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

Yasunori Sato, PhD,† Noboru Yamamoto, MD,‡ Hideo Kunitoh, MD,‡§ Yuichiro Ohe, MD,‡ Hironobu Minami, MD,‖¶ Nan M. Laird, PhD,† Noriko Katori, PhD,# Yoshiro Saito, PhD,** Sumiko Ohnami, BS,* Hiromi Sakamoto, PhD,* Jun-ichi Sawada, PhD,†† Nagahiro Saijo, MD, PhD,‡‡ Teruhiko Yoshida, MD, PhD,* and Tomohide Tamura, MD, PhD‡*

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.

(*J Thorac Oncol.* 2011;6: 132–138)

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-

*Genetics Division, National Cancer Center Research Institute, Tokyo, Japan; †Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; ‡Division of Internal Medicine, National Cancer Center Hospital; §Department of Respiratory Medicine, Mitsui Memorial Hospital, Tokyo; ‖Division of Internal Medicine, National Cancer Center Hospital East, Chiba; ¶Division of Oncology/Hematology, Kobe University Graduate School of Medicine, Kobe; #Divisions of Drugs, **Medicinal Safety Science, and ††Functional Biochemistry and Genomics, National Institute of Health Sciences, Tokyo; and ‡‡National Cancer Center Hospital East, Chiba, Japan.

Disclosure: Dr. Minami has received honororia from Bristol-Myers Squibb KK. The other authors declare no conflicts of interest.

Address for correspondence: Teruhiko Yoshida, MD, Genetics Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: tyoshida@ncc.go.jp

The first two authors contributed equally to this work.

ISSN: 1556-0864/11/0601-0132

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.

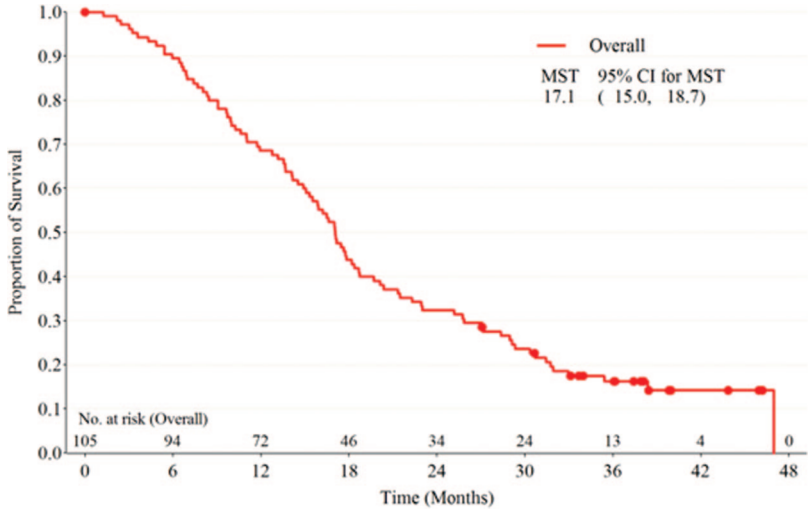

FIGURE 1. Kaplan-Meier plot for overall survival.

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

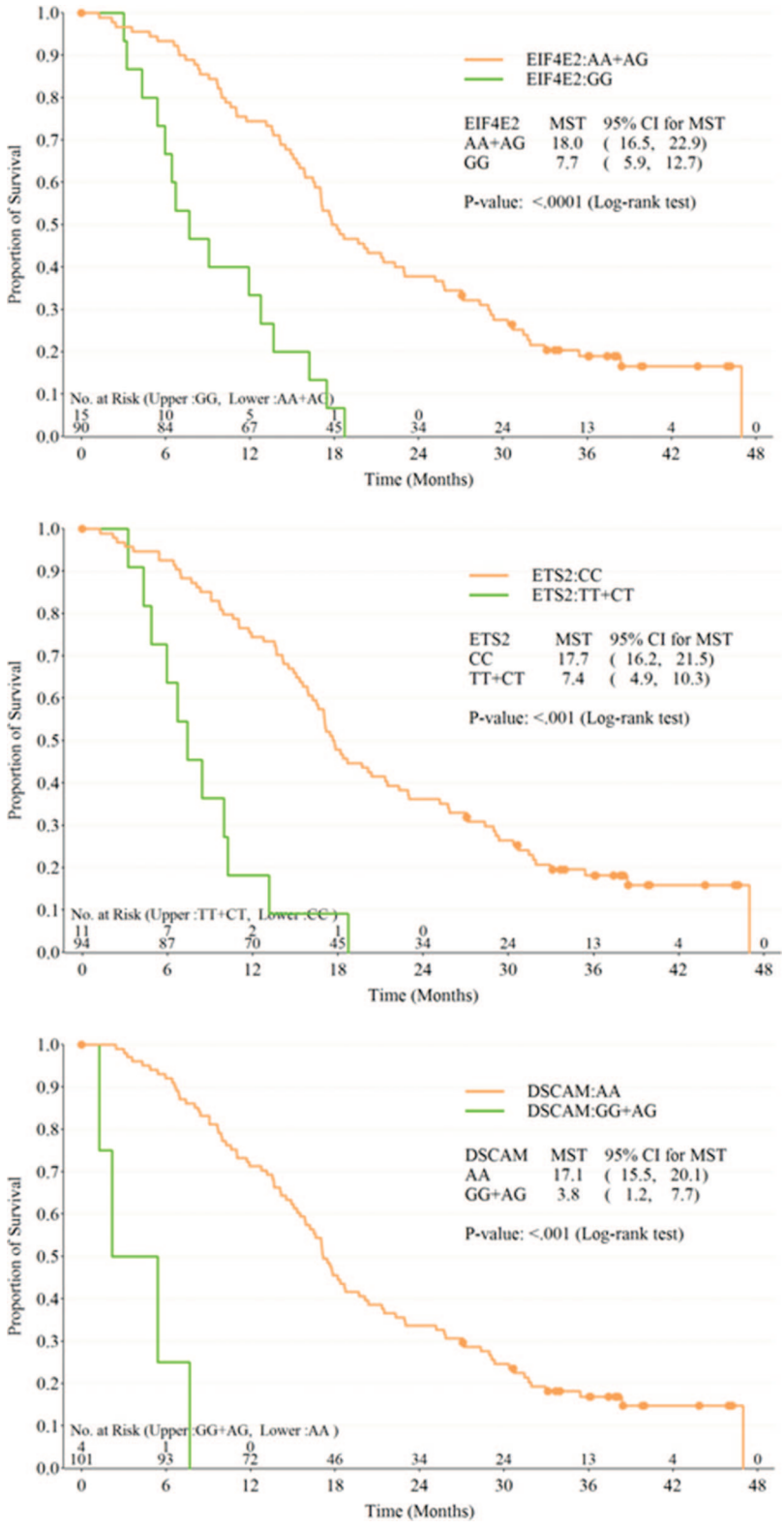

FIGURE 2. Overall survival stratified for the single-nucleotide polymorphism genotype.

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.

ACKNOWLEDGMENTS

Supported by the Program for the Promotion of Fundamental Studies in Health Sciences from National Institute of Biomedical Innovation (ID 05-41).

REFERENCES

1. Cancer Statistics in Japan 2008: The Editorial Board of the Cancer Statistics in Japan. Tokyo, Japan: Foundation for Promotion of Cancer Research 2008. Available at: http://www.fpcr.or.jp/publication/statistics.html. Accessed March 3, 2010.
2. Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomized clinical trials. *BMJ* 1995;311:899–909.
3. Fukuoka M, Niitani H, Suzuki A, et al. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J Clin Oncol* 1992;10:16–20.
4. Rowinsky EK, Donehower RC. Paclitaxel (taxol). *N Engl J Med* 1995;332:1004–1014.
5. Gelmon K. The taxoids: paclitaxel and docetaxel. *Lancet* 1994;344:1267–1272.
6. Hertel LW, Border GB, Kroin JS, et al. Evaluation of the antitumor activity of gemcitabine. *Cancer Res* 1990;50:4417–4422.
7. Binet S, Fellous A, Lataste H, et al. Biochemical effects of navelbine on tubulin and associated proteins. *Semin Oncol* 1989;16:9–14.
8. Petris L, Crino L, Scagliotti GV, et al. Treatment of advanced non-small cell lung cancer. *Ann Oncol* 2006;17(Suppl 2):ii36–ii41.
9. Kubota K, Kawahara M, Ogawara M, et al. Vinorelbine plus gemcitabine followed by docetaxel versus carboplatin plus paclitaxel in patients with advanced non-small-cell lung cancer: a randomised, open-label, phase III study. *Lancet Oncol* 2008;9:1135–1142.
10. Bepler G. Using translational research to tailor the use of chemotherapy in the treatment of NSCLC. *Lung Cancer* 2005;50(Suppl 1):S13–S14.

11. Rosell R, Cobo M, Isla D, et al. Applications of genomics in NSCLC. *Lung Cancer* 2005;50:S33–S40.
12. Nakajima Y, Yoshitani T, Fukushima-Uesaka H, et al. Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. *Clin Pharmacol Ther* 2006;80:179–191.
13. Saito Y, Katori N, Soyama A, et al. CYP2C8 haplotype structures and their influence on pharmacokinetics of paclitaxel in a Japanese population. *Pharmacogenet Genomics* 2007;17:461–471.
14. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.
15. Cox, DR. Regression models and life tables. *J R Stat Soc* 1972;34:187–220.
16. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York, NY: John Wiley and Sons, 1980.
17. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1979;6:65–70.
18. Gurubhagavatula S, Liu G, Park S, et al. XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. *J Clin Oncol* 2004;22:2594–2601.
19. Isla D, Sarries C, Rosell R, et al. Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann Oncol* 2004;15:1194–1203.
20. Ryu JS, Hong YC, Han HS, et al. Association between polymorphisms of ERCC1 and XPD and survival in non-small cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004; 44:311–316.
21. de las Penas R, Sanchez-Ronco M, Alberola V, et al. Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small-cell lung cancer patients. *Ann Oncol* 2006;17:668–675.
22. Booton R, Ward T, Heighway J, et al. Xeroderma pigmentosum group D haplotype predicts for response, survival, and toxicity after platinum-based chemotherapy in advanced nonsmall cell lung cancer. *Cancer* 2006;106:2421–2427.
23. Spitz MR, Wu X, Wang Y, et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001;61:1354–1357.
24. Duell EJ, Wiencke JK, Cheng TJ, et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis* 2000;21:965–971.
25. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 2001;22:1437–1445.
26. Bosken CH, Wei Q, Amos CI, et al. An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer. *J Natl Cancer Inst* 2002;94:1091–1099.
27. Wei Q, Wang X, Shen H. DNA repair gene XPD polymorphism and lung cancer risk: a meta-analysis. *Lung Cancer* 2004;46:1–10.
28. Chen J, Larochelle S, Li X, et al. Xpd/Ercc2 regulates CAK activity and mitotic progression. *Nature* 2003;424:228–232.
29. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005;6:95–108.
30. Nordborg M, Tavaré B. Linkage disequilibrium: what history has to tell us. *Trends Genet* 2002;18:83–90.
31. Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* 1999;68:913–963.
32. Gross JD, Moerke NJ, von der Haar T, et al. Ribosome loading onto the mRNA cap is driven by conformational coupling between eIF4G and eIF4E. *Cell* 2003;115:739–750.
33. Joshi B, Cameron A, Jagus R. Characterization of mammalian eIF4E-family members. *Eur J Biochem* 2004;271:2189–2203.
34. Okumura F, Zou W, Zhang DE. ISG15 modification of the eIF4E cognate 4EHP enhances cap structure-binding activity of 4EHP. *Genes Dev* 2007;21:255–260.
35. Rom E, Kim HC, Gingras AC, et al. Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. *J Biol Chem* 1998;273:13104–13109.
36. Ramaswamy S, Ross KN, Lander ES, et al. A molecular signature of metastasis in primary solid tumors. *Nat Genet* 2003;33:49–54.
37. Foos G, Hauser CA. Altered Ets transcription factor activity in prostate tumor cells inhibits anchorage-independent growth, survival, and invasiveness. *Oncogene* 2000;19:5507–5516.
38. Yamakawa K, Huo Y-K, Haendel MA, et al. DSCAM: a novel member of the immunoglobulin superfamily maps in a Down syndrome region and is involved in the development of the nervous system. *Hum Mol Genet* 1998;**7**:227–237.
39. Wojtowicz WM, Flanagan JJ, Millard S, et al. Alternative splicing of *Drosophila* Dscam generates axon guidance receptors that exhibit isoform-specific homophilic binding. *Cell* 2004;118:619–633.
40. Fuerst PG, Koizumi A, Masland RH, et al. Neurite arborization and mosaic spacing in the mouse retina require DSCAM. *Nature* 2008;451: 470–474.
41. Coe BP, Lockwood WW, Girard L, et al. Differential disruption of cell cycle pathways in small cell and non-small cell lung cancer. *Br J Cancer* 2006;94:1927–1935.