficult to establish a common guideline for NSCLC among Asian countries at the present time because of the differences in medical care in each country as well as the drug lag seen in some countries. Asian collaborative trials on treatment of NSCLC need to be started at an early date to generate Asian data.

EARLY-STAGE LUNG CANCER

Some differences are seen between Asia and Europe and the USA in regard to early-stage lung cancer. Based on clinical practice, it is found that the results of surgery for early-stage lung cancer are better in Asia than in the West. There are also differences with regard to the value of adjuvant chemotherapy. For example, for Stage I, adjuvant chemotherapy is not used in China, whereas in the US and Europe adjuvant chemotherapy is recommended for Stage IB lung cancer. One problem is how to treat patients with early-stage lung cancer with EGFR mutation, which occurs at a much higher incidence of about 30% in Asian populations. Asian clinical trials are needed to answer this.

LOCALLY ADVANCED NSCLC

In regard to locally advanced NSCLC, it is accepted that concurrent chemoradiation therapy (CRT) should be accepted as standard treatment. However, there are several questions regarding the drug to be used in Asian populations: the type of drug, dosage and schedule that will be suitable. As reported, chemotherapy toxicity is higher in Asian populations, but the response and survival are better than in the West. The radiation technique used in CRT has mostly been 3D conformal irradiation. However, this may not be possible in all Asian countries, so further investigation is needed regarding the radiation technique to be used concurrently with chemotherapy. Induction chemotherapy or CRT prior to surgery also needs to be studied in Asia, as does surgery for locally advanced NSCLC. A third point regarding locally advanced NSCLC is maintenance therapy, especially tyrosine kinase inhibitors (TKIs). Detrimental effects were reported in an American population administered maintenance TKI. However, because of the high incidence of EGFR mutation in Asians, it is not known whether maintenance therapy with TKIs will benefit the patient or not. In the West most population studies were based on PET CT, whereas in most Asian countries, especially Southeast Asia, the method is usually only CT scan. Thus, there are various problems remaining in Asian populations with regard to locally advanced NSCLC.

ADVANCED NSCLC

Three aspects of management of advanced NSCLC in the Asian region need to be addressed. First, there are some epidemiological differences, especially the incidence of NSCLC mortality. Second, there seem to be some differences in the etiological factors implicated in lung cancer in the East compared with the West. In the East, there are more cases that are not directly associated with smoking, meaning that lung cancer non-smokers are more prevalent, especially in East Asian women. Third, there is increasing evidence in support of major differences in treatment of advanced NSCLC in terms of the efficacy and toxicity, especially with TKIs. Asian patients derive much greater benefit from TKIs compared with Caucasian people. In fact, some of the Korean consensus guidelines suggest broader recommendation of TKIs even to patients with a poor performance status.

Cytotoxic agents are usually relatively or absolutely contraindicated for poor PS patients, but TKIs are much more convenient to administer and much less toxic than cytotoxic agents. Thus, TKIs can be recommended to a broader range of patients with a poor performance status. There are also recent data that indicate possible benefit from TKIs even in the first-line setting, without any prior chemotherapy.

In summary, there is mounting evidence of differences between Asian and Caucasian lung cancer patients in many aspects, including epidemiology, etiology and treatment outcomes and toxicities. Asia truly needs its own region-specific clinical trials to address each of these issues in regard to NSCLC.

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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.

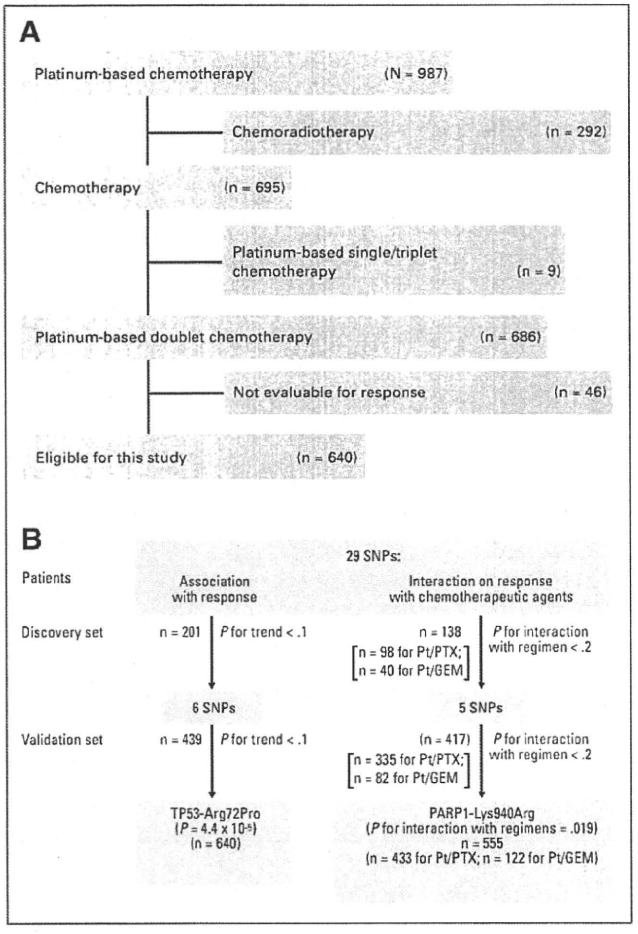

Fig 1. Patients and strategy. (A) Selection of eligible cases. (B) A two-phase screening of single nucleotide polymorphisms (SNPs) associated with responses to platinum-based doublet chemotherapy. Pt, platinum; PTX, paclitaxel; GEM, gemcitabine.

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			Discovery Set*		Validation Set	
	No.	%	95% CI	No.	%	No.	%
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

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

		Nonresponders		Responders						
NSCLC	Genotype	No.	%	No.	%	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	.87§	
	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	.14§	
	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.

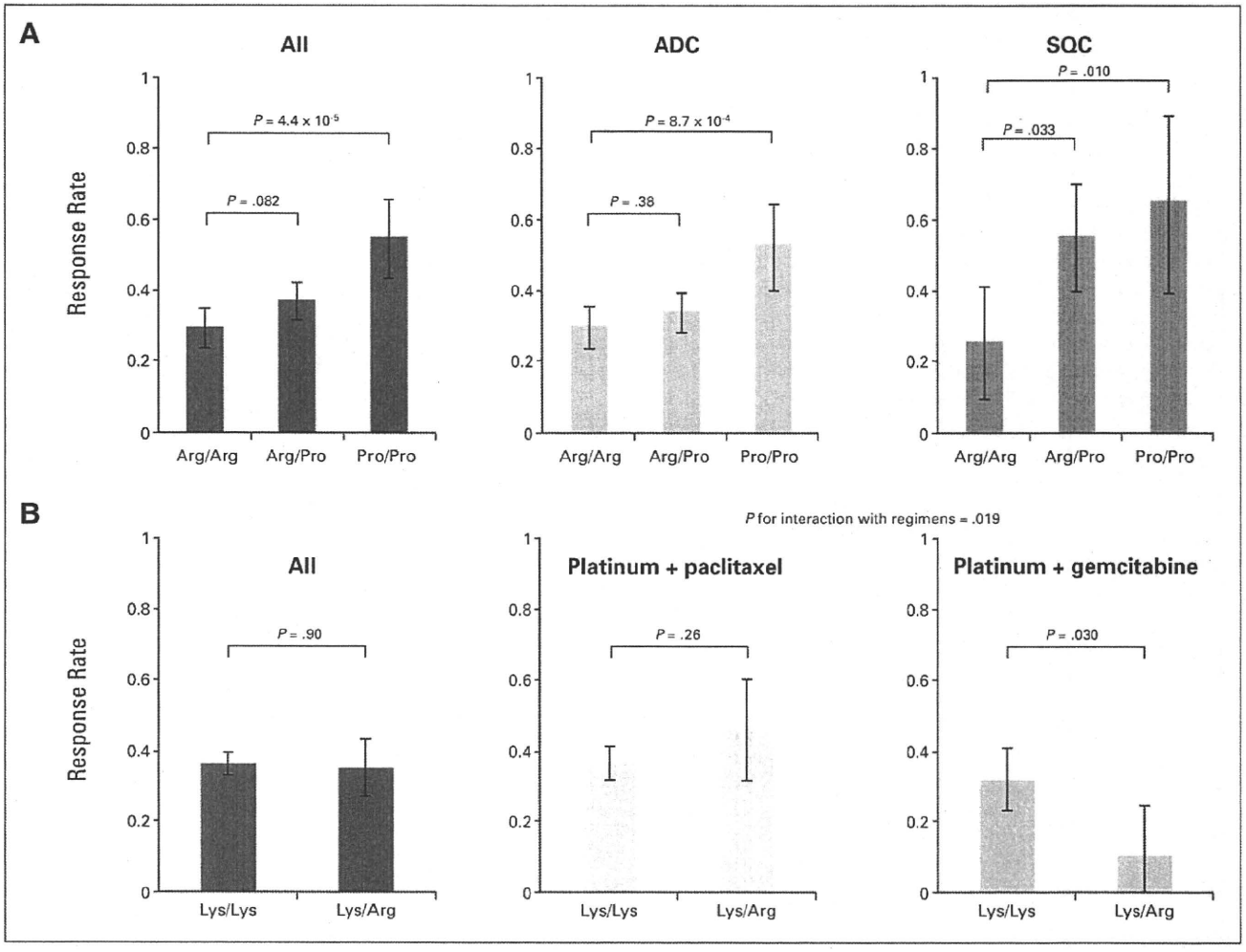

Fig 2. (A) Response rates according to *TP53* genotypes in (left) all patients and those with (middle) aenocarcinoma (ADC) and (right) squamous cell carcinoma (SQC). (B) Response rates according to *PARP1* genotypes in (left) all patients and those treated with (middle) platinum plus pacliteaxel or (right)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

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).

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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Genome-Wide Association Study on Overall Survival of Advanced Non-small Cell Lung Cancer Patients Treated with Carboplatin and Paclitaxel

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Purpose: Our goal was to identify candidate polymorphisms that could influence overall survival (OS) in advanced non-small cell lung cancer (NSCLC) patients treated with carboplatin (CBDCA) and paclitaxel (PTX).

Methods: Chemotherapy-naïve stage IIIB or IV NSCLC patients treated with CBDCA (area under the curve = 6 mg/mL/min) and PTX (200 mg/m^2, 3-hour period) were eligible for this study. The DNA samples were extracted from peripheral blood mononuclear cells before treatment, and genotypes at approximately 110,000 gene-centric single-nucleotide polymorphisms (SNPs) were obtained by Illumina's Sentrix Human-1 Genotyping BeadChip. Statistical analyses were performed by the log-rank test and Cox proportional hazards model.

Results: From July 2002 to May 2004, 105 patients received a total of 308 cycles of treatment. The median survival time (MST) of 105 patients was 17.1 months. In the genome-wide association study, three SNPs were associated significantly with shortened OS after multiple comparison adjustment: rs1656402 in the *EIF4E2* gene (MST was 18.0 and 7.7 months for AG [n = 50] + AA [n = 40] and GG [n = 15], respectively; $p = 8.4 \times 10^{-8}$), rs1209950 in the *ETS2* gene (MST = 17.7 and 7.4 months for CC [n = 94] and CT [n = 11] + TT [n = 0]; $p = 2.8 \times 10^{-7}$), and rs9981861 in the *DSCAM* gene (MST = 17.1 and 3.8 months for AA [n = 75] + AG [n = 26] and GG [n = 4]; $p = 3.5 \times 10^{-6}$).

Conclusion: Three SNPs were identified as new prognostic biomarker candidates for advanced NSCLC treated with CBDCA and PTX. The agnostic genome-wide association study may unveil unexplored molecular pathways associated with the drug response, but our findings should be replicated by other investigators.

Key Words: Advanced non-small lung cancer, Carboplatin, Paclitaxel, Genome-wide association study, Single-nucleotide polymorphisms.

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Lung cancer is the leading cause of cancer death in Japan and worldwide for both men and women.[1] Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases. Several third-generation agents are available for the treatment of NSCLC, including docetaxel, paclitaxel (PTX), gemcitabine, and vinorelbine, and the combination of one of these agents with a platinum compound has been considered the standard treatment option for advanced NSCLC.[2–9]

Despite these advances, survival prospects still remain disappointingly low for most patients. To seek further improvements in response rate and survival time, the conventional treatment approach to NSCLC is beginning to shift toward the application of specific strategies and techniques, such as pharmacogenomics to tailor treatment to individual patients.[10,11]

To identify the clinical predictors of outcome, it is critically important to observe individual differences in drug response and the role of genetic polymorphisms that are relevant to the pathways of drug metabolism and/or the biology of drug responses. However, genetic polymorphisms that are associated with overall survival (OS) or antitumor effect have not yet been fully elucidated.

With this as background, this prospective study employed a genome-wide association study (GWAS) to identify candidate polymorphisms that could influence OS in advanced NSCLC patients treated with carboplatin (CBDCA) and PTX. Possible associations with toxicities and pharma-

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cokinetic (PK) parameters were also tested to complement our previous candidate gene approach focusing on CYP3A4[12] and CYP2C8.[13]

PATIENTS AND METHODS

Patient Recruitment and Treatment Schedule

Patients with histologically and/or cytologically documented NSCLC were eligible for participation in the study and treated with CBDCA and PTX at the National Cancer Center Hospital and National Cancer Center Hospital East. Each patient had to meet the following criteria: clinical stage IIIB or IV, no prior chemotherapy, no prior surgery and/or radiotherapy for the primary site, age older than 20 years, and Eastern Cooperative Oncology Group performance status[14] between 0 and 2. This study was approved by the Ethics Review Committees of the National Cancer Center and National Institutes of Health Sciences, and written informed consent was obtained from all patients before study entry.

One hundred five patients received 200 mg/m^2 of PTX (Bristol-Myers K.K., Tokyo, Japan) over a 3-hour period followed by carboplatin at a dose calculated to produce an area under the concentration time curve of 6.0 mg/mL/min on day 1, with the cycle being repeated every 3 weeks. In addition, to prevent hypersensitivity reactions, all patients received short-term premedication including dexamethasone, ranitidine, and an antiallergic agent (diphenhydramine or chlorpheniramine maleate).

Monitoring, Response and Toxicity Evaluation, and Follow-Up

A complete medical history and data on physical examinations were recorded before the CBDCA and PTX combination therapy. Complete blood cell and platelet counts as well as blood chemistry were measured once a week during the first 2 months of the treatment. Response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), except that tumor markers were excluded from the criteria. Toxicity grading criteria in National Cancer Institute Common Toxicity Criteria Version 2.0 were used to evaluate toxicity. Patients were followed by direct evaluation or resident registration until death or up to 5 years after treatment. OS was calculated from the date of patient enrollment in this study to the date of death or the last follow-up.

Pharmacokinetic Sampling and Analysis

For PTX PK analysis, 5 ml of heparinized blood was sampled before the first PTX administration and at 0, 1, 3, and 9 hours after the termination of the infusion. The area under the curve (AUC) and clearance (CL m^{-2}) were calculated by a curve fitting method using the model of two compartments with constant infusion using WinNonlin ver. 3.3 (Pharsight Corporation, Mountain View, CA). The PK data were used in our previous pharmacogenetic analyses.[12,13]

DNA Extraction and Genotyping

Whole blood was collected from patients at the time of enrollment, and DNA was extracted from peripheral lymphocytes using a proteinase-K phenol chloroform method or Qiagen FlexiGene DNA isolation kit (QIAGEN Inc., Valencia, CA). All samples were assayed with the Illumina Infinium Human-1 BeadChip (Illumina Inc., San Diego, CA), which assays 109,365 gene-centric single-nucleotide polymorphisms (SNPs). If a genotyping call rate on all SNPs was found to be less than 95%, the sample was excluded from the analysis.

Statistical Analysis

As a quality control for genotyping, Hardy-Weinberg equilibrium testing was applied. To estimate the association between OS and genotypes, hazard ratios (HRs) and 95% confidence intervals were calculated using univariate or multivariate Cox proportional hazards models[15,16] and assessed using the log-rank test. Survival curves were drawn using the Kaplan-Meier method.[14] Statistical significance level was set to 0.05, two sided, after Holm's adjustment for a multiple testing.[17] All statistical analyses were performed with the use of SAS software, version 9.1.3 (SAS Institute Inc., Cary, NC). All statistical analyses were planned before the study.

RESULTS

Patient Characteristics, Survival, Response, and Toxicity

From July 2002 to May 2004, 239 patients treated with PTX were enrolled. Among them, 110 chemotherapy-naïve advanced NSCLC patients treated with CBDCA (AUC = 6 mg/mL/min) and PTX (200 mg/m^2, 3-hour period) were eligible in this study, but five patients were excluded from the analysis because genotyping data were not available. Their characteristics are shown in Table 1. All patients were followed up for more than 2.5 years, and the median follow-up time among censored observations was 38 months (range, 27–46 months), with 89 patients deceased (85%) as of November 2006. The median survival time (MST) of the 105 patients was 17.1 months (95% confidence interval: 15.0–18.7) (Figure 1). The 1- and 3-year survival probabilities were 68% and 16%, respectively.

Of the 105 patients, changes in tumor measurements were partial response in 43 (41%) patients, stable disease in 47 (45%), progressive disease in 11 (10%), and not evaluated in 4 (4%). There were no cases with a complete response.

All patients were evaluated for toxicity. Hematologic toxicity and nonhematologic toxicity are summarized in Table 2. Grade 3 or 4 nonhematologic toxicity occurred in 15

TABLE 1. Patient Characteristics

Assessable patients	105
Gender (male/female)	76/29
Age, median (range)	61 (29–80)
PS (0/1/2)	20/82/3
Stage (IIIB/IV)	46/59
No. of treatment cycles	
Mean	2.93
Range	1.0–6.0

PS, performance status.

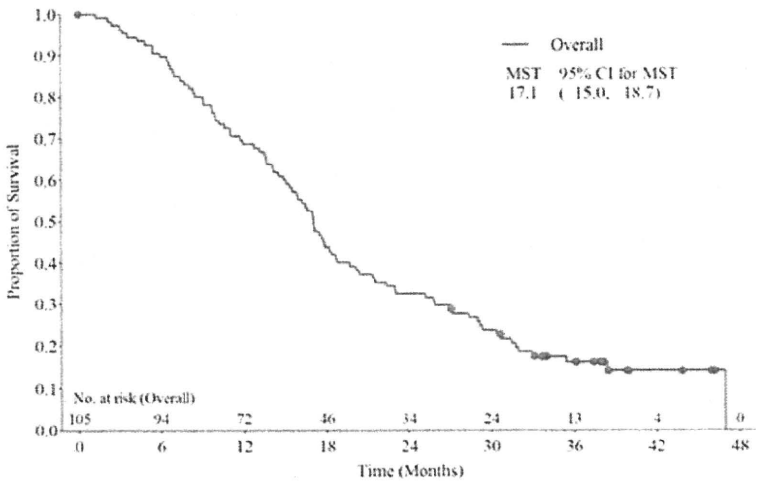

FIGURE 1. Kaplan-Meier plot for overall survival.

TABLE 2. Incidence of Hematologic and Nonhematologic Toxicities After the First Cycle

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Total
Leukopenia	40	34	9	0	101
Neutropenia	8	22	39	18	105
Anemia	73	16	2	0	105
Thrombocytopenia	16	3	0	0	102
Febrile neutropenia	0	0	5	0	105
Nausea	7	3	0	0	105
Vomiting	8	4	3	0	105
Diarrhea	5	6	0	1	105
Arthralgia	58	12	2	0	105
Myalgia	47	10	1	0	105
Hyperbilirubinemia	33	10	0	0	105
AST (GOT) increase	38	1	0	0	105
ALT (GPT) increase	38	3	1	0	105
ALP increase	32	5	0	0	105
Neuropathy, sensory	65	6	1	0	105
Neuropathy, motor	1	0	0	1	105

AST, aspartate transaminase; GOT, glutamic oxaloacetic transaminase; ALT, alanine aminotransferase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase.

(14%) patients, suggesting that nonhematologic toxicity was generally mild; but grade 4 motor neuropathy occurred in one patient and grade 4 diarrhea occurred in another. On the other hand, grade 3 or 4 hematologic toxicity occurred in 57 (53%) patients. Grade 4 neutropenia occurred in 18 (17%) patients. Febrile neutropenia (grade 3) occurred in five patients.

Effects of Patients' Background on Overall Survival

The effects of patients' background on OS were analyzed as summarized in Table 3. The effects of gender, Eastern Cooperative Oncology Group performance status, and tumor response showed significant associations with OS, but age, stage, and number of cycles did not show a significant association.

TABLE 3. Univariate Analysis of Patients' Characteristics

	Overall Survival		
Variable	Crude HR	95% CI for HR	*p*
Age			
≥65 vs. <65	1.12	0.72–1.71	0.61
Gender			
Male vs. female	2.06	1.26–3.39	0.0039
PS			
2 vs. 0–1	7.68	2.28–25.8	0.0010
Stage			
IV vs. IIIB	1.19	0.78–1.83	0.40
No. of cycles	0.92	0.74–1.13	0.42
Tumor response			
PR vs. PD	0.199	0.098–0.403	<.0001
NC vs. PD	0.216	0.108–0.434	<.0001

CI, confidence interval; HR, hazard ratio; PR, partial response; PD, progressive disease; NC, no change.

Pharmacogenomic Analyses

Table 4 lists 10 SNPs, showing the least *p* values for log-rank test. The following three SNPs were associated significantly with shortened OS after multiple comparison adjustment: rs1656402 in the *EIF4E2* gene (MST for AG [$n = 50$] + AA [$n = 40$] and GG [$n = 15$] were 18.0 and 7.7 months, respectively; $p = 8.4 \times 10^{-8}$, HR = 4.22 [2.32–7.66]), rs1209950 in the *ETS2* gene (MST for CC [$n = 94$] and CT [$n = 11$] + TT [$n = 0$] were 17.7 and 7.4 months, respectively; $p = 2.8 \times 10^{-7}$, HR = 4.96 [2.52–9.76]), and rs9981861 in the *DSCAM* gene (MST for GG [$n = 75$] + AG [$n = 26$] and AA [$n = 4$] were 17.1 and 3.8 months, respectively; $p = 3.5 \times 10^{-6}$, HR = 16.1 [5.38–51.2]). In Figure 2, the Kaplan-Meier plots were drawn with subjects stratified into subgroups according to each significant polymorphism in either dominant or recessive model. Two (rs1656402 and rs9981861) of these significant SNPs were associated with tumor response and AUC 6α-,C3′-*p*-dihydroxy-PTX as shown

TABLE 4. Ten SNPs Associated with OS in GWAS

		SNP Information			Patients						
Chr #	Rs #	Gene Symbol	Genotype	Frequency	Total	Events	MST (95% CI)	HR (95% CI)	p^a	p^b	p^c
2	rs1656402	*EIF4E2*	AA	0.145	40	37	15.6 (13.5–17.0)	Ref	8.4×10^{-8}	4.5×10^{-7}	0.0046
			AG	0.461	50	37	24.4 (18.6–30.3)	0.42 (0.26–0.67)			
			GG	0.393	15	15	7.69 (5.95–12.7)	2.73 (1.46–5.10)			
21	rs1209950	*ETS2*	CC	0.938	94	78	17.6 (16.2–21.4)	Ref	2.8×10^{-7}	6.5×10^{-5}	0.015
			CT	0.059	11	11	7.39 (4.86–10.2)	4.96 (2.52–9.76)			
			TT	0.002	—	—	—	NA			
21	rs9981861	*DSCAM*	AA	0.652	75	61	17.8 (15.3–21.4)	Ref	3.5×10^{-6}	9.2×10^{-7}	0.050
			AG	0.314	26	24	16.5 (2.14–18.1)	1.33 (0.82–2.15)			
			GG	0.034	4	4	3.78 (2.14–7.69)	18.0 (5.78–56.2)			
2	rs10496036	*RTN4*	GG	0.701	84	70	17.6 (15.9–21.4)	Ref	2.4×10^{-5}	0.00063	1.00
			AG	0.270	18	2	14.1 (9.63–19.6)	1.52 (0.87–2.62)			
			AA	0.030	3	0	4.30 (2.43–5.95)	22.2 (5.72–86.2)			
6	rs1547633		GG	0.678	69	60	16.9 (13.6–18.3)	Ref	2.3×10^{-5}	7.7×10^{-6}	1.00
			GT	0.283	33	26	21.4 (16.2–27.0)	0.76 (0.48–1.21)			
			TT	0.039	3	3	3.58 (3.02–4.30)	29.7 (6.47–136)			
6	rs1570070	*IGF2R*	GG	0.553	66	57	18.2 (15.8–21.4)	Ref	2.2×10^{-5}	0.00010	1.00
			GA	0.388	33	27	16.4 (11.4–17.7)	1.01 (0.63–1.62)			
			AA	0.059	4	4	4.67 (2.17–7.39)	10.5 (3.85–28.9)			
7	rs2711095		GG	0.655	70	59	17.3 (15.9–19.6)	Ref	2.3×10^{-5}	5.0×10^{-5}	1.00
			AG	0.303	30	25	17.3 (11.7–27.0)	1.33 (0.88–2.00)			
			AA	0.042	5	5	5.39 (1.25–9.63)	10.2 (3.8–27.1)			
16	rs4313828	*CNTNAP4*	AA	0.947	99	83	17.4 (15.8–20.4)	Ref	2.2×10^{-5}	8.2×10^{-5}	1.00
			AG	0.050	6	6	7.51 (3.22–9.92)	7.12 (2.87–17.6)			
			GG	0.003	—	—	—	NA			
6	rs894817	*IGF2R*	AA	0.560	65	56	18.3 (15.8–22.3)	Ref	2.8×10^{-5}	0.00012	1.00
			AG	0.379	36	29	16.2 (10.2–17.7)	1.09 (0.69–1.71)			
			GG	0.061	4	4	4.67 (2.17–7.39)	14.3 (4.57–44.9)			
7	rs959494	*SCIN*	AA	0.659	70	56	17.5 (15.9–21.4)	Ref	3.1×10^{-5}	0.00043	1.00
			AG	0.299	30	28	16.0 (8.44–20.3)	1.53 (0.97–2.42)			
			GG	0.042	4	4	5.08 (2.43–9.07)	12.0 (3.97–36.7)			

[a] p values were calculated by univariate Cox proportional hazards model.
[b] p values were calculated by multivariate Cox proportional hazards model including gender and PS as covariates.
[c] p values were adjusted for multiple testing by using the Holm's method.
MST, median survival time; CI, confidence interval; HR, hazard ratio.

in Supplementary Tables 1 (http://links.lww.com/JTO/A43) and 2 (http://links.lww.com/IGC/A24), respectively.

The following PK parameters were measured in this study: AUC PTX (h*/μg/mL), AUC 6-α-hydroxy-PTX (6-α-OH-PTX) (h/μg/ml), AUC C3′-*p*-hydroxy-PTX (3′-*p*-OH-PTX) (h*/μg/mL), AUC 6α-,C3′-*p*-dihydroxy-PTX (diOH-PTX) (h*/μg/mL), AUC Cremophor EL (μl*/h/mL), CL PTX (L/h/m^2). However, no significant association was detected between the PK parameters and the SNPs by a multiple testing correction (data not shown). For reference, we showed the results of association between top 10 SNPs and PK parameters in Supplementary Table 2. This GWAS neither detected a statistically significant association with any of the grade 3/4 adverse reactions (data not shown), probably due to their low incidence, except for neutropenia (Table 2).

DISCUSSION

Cytotoxic chemotherapy continues to be the mainstay for initial treatment of patients with advanced NSCLC. Individualizing chemotherapy to deliver the most active and least toxic agent to each patient could provide an important improvement in patient care.[11] Previous pharmacogenetic studies have identified biomarkers for survival of patients with advanced NSCLC treated with platinum-based chemotherapy.[18–22] Among these are the *XRCC1*, *XRCC3*, and *XPD* genes, which play an important role in DNA repair.[23–28] Similar to previous studies of platinum-based chemotherapy, Gurubhagavatula et al.[18] observed a trend toward decreased survival for patients with variant *XPD* or *XRCC1* genotype and improved survival for patients with variant *XRCC3* genotype.

These genetic polymorphisms were identified by candidate gene approach, which relies on an a priori selection of small numbers of candidate genes based on the existing information or hypothesis. Although successful in several examples, this candidate gene approach may not be able to capture all the genetic factors, which influence a drug response in a complex interplay with multiple unknown as well

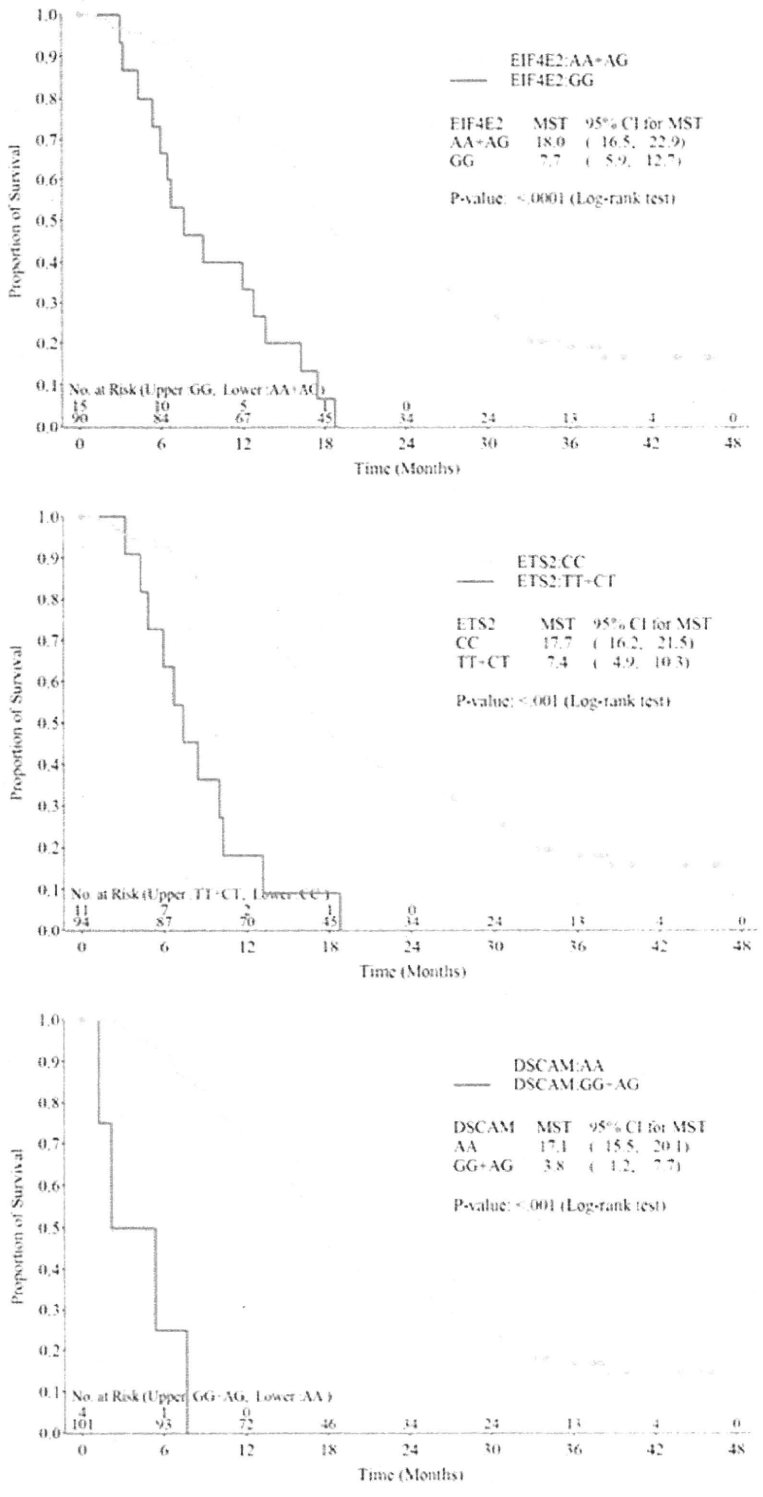

FIGURE 2. Overall survival stratified for the single-nucleotide polymorphism genotype.

as known factors such as disease phenotypes, genetic factors, and the variability in drug target response. GWAS, which makes no assumptions about the genomic location of the causal variants but surveys the whole genome,[29,30] is expected to complement the candidate gene approach. According to our findings from a gene-centric GWAS, three poly-

morphisms were associated with shortened OS in advanced NSCLC with CBDCA and PTX. The three SNPs have not been previously investigated for an association with NSCLC risk or drug response. On the other hand, the SNPs implicated in the prognosis of NSCLC by the previous candidate gene approach[18] were not detected in the GWAS, because the Human-1 BeadChip does not harbor the identical SNPs analyzed before and/or their p values were not sufficiently small in the context of the genome scan.

The first candidate SNP for the OS association, rs1656402, is in the third intron of the gene, *EIF4E2*, encoding for the translational factor eukaryotic initiation factor 4E, which is a central component in the initiation and regulation of translation in eukaryotic cells. Through its interaction with the 5′ cap structure of mRNA, eIF4E functions to recruit mRNAs to the ribosome.[31–34] Prototypical eIF4E-2 is expressed ubiquitously,[33,35] but in metastatic tumors, its expression was increased,[36] suggesting that eIF4E-2 plays an active role in the prognosis of NSCLC.

The second candidate SNP is located at the 4321 bp upstream of the *ETS2* gene. The Ets family of transcription factors includes important downstream targets in cellular transformation. For instance, alteration of Ets activity has been found to reverse the transformed phenotype of ras-transfected mouse fibroblasts and of several human tumor cell lines. It has been reported that Ets factor activity can strongly influence the transformed and invasive phenotype of a human prostate tumor cell line.[37]

The third candidate rs9981861 is in the 31st intron of the 33-exon *DSCAM* gene, which encodes Down syndrome cell adhesion molecule, a member of the immunoglobulin superfamily. The gene was cloned from the Down syndrome region on chromosome 21q22 and found to be expressed widely in the developing nervous system.[38] Mouse *DSCAM* has been shown to mediate arborization of neurite processes and spacing of neuronal cell bodies.[39,40] Expression of the *DSCAM* gene has been upregulated in small cell lung cancer compared with NSCLC.[41]

Because a GWAS is based on a linkage disequilibrium (LD) mapping of a disease locus by use of SNPs as markers, the particular SNPs per se identified in this study may not be functionally responsible for the observed effect on survival time. In fact, LD maps drawn by the HapMap data around the three SNPs indicate that at least the SNPs of the *EIF4E2* and *ETS2* genes are embedded in extended LD blocks (Supplementary Figure 1, http://links.lww.com/IGC/A25); it may be then difficult to narrow down the regions of interest further for these SNPs by statistical genetics alone, at least in the Asian population.

In summary, a hypothesis-free GWAS detected previously unrecognized associations between polymorphisms of the three genes and shortened OS in advanced NSCLC treated with CBDCA and PTX. Additionally, these three SNPs on the three genes were significant after a multiple testing adjustment. In considering a multiple testing problem, we assume the existence of about 10,000 linkage disequilibrium blocks within 100,000 gene-centric SNPs, which are concentrated in about 2% of the human genome (i.e., average interval of two SNPs is 600 bp). It follows that the p value cutoff is set at 5.0×10^{-6} if the Bonferroni correction is applied. However, in the first screening, such correction for a multiple testing is often too conservative, failing to detect many drug-response SNPs; therefore, we showed top 10 SNPs in Table 4. In addition, to facilitate the second screening or replication studies by other investigators, statistics of association between OS, PK parameters, toxicity, and all SNPs analyzed in this study are available at Genome Medicine Database of Japan (http://gemdbj.nibio.go.jp).

The ultimate goal of this work is better clinical management of patients after the assessment of genotype risk on OS. To this end, however, we need to identify genetic polymorphisms that can differentiate patients' response and outcome to different chemotherapeutic agents. Although our work may contribute as the first step to establish such a predictive factor, especially the survival-related SNPs that also influence pharmacokinetics, the current single-arm prospective study does not provide definite evidence of pharmacogenomic profiling for a platinum-based chemotherapy. Several targeted therapies for NSCLC are in clinical development, and it is hoped that this line of pharmacogenetic studies will eventually help clinicians to choose platinum or nonplatinum doublets as the first-line regimen, for instance. Further studies of NSCLC would stratify patients according to the SNP status to tailor treatment to individual patients. The results of a single association study should be validated by independent studies by other investigators as well as biologic functional analyses.

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Genetic polymorphisms and haplotypes of *POR* encoding cytochrome P450 oxidoreductase in a Japanese population

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Footnotes

On September 17, 2010, these variations were not found on the homepage of the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB (http://www.pharmgkb.org/do/) database.

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