Published OnlineFirst March 3, 2011; DOI:10.1158/1078-0432.CCR-10-2638

producing human small cell lung cancer H146, based on angiogenesis inhibition. Int J Cancer 2008;122:664–71.
12. Matsui J, Funahashi Y, Uenaka T, Watanabe T, Tsuruoka A, Asada M. Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. Clin Cancer Res 2008;14:5459–65.
13. Ikuta K, Yano S, Trung VT, Hanibuchi M, Goto H, Li Q, et al. E7080, a multi-tyrosine kinase inhibitor, suppresses the progression of malignant pleural mesothelioma with different proangiogenic cytokine production profiles. Clin Cancer Res 2009;15:7229–39.
14. Chang YS, Adnane J, Trail PA, Levy J, Henderson A, Xue D, et al. Sorafenib (BAY 43–9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. Cancer Chemother Pharmacol 2007;59:561–74.
15. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, et al. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003;9:327–37.
16. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
17. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 2003;13:176–81.
18. Tsuchida Y, Therasse P. Response evaluation criteria in solid tumors (RECIST): new guidelines. Med Pediatr Oncol 2001;37:1–3.
19. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. Nat Rev Cancer 2005;6:835–45.
20. Duda DG, Cohen KS, di Tomaso E, Au P, Klein RJ, Scadden DT, et al. Differential CD146 expression on circulating versus tissue endothelial cells in rectal cancer patients: implications for circulating endothelial and progenitor cells as biomarkers for antiangiogenic therapy. J Clin Oncol 2006;24:1449–53.
21. Kimura H, Kasahara K, Sekijima M, Tamura T, Nishio K. Plasma MIP-1beta levels and skin toxicity in Japanese non-small cell lung cancer patinets treated with the EGFR-targeted tyrosine kinase inhibitor, gefitinib. Lung Cancer 2005;50:393–9.
22. Drevs J, Siegert P, Medinger M, Mross K, Strecker R, Zirrgiebel U, et al. Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. J Clin Oncol 2007;25:3045–54.
23. Keizer RJ, Gupta A, Mac Gillavry MR, Jansen M, Wanders J, Beijnen JH, et al. A model of hypertension and proteinuria in cancer patients treated with the anti-angiogenic drug E7080. J Pharmacokinet Pharmacodyn 2010;37:347–63.
24. Robinson ES, Matulonis UA, Ivy P, Berlin ST, Tyburski K, Penson RT, et al. Rapid development of hypertension and proteinuria with cediranib, an oral vascular endothelial growth factor receptor inhibitor. Clin J Am Soc Nephrol 2010;5:477–83.
25. Zhu X, Wu S, Dahut WL, Parikh CR. Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. Am J Kidney Dis 2007;49:186–93.
26. Ronzoni M, Manzoni M, Mariucci S, Loupakis F, Brugnatelli S, Bencardino K, et al. Circulating endothelial cells and endothelial progenitors as predictive markers of clinical response to bevacizumab-based first-line treatment in advanced colorectal cancer patients. Ann Oncol 2010;21:2382–9.
27. Matsui J, Wakabayashi T, Asada M, Yoshimatsu K, Okada M. Stem cell factor/c-kit signaling promotes the survival, migration, and capillary tube formation of human umbilical vein endothelial cells. J Biol Chem 2004;279:18600–7.
28. Dentelli P, Rosso A, Balsamo A, Colmenares Benedetto S, Zeoli A, Pegoraro M, et al. C-KIT, by interacting with the membrane-bound ligand, recruits endothelial progenitor cells to inflamed endothelium. Blood 2007;109:4264–71.
29. Kawaishi M, Fujiwara Y, Fukui T, Kato T, Yamada K, Ohe Y, et al. Circulating endothelial cells in non-small cell lung cancer patients treated with carboplatin and paclitaxel. J Thorac Oncol 2009;4:208–13.
30. Calleri A, Bono A, Bagnardi V, Quarna J, Mancuso P, Rabascio C, et al. Predictive potential of angiogenic growth factors and circulating endothelial cells in breast cancer patients receiving metronomic chemotherapy plus bevacizumab. Clin Cancer Res 2009;15:7652–7.
31. Takahashi M. Role of the SDF-1/CXCR4 system in myocardial infarction. Circ J 2010;74:418–23.
32. Jain RK, Duda DG, Willett CG, Sahani DV, Zhu AX, Loeffler JS, et al. Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol 2009;6:327–38.
33. Glen H, Boss DR, Morrison R, et al. A phase I study of E7080 in patients (pts) with advanced malignancies. J Clin Oncol 2008;26:abstr 3526.
34. Hong DS, Koetz BS, Kurzrock R, et al. Phase I dose-escalation study of E7080, a selective tyrosine kinase inhibitor, administered orally to patients with solid tumors. J Clin Oncol 2010;28:abstr 2540.
35. Gupta A, Koetz B, Hanekom W. Population pharmacokinetics and exposure/ response relationship of the receptor tyrosine kinase inhibitor E7080 in phase I studies. Presented at the 22nd EORTC-NCI-AACR symposium on "Molecular targets and Cancer Therapeutics, 2010, abstr 453.

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.

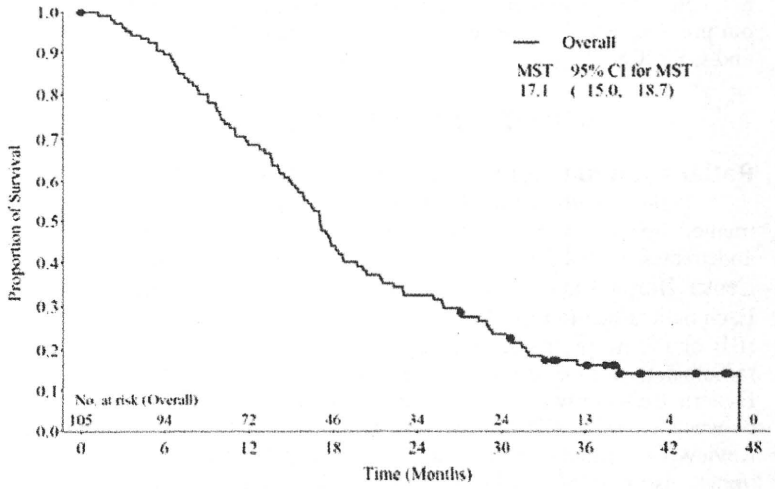

FIGURE 1. Kaplan-Meier plot for overall survival.

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

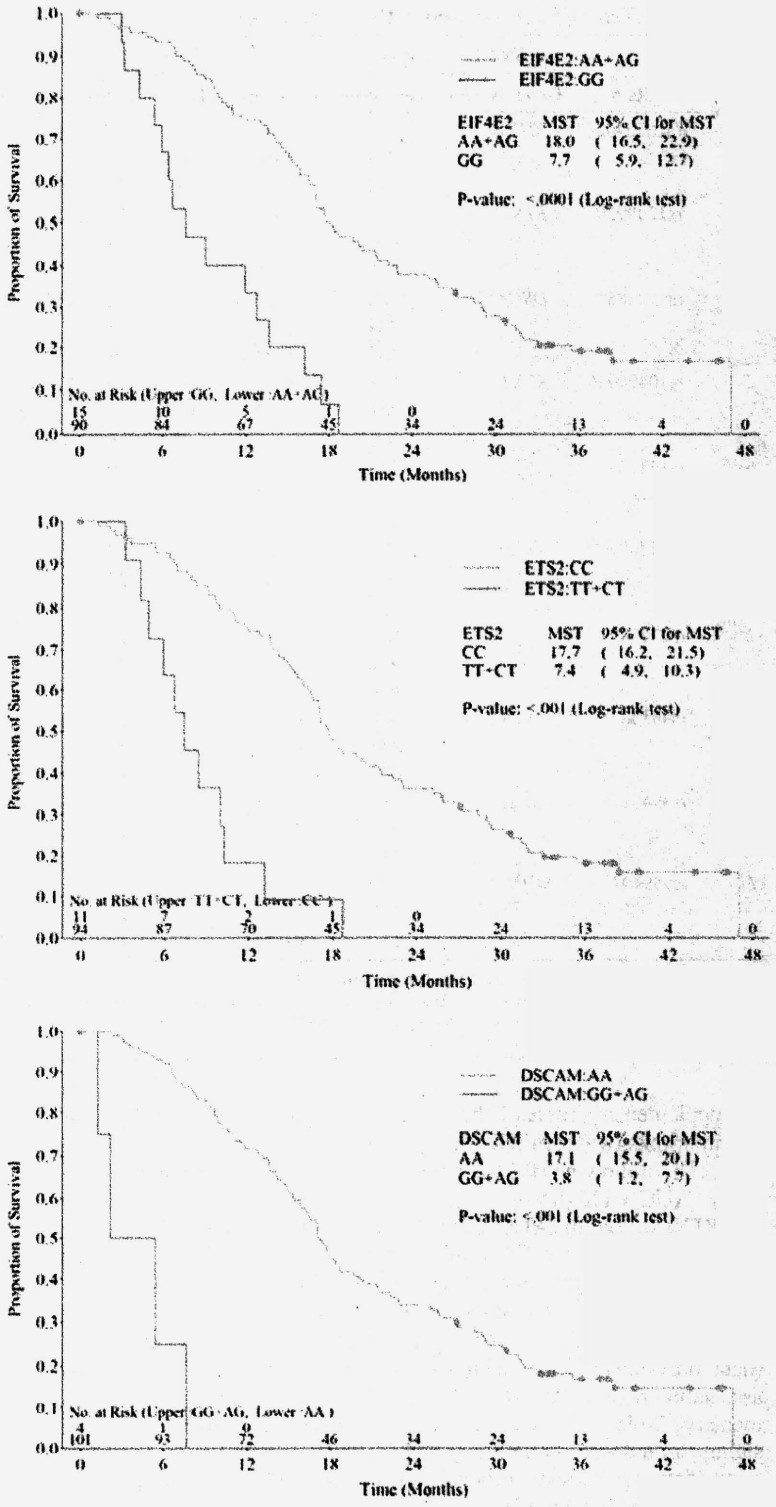

FIGURE 2. Overall survival stratified for the single-nucleotide polymorphism genotype.

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.

Cancer Science
The official journal of the Japanese Cancer Association JCA

Innovator and generic cisplatin formulations: Comparison of renal toxicity

Ikuo Sekine,[1] Kaoru Kubota, Yosuke Tamura, Hajime Asahina, Kazuhiko Yamada, Hidehito Horinouchi, Hiroshi Nokihara, Noboru Yamamoto and Tomohide Tamura

Division of Internal Medicine and Thoracic Oncology, National Cancer Center Hospital, Tokyo, Japan

(Received August 18, 2010/Revised September 27, 2010/Accepted September 28, 2010/Accepted manuscript online October 10, 2010/Article first published online November 5, 2010)

To compare the incidence and degree of renal toxicity associated with innovator and generic cisplatin formulations, increase in the serum creatinine (CRN) levels (mg/dL) and incidence of grade 2–3 CRN elevation during the first and all cycles of chemotherapy were retrospectively evaluated in patients treated with innovator (group 1, $n = 296$) and generic (group 2, $n = 321$) cisplatin formulations. There were no differences in the sex, age, performance status or number of chemotherapy cycles between groups 1 and 2. The median increases in CRN levels during the first cycle were 0.20 mg/dL regardless of the sex or group. There was no difference in the incidence of grade 2–3 CRN elevation between groups 1 and 2 among female or male patients. The median increases in CRN levels during all cycles were 0.2 (0–1.0) and 0.3 (0–1.8) in the female patients of groups 1 and 2, respectively ($P = 0.68$), and 0.3 (0–2.1) and 0.5 (0–3.6) in the male patients of groups 1 and 2, respectively ($P < 0.001$). Grade 2–3 CRN elevation was observed in 18.1% and 24.7% of the female patients in groups 1 and 2, respectively ($P = 0.33$), and 9.4% and 20.9% of the male patients in groups 1 and 2, respectively ($P < 0.001$). Renal toxicity was slightly more severe in patients treated with the generic cisplatin formulation than in those treated with the innovator formulation, especially among the male patients. (*Cancer Sci* 2011; 102: 162–165)

Cisplatin, despite its severe toxicity, has been used in cancer chemotherapy for more than 30 years because of its significant therapeutic efficacy.[1] Although carboplatin, an analog of cisplatin with a milder toxicity profile, was also introduced for clinical use, randomized trials and meta-analyses showed that cisplatin-based chemotherapy was slightly superior to carboplatin-based chemotherapy in terms of the response rate and survival, at least in certain subgroups, without any significant increase in the incidence of severe toxicities among patients with germ cell tumor,[2] head and neck cancer,[3] and non-small-cell lung cancer.[4] In addition, cisplatin was shown to have a significant role in the treatment of bladder cancer, cervical cancer, esophageal cancer, ovarian cancer and small cell lung cancer, although carboplatin is being used increasingly in the treatment of some of these cancers as an alternative chemotherapeutic agent.[5] Thus, cisplatin still plays a pivotal role in the systemic treatment of a variety of solid tumors.

Renal toxicity is a major dose-limiting factor of cisplatin in most drug administration schedules.[6] Although the exact mechanism is unclear, the greatest concentration of platinum and widespread necrosis are reportedly observed in the proximal tubules of the kidney. This tubular impairment leads to a secondary reduction of renal blood flow and the glomerular filtration rate, potentiating the primary tubular damage. This vicious cycle causes delayed deterioration of renal function, as an increase in the serum creatinine (CRN) level typically appears 6–7 days after cisplatin administration in humans.[6] The standard prophylaxis for cisplatin nephrotoxicity is infusion of 1–4 L of normal saline on the day of cisplatin administration.[6] Although this vigorous hydration diminishes life-threatening renal toxicity, 7–40% of patients still develop a mild to moderate increase of serum CRN levels, which influences the subsequent cisplatin therapy.[7,8]

Generic substitutes serve as lower-cost alternatives to the more costly brand-name drugs for patients.[9] If it can be shown that a generic formulation is "essentially similar" in qualitative and quantitative composition to an innovator preparation, then the formulation can be marketed as "generic" without the need for expensive regulatory clinical trials. However, whether generic cisplatin formulations are truly therapeutically identical and interchangeable with innovator formulation of the drug has not yet been investigated. The objective of this study was to compare the severity of renal toxicity between an innovator cisplatin formulation and a generic substitute.

Patients and Methods

Patient selection. Patients were retrospectively selected for this study according to the following criteria: (i) a histological or cytological diagnosis of thoracic malignancy; (ii) no prior chemotherapy, except for a combination of uracil and ftorafur (UFT) as adjuvant chemotherapy after surgery; (iii) chemotherapy with a regimen that included 80 mg/m^2 of cisplatin; and (iv) receiving treatment as an inpatient at the National Cancer Center Hospital between November 2000 and March 2009. In this period, the innovator cisplatin formulation was administered between November 2000 and May 2004, and CISPLATIN for I.V. infusion (MARUKO), a generic cisplatin formulation, was administered thereafter. Patients with abnormal elevation of serum CRN before the start of chemotherapy were excluded from the current study.

Cisplatin administration. Cisplatin was administered at a dose of 80 mg/m^2 by intravenous infusion over 60–120 min on day 1 in combination with other chemotherapeutic agents, 40 g of mannitol and 3000 mL of hydration. On days 2–5, 2000 mL of intravenous infusion fluids were administered over 8 h. Antiemetic prophylaxis consisted of a $5HT_3$-antagonist and 16 mg of dexamethasone on day 1, followed by 8 mg of dexamethasone on days 2 and 3, 4 mg on day 4 and 2 mg on day 5. These treatments were repeated every 3–4 weeks. This sequence of administration was consistently maintained during the study period.

Data collection and statistical analyses. The patients' baseline characteristics, including age, sex and performance status (PS), pretreatment CRN level (CRNpre), chemotherapy regimen received, number of chemotherapy cycles administered and the maximum CRN level (CRNmax) during the first cycle and all cycles of chemotherapy were retrospectively obtained from medical charts. The patients' list was encoded and anonymized. The median CRNmax, median increase in the serum CRN levels (difference between CRNpre and CRNmax), and the Common

[1]To whom correspondence should be addressed. E-mail: isekine@ncc.go.jp

doi: 10.1111/j.1349-7006.2010.01764.x

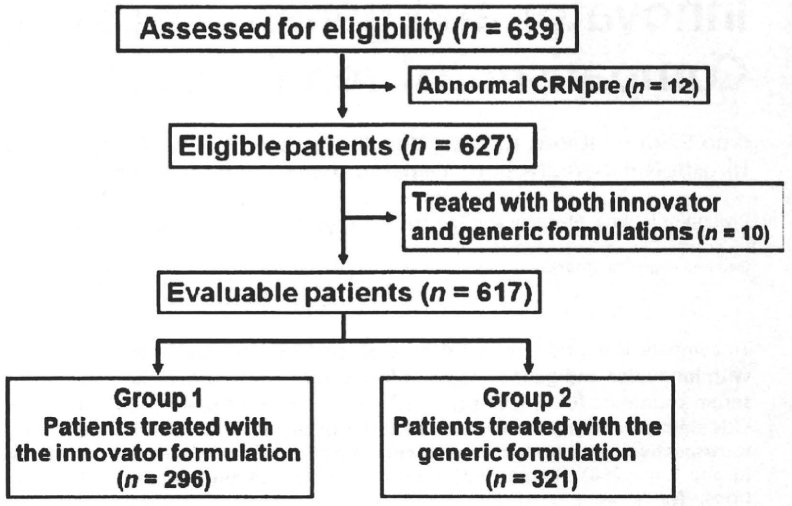

Fig. 1. Flow of patients. In the present study there was a total of 296 patients treated with an innovator cisplatin formulation (group 1) and 321 patients treated with a generic cisplatin formulation (group 2). CRNpre, pretreatment serum creatinine level.

Toxicity Criteria–Adverse Event (CTC-AE ver. 3.0) grades of the CRNmax were compared between patients treated with the innovator cisplatin formulation (group 1) and those treated with the generic formulation (group 2); these evaluations were performed for the entire study population, as well as separately for the female and male patients, because the normal range of the serum CRN level differs between the sexes. Mann–Whitney tests were used to evaluate continuous variables, and Chi-squared tests for categorical variables. The Dr SPSS II 11.0 for Windows software (SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

This study was approved by the president of the National Cancer Center Hospital, Tokyo, Japan. The institutional review board and ethical review committee decided to exempt this study from the usual review process because of its retrospective nature.

Results

Of the 639 patients assessed for eligibility in this study, 627 patients met the inclusion criteria; 12 patients were excluded because of abnormal CRNpre levels. An additional 10 patients were excluded because they were treated with the innovator cisplatin formulation in the first cycle of chemotherapy, but with the generic formulation in subsequent cycles. Thus, a total of 617 patients were included as the subjects of this study. Of these, 296 patients were treated with the innovator cisplatin formulation (group 1) and 321 were treated with the generic formulation (group 2) (Fig. 1). The median age of the patients was 60 years in both groups, and female patients accounted for 24% of all patients (Table 1). There were no meaningful differences in the PS, CRNpre levels, height, weight or number of chemotherapy cycles between the groups.

The median (range) CRNmax levels during the first cycle of chemotherapy were 0.7 (0.5–1.8) mg/dL and 0.7 (0.5–1.6) mg/dL in the female patients of group 1 and group 2, respectively ($P = 0.25$), while they were 1.0 (0.5–4.5) mg/dL and 1.1 (0.6–4.2) mg/dL, respectively, in the male patients of group 1 and group 2, respectively ($P = 0.016$). These differences were even more pronounced when the CRNmax levels during all cycles of chemotherapy were evaluated. The median (range) CRNmax level in the female patients did not differ between groups 1 and 2 (0.8 [0.5–1.8] mg/dL *vs* 0.9 [0.6–2.5] mg/dL, $P = 0.22$), whereas that in the male patients was higher in group 2 than in group 1 (1.1 [0.5–4.5] mg/dL *vs* 1.3 [0.7–4.2] mg/dL, $P < 0.001$).

Table 1. Patient characteristics

Characteristics	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Sex			
Females	72 (24)	77 (24)	0.93
Males	224 (76)	244 (76)	
Age (years)			
Median (range)	59.5 (18–77)	60.0 (18–75)	0.85
Height (cm)			
Median (range)	164 (146–181)	165 (143–189)	0.03
Weight (kg)			
Median (range)	60.6 (35.3–102)	60.3 (33.9–106)	0.76
PS			
0–1	294 (99)	317 (99)	0.69
2–3	2 (1)	4 (1)	
CRNpre			
Median (range)	0.7 (0.3–1.1)	0.7 (0.3–1.1)	0.17
No. cycles			
1–2	121 (41)	123 (38)	0.56
3–5	175 (59)	198 (62)	
Median (range)	3 (1–5)	3 (1–4)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CRNpre, pretreatment serum creatinine level.

The increases in the serum CRN levels (differences between the CRNmax and CRNpre levels) during the first cycle of chemotherapy are summarized in Table 2. The median increase of the serum CRN levels did not differ between the female patients of groups 1 and 2, while it was higher in the male patients of group 2 than in those of group 1. However, the percentage of patients with a significant increase of serum CRN levels (0.7 mg/dL or higher) did not differ between groups 1 and 2 in the entire subject population, in the female patients alone, or in the male patients alone. The clinical significance of the increase in serum CRN levels was assessed using the CTC-AE grade of serum CRN. The CRN CTC-AE grades during the first cycle of chemotherapy did not differ between groups 1 and 2 in the entire subject population, in the females alone, or in the males alone (Table 2).

There was a definite increase in the serum CRN levels during all cycles of chemotherapy (Table 3). The median increase in the serum CRN levels did not differ between the female patients

Table 2. Increase in serum creatinine levels and toxicity grades during the first cycle of chemotherapy

	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Increase in serum creatinine levels (mg/dL)			
Total			
0–0.3	242 (81.8)	238 (74.1)	0.070
0.4–0.6	35 (11.8)	51 (15.9)	
≥0.7	19 (6.4)	32 (10.0)	
Median (range)	0.2 (0–1.0)	0.2 (0–1.2)	0.054
Female			
0–0.3	64 (88.9)	66 (85.7)	0.76
0.4–0.6	5 (6.9)	8 (10.4)	
≥0.7	3 (4.2)	3 (3.9)	
Median (range)	0.2 (0–1.0)	0.2 (0–1.2)	0.90
Male			
0–0.3	178 (79.5)	172 (70.5)	0.070
0.4–0.6	30 (13.4)	43 (17.6)	
≥0.7	16 (7.1)	29 (11.9)	
Median (range)	0.2 (0–2.1)	0.2 (0–3.6)	0.027
CTC-AE grade			
Total			
0	211 (71.3)	208 (64.8)	0.20
1	69 (23.3)	87 (27.1)	
2–3	16 (5.4)	26 (8.1)	
Female			
0	43 (59.3)	40 (51.9)	0.60
1	23 (31.9)	28 (36.4)	
2–3	6 (8.3)	9 (11.7)	
Male			
0	168 (75.0)	168 (68.9)	0.29
1	46 (20.5)	59 (24.2)	
2–3	10 (4.5)	17 (7.0)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CTC-AE, Common Toxicity Criteria–Adverse Event ver. 3.0.

Table 3. Increase in serum creatinine levels and toxicity grades during all cycles of chemotherapy

	Group 1† (n = 296)	Group 2‡ (n = 321)	P-value
	N (%)	N (%)	
Increase in serum creatinine levels (mg/dL)			
Total			
0–0.3	177 (59.8)	146 (45.5)	<0.001
0.4–0.6	71 (24.0)	85 (26.5)	
≥0.7	48 (16.2)	90 (28.0)	
Median (range)	0.3 (0–2.1)	0.4 (0–3.6)	<0.001
Female			
0–0.3	49 (68.1)	52 (67.5)	0.99
0.4–0.6	15 (20.8)	16 (20.8)	
≥0.7	8 (11.1)	9 (11.7)	
Median (range)	0.2 (0–1.0)	0.3 (0–1.8)	0.68
Male			
0–0.3	128 (57.1)	94 (38.5)	<0.001
0.4–0.6	56 (25.0)	69 (28.3)	
≥0.7	40 (17.9)	81 (33.2)	
Median (range)	0.3 (0–2.1)	0.5 (0–3.6)	<0.001
CTC-AE grade			
Total			
0	160 (54.1)	122 (38.0)	<0.001
1	102 (34.5)	129 (40.2)	
2–3	34 (11.5)	70 (21.8)	
Female			
0	30 (41.7)	24 (31.2)	0.44
1	29 (40.3)	34 (44.2)	
2–3	13 (18.1)	19 (24.7)	
Male			
0	130 (58.0)	98 (40.2)	<0.001
1	73 (32.6)	95 (38.9)	
2–3	21 (9.4)	51 (20.9)	

†Patients treated with innovator formulation. ‡Patients treated with generic formulation. CTC-AE, Common Toxicity Criteria–Adverse Event ver. 3.0.

of groups 1 and 2, while it was higher in the male patients of group 2 than in those of group 1. The percentage of patients with an increase in CRN levels of ≥0.7 mg/dL did not differ between the female patients of groups 1 and 2, but was higher in the male patients of group 2 than in those of group 1 (33.2% *vs* 17.9%, $P < 0.001$). The percentage of patients with grade 2–3 CRN CTC-AE in the male patients was 20.9% in group 2 and 9.4% in group 1 ($P < 0.001$), although no significant difference was noted between the female patients of groups 1 and 2 (Table 3).

Since we unexpectedly found a distinct trend of increase in the CRN levels in the female and male patients, we studied the gender difference (Table 4). The increase in CRN levels was higher in the male than female patients, while the CRN CTC-AE grades were more severe in the female than male patients.

Discussion

This study showed that the renal toxicity was slightly more severe in the patients who were treated with the generic cisplatin formulation than in those treated with the innovator formulation, especially among male patients. This result was not attributable to biased prognostic factors for cisplatin-induced renal toxicity, including age, PS, dose of cisplatin or the number of chemotherapy cycles pointed out previously,[7,8] because these variables were distributed equally between patients who were treated with the generic and innovator cisplatin formulations. Higher CTC-AE grades as well as increased serum CRN levels during chemotherapy were observed in patients treated with the generic cisplatin formulation, and therefore this renal toxicity can be as severe as it influences subsequent therapies of the patients.

The generic drug is exactly the same as the innovator drug in its basic composition and property, including cisplatin content, solvent and pH and osmotic pressure of the solution, but the additives to stabilize the solution may not be identical. Thus, this small difference is considered to result in the increased renal toxicity of the generic drug. Unlike the basic composition and property of drugs, the influence of additives can not be easily examined. It is much simpler to use the same additives in the manufacturing process of generic drugs.

We never expected that the association between increased renal toxicity and administration of the generic cisplatin formulation would be more evident in male patients, and this result prompted us to compare the renal toxicity between the sexes. A rise in the serum CRN levels during chemotherapy was more frequent in male patients, while the CTC-AE grades were more severe in the female than male patients. This is probably because men generally have a larger and more muscular physique that leads to higher CRN production and a higher upper limit of the normal range of the serum CRN level in men than in women. In previous studies, a rise in serum CRN during cisplatin-containing chemotherapy was found to be slightly more frequent in female patients, but the difference was only mild and of no clinical significance. Thus, there seems to be only a small difference, if any, in the responses to nephrotoxic agents between the sexes, and males are unlikely to be more vulnerable to cisplatin. Another possible explanation for the current results might be the

doi: 10.1111/j.1349-7006.2010.01764.x

Table 4. Gender differences in the increase of serum creatinine levels and toxicity grades during the first and all cycles of chemotherapy

	Females (n = 149)	Males (n = 468)	P-value
	N (%)	N (%)	
During the first cycle of chemotherapy			
An increase in CRN (mg/dL)			
0–0.3	130 (87.2)	350 (74.8)	0.006
0.4–0.6	13 (8.7)	73 (15.6)	
≥0.7	6 (4.0)	45 (9.6)	
Median (range)	0.20 (0–1.2)	0.20 (0–3.6)	<0.001
CTC-AE grade			
0	83 (55.7)	336 (71.8)	0.001
1	51 (34.2)	105 (22.4)	
2–3	15 (10.1)	27 (5.8)	
During all cycles of chemotherapy			
An increase in CRN (mg/dL)			
0–0.3	101 (67.8)	222 (47.4)	0.001
0.4–0.6	31 (20.8)	125 (26.7)	
≥0.7	17 (11.4)	121 (25.9)	
Median (range)	0.20 (0–1.8)	0.40 (0–3.6)	<0.001
CTC-AE grade			
0	54 (36.2)	228 (48.7)	0.023
1	63 (42.3)	168 (35.9)	
2–3	32 (21.5)	72 (15.4)	

CTC-AE, Common Toxicity Criteria–Adverse Event ver. 3.0.

relatively low volume of hydration in male patients, because the infusion volume administered was the same in male and female patients despite the larger physique of male patients. However, the explanation attributed to this small difference in the infusion volume might not be plausible, because the volume of hydration was not clearly associated with cisplatin-induced renal toxicity in a previous study.[8]

Because this study has suggested that generic cisplatin might be slightly more toxic to the kidneys, some kind of countermeasures are necessary. First, the content of hydration and timing of mannitol administration should be reconsidered to avoid renal toxicity. We had not included magnesium in the hydration fluid, but recent randomized trials showed that addition of magnesium was effective in reducing cisplatin-induced renal toxicity.[10,11] We have administered mannitol after cisplatin infusion, but the National Cancer Center Hospital (NCCH) guideline recommends using mannitol before cisplatin infusion. If these countermeasures fail to reduce renal toxicity, then the use of generic cisplatin would not be recommended.

A major limitation of the present study is that the generic and innovator cisplatin formulations were not allocated in a randomized fashion to the study population, but was determined by the period of the treatment. Although cisplatin administration in our hospital was consistent throughout the study period, there might be unknown factors associated with renal toxicity influenced by the study period.

Generic formulations are approved without clinical trials in Japan, as well as in other countries. This system has worked well to reduce drug costs safely, provided that the drug has a potentially low toxicity profile. However, the results of this study suggest that more attention should be given to the developmental system of generic formulations, especially in relation to anticancer agents, which might have severe and life-threatening toxicities. One possibility is disclosure of the drug manufacturing process so that drug companies can strictly follow the process when they develop a generic formulation of the original drug. Utmost importance should also be given to post-marketing surveys. A survey of at least 1000 treated patients can define the toxicity profile of a new formulation. These revisions of the developmental process might offer safer generic formulations to patients without further increase of the medical costs.

In conclusion, renal toxicity was slightly more severe in patients treated with a generic cisplatin formulation than in those treated with an innovator formulation, especially among male patients. This result suggests that more attention should be given to the developmental system of generic formulations, especially in those drugs that have a narrow therapeutic window, such as anticancer agents.

Acknowledgment

The authors thank Mika Nagai for her assistance in the preparation of this manuscript.

Disclosure Statement

The authors indicate no potential conflict of interest.

References

1 Reed E. Cisplatin and its analogues. In: DeVita V, Lawrence T, Roesnberg S, eds. *Cancer: Principles & Practice of Oncology*, 8th edn. Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins, 2008; 419–26.
2 Horwich A, Sleijfer DT, Fossa SD *et al.* Randomized trial of bleomycin, etoposide, and cisplatin compared with bleomycin, etoposide, and carboplatin in good-prognosis metastatic nonseminomatous germ cell cancer: a Multiinstitutional Medical Research Council/European Organization for Research and Treatment of Cancer Trial. *J Clin Oncol* 1997; **15**: 1844–52.
3 De Andres L, Brunet J, Lopez-Pousa A *et al.* Randomized trial of neoadjuvant cisplatin and fluorouracil versus carboplatin and fluorouracil in patients with stage IV-M0 head and neck cancer. *J Clin Oncol* 1995; **13**: 1493–500.
4 Ardizzoni A, Boni L, Tiseo M *et al.* Cisplatin- versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst* 2007; **99**: 847–57.
5 Go RS, Adjei AA. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J Clin Oncol* 1999; **17**: 409–22.
6 Cornelison TL, Reed E. Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. *Gynecol Oncol* 1993; **50**: 147–58.
7 de Jongh FE, van Veen RN, Veltman SJ *et al.* Weekly high-dose cisplatin is a feasible treatment option: analysis on prognostic factors for toxicity in 400 patients. *Br J Cancer* 2003; **88**: 1199–206.
8 Stewart DJ, Dulberg CS, Mikhael NZ *et al.* Association of cisplatin nephrotoxicity with patient characteristics and cisplatin administration methods. *Cancer Chemother Pharmacol* 1997; **40**: 293–308.
9 Haas JS, Phillips KA, Gerstenberger EP, Seger AC. Potential savings from substituting generic drugs for brand-name drugs: medical expenditure panel survey, 1997–2000. *Ann Intern Med* 2005; **142**: 891–7.
10 Willox JC, McAllister EJ, Sangster G *et al.* Effects of magnesium supplementation in testicular cancer patients receiving cis-platin: a randomised trial. *Br J Cancer* 1986; **54**: 19–23.
11 Bodnar L, Wcislo G, Gasowska-Bodnar A *et al.* Renal protection with magnesium subcarbonate and magnesium sulphate in patients with epithelial ovarian cancer after cisplatin and paclitaxel chemotherapy: a randomised phase II study. *Eur J Cancer* 2008; **44**: 2608–14.

Drug Metab. Pharmacokinet. 26 (1): 107–116 (2011).

SNP Communication

Genetic Polymorphisms and Haplotypes of *POR*, Encoding Cytochrome P450 Oxidoreductase, in a Japanese Population

Yoshiro Saito[1,2,*], Noboru Yamamoto[3], Noriko Katori[1,4], Keiko Maekawa[1,2], Hiromi Fukushima-Uesaka[1], Daisuke Sugimoto[5], Kouichi Kurose[1,2], Kimie Sai[1,5], Nahoko Kaniwa[1,2], Jun-ichi Sawada[1,6,**], Hideo Kunitoh[3,†], Yuichiro Ohe[3], Teruhiko Yoshida[7], Yasuhiro Matsumura[8], Nagahiro Saijo[9,††], Haruhiro Okuda[1,6] and Tomohide Tamura[3]

[1]*Project team for Pharmacogenetics, National Institute of Health Sciences, Tokyo, Japan*
[2]*Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan*
[3]*Thoracic Oncology Division, National Cancer Center Hospital, Tokyo, Japan*
[4]*Division of Drugs, National Institute of Health Sciences, Tokyo, Japan*
[5]*Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, Tokyo, Japan*
[6]*Division of Organic Chemistry, National Institute of Health Sciences, Tokyo, Japan*
[7]*Genetics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan*
[8]*Investigative Treatment Division, Research Center for Innovative Oncology, Kashiwa, Japan*
[9]*Deputy Director, National Cancer Center Hospital East, Kashiwa, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Cytochrome P450 oxidoreductase (POR) transfers electrons from NADPH to all microsomal cytochrome P450 (CYP) enzymes and is necessary for microsomal CYP activities. In this study, to find genetic variations and to elucidate the haplotype structures of *POR*, we comprehensively screened the genetic variations in the 5′-flanking region, all the exons and their flanking introns of *POR* for 235 Japanese subjects. Seventy-five genetic variations including 26 novel ones were found: 7 were in the 5′-flanking region, 2 in the 5′-untranslated region (5′-UTR, non-coding exon 1), 16 in the coding exons (10 nonsynonymous and 6 synonymous), 45 in the introns, 4 in the 3′-UTR and 1 in the 3′-flanking region. Of these, 4 novel nonsynonymous variations, 86C>T (T29M), 1648C>T (R550W), 1708C>T (R570C) and 1975G>A (A659T), were detected with allele frequencies of 0.002. We also detected known non-synonymous SNPs 683C>T (P228L), 1237G>A (G413S), 1453G>A (A485T), 1508C>T (A503V), 1510G>A (G504R) and 1738G>C (E580Q) with frequencies of 0.002, 0.009, 0.002, 0.434, 0.002 and 0.002, respectively. Based on the linkage disequilibrium (LD) profiles, the analyzed region could be divided into two LD blocks. For Blocks 1 and 2, 14 and 46 haplotypes were inferred, respectively, and 2 and 6 common haplotypes found in more than 0.03 frequencies accounted for more than 81% of the inferred haplotypes. This study provides fundamental and useful information for the pharmacogenetic studies of drugs metabolized by CYPs in the Japanese population.

Keywords: *POR*; genetic polymorphism; haplotype; Japanese; nonsynonymous variation

Received; September 19, 2010, Accepted; October 18, 2010, J-STAGE Advance Published Date: November 12, 2010
**To whom correspondence should be addressed:* Yoshiro Saito, Ph.D., *Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-3700-9528, Fax. +81-3-3700-9788, E-mail: yoshiro@nihs.go.jp*
***Present address: Pharmaceuticals and Medical Devices Agency, Shin-Kasumigaseki Building, 3-3-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, Japan.*
†*Present address: Department of Respiratory Medicine, Mitsui Memorial Hospital, 1 Kandaizumi-cho, Chiyoda-ku, Tokyo 101-8643, Japan.*
††*Present address: Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan.*
On September 17, 2010, these variations were not found in the Japanese Single Nucleotide Polymorphisms (JSNP) database (http://snp.ims.u-tokyo.ac.jp/), dbSNP database in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or the PharmGKB (http://www.pharmgkb.org/do/) database.
This study was supported in part by the program for the Promotion of Fundamental Studies in Health Sciences from the National Institute of Biomedical Innovation, by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan, and by KAKENHI (22590054) from the Japan Society for the Promotion of Science (JSPS).

Introduction

Cytochrome P450 oxidoreductase (POR) is a flavoprotein that transfers electrons from NADPH to all microsomal cytochrome P450 (CYP) enzymes.[1] The human genome contains 50 microsomal CYP enzymes including 15 genes principally for drug metabolism, and all of these microsomal CYPs require POR activity for catalysis. Mutations leading to disrupt ion of POR activities have been known to cause autosomal recessive genetic diseases, ambiguous genitalia, congenital adrenal hyperplasia, Antley-Bixler syndrome, and polycystic ovary syndrome.[2–4]

Human POR, a 77 kDa protein with 680 amino acids contains one flavin adenine dinucleotide (FAD) and one flavin adenine mononucleotide (FMN) molecules. Electrons from NADPH pass through the FAD to the FMN, and then to the CYP.[1] POR-CYP interaction is formed by electrostatic power: the CYP-interacting surface of POR is charged negatively by acidic amino acids, and the POR-binding site of CYP is positively charged by basic residues (Lys and Arg).

The POR gene consists of 16 exons (including non-coding exon 1) spanning approximately 72 kb at chromosome 7q11.2.[5] Using a human liver bank, 18.3, 16.5 and 3.39-fold interindividual variations were observed in POR mRNA levels, protein levels and cytochrome C reductase activities, respectively.[6] Since its activity is necessary for CYP functions, genetic variations in *POR* might affect the functions of broad ranges of CYPs that are involved in drug metabolism. Many genetic variations have been already reported in the *POR* gene for diverse populations,[6–8] and 41 alleles/haplotypes were publicized in Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (http://www.cypalleles.ki.se/por.htm). Huang *et al.*[9] sequenced the *POR* gene of 218 African-Americans, 260 European-Americans, 179 Chinese-Americans and 185 Mexican-Americans, and detected 140 distinct nucleotide variations including 15 nonsynonymous ones.[9] They also examined the functional significance of these 15 variations using bacterial expression systems. Six and five variations were associated with >50% reduced V_{max}/K_m values for cytochrome C reduction activities and NADPH oxidation activities, respectively, when compared to those of the wild-type. But when assayed with expressed P450c17, only four variations decreased 17α-hydroxylase and 17,20-lyase activities by >50%. The authors also extended their study for effects of 35 POR nonsynonymous variations on CYP1A2 and CYP2C19 activities, in which 10 POR variant proteins were associated with no detectable catalytic activities and 8 including P228L had >50% reduced V_{max}/K_m values for both enzymes, when compared to those of the wild-types.[10] Recently, Gomes *et al.*[6] comprehensively screened the effects of POR and CYP genetic variations as well as patients' non-genetic factors on 10 CYP catalytic activities using 150 Caucasian surgical liver samples, and found that three intronic polymorphisms were significantly associated with the altered CYP3A4 activity (IVS3+88G>A), CYP1A2, CYP2C8, CYP2C19 and CYP3A4 activities (IVS4+89C>T), and CYP2C19 and CYP3A4 activities (IVS11+20G>A), although their mechanisms for activity changes have not been revealed.[6] The most common polymorphism A503V (minor allele frequency [MAF] = 0.303) had negligible functional effects,[6] as reported previously.[9,10]

Although many studies have been conducted as above, reports are lacking on *POR* genetic polymorphisms in the Japanese population. Here we sequenced the 5′-flanking region, all exons and their flanking regions of *POR* from 235 Japanese subjects.

Materials and Methods

Human genomic DNA samples: A total of 235 unrelated Japanese cancer patients administered paclitaxel were participated in this study. The ethical review boards of the National Cancer Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all participating patients. Genomic DNA for sequencing analysis was extracted from blood leukocytes collected from the subjects prior to the paclitaxel administration.

PCR conditions for sequencing: The GenBank accession numbers, NT_079595.2 (genome) and NM_000941.2 (mRNA) were used for primer design and as the reference sequences. For sequencing, a set of four multiplex long-range PCR was performed to amplify all 16 exons from 150 ng of genomic DNA using 0.04 units/μl of LA-Taq in GC buffer I (Takara Bio Inc., Shiga, Japan) using primer sets (0.2 μM) designed in the 5′-flanking or intronic regions as listed in "1st PCR" of **Table 1**. The first PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. Next, short targeted regions for sequences, except for 5′-flanking region and exon 1, were amplified separately in the 2nd PCR using the 1st PCR product as a template by Ex-Taq (0.02 units/μl, Takara Bio Inc.) with the primers (0.2 μM) listed in "2nd PCR" in **Table 1**. Because of a high GC content, the 5′-flanking region and exon 1 were amplified using 0.04 units/μl of LA-Taq in GC buffer I with 0.2 μM of the primers listed in **Table 1**. The 2nd PCR conditions were same as the 1st PCR. Thereafter, the PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) with the primers listed in "Sequencing" of **Table 1**. The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3730xl DNA Analyzer (Applied Biosystems). All the rare detected variations were confirmed by repeating the PCR from the genomic DNA and sequencing the newly generated PCR products.

Table 1. Primer sequences used for amplification and sequencing of *POR*

Usage	Region	Forward primer	Reverse primer	Amplified length
1st PCR	5′-Flanking–Exon 1	CTTCTTTGGACAGGTATTGTGCCC	TAGGACCATTTCAGACAAGTGCCA	5,943
	Exon 2	AGTCAGTGGCTGTCATTTTCTCTG	ATACCTGACCTCTCCCAAACGAAA	8,577
	Exon 3	TCATCCTCCTCCATCGGGTAATCA	TGAGTTAGAGGGAGGGTCTGTTCT	9,986
	Exons 4–16	GGATGTAAGTATCTATGGGAGGTC	AAATGCCTCCTCCCTGCTTAGTTC	8,345
2nd PCR	5′-Flanking*	AAAAGATGGAGAAGGGGCTCTG	GCGAAAGATAGCACTCACCG	1,195
	Exon 1*	CCACGCACTTTCATTTCTCT	ATACCGAGCCCTAACCCTCA	556
	Exon 2	TGAGTGAGCCCCTTCTCCTA	CCCAAGAGTCACCCCAAAAT	463
	Exon 3	AGCCCTGGTGTTGGATTAGA	GTTAGGCAAGAATGACTCCC	399
	Exon 4	ACAGTGAGAAGCAAGTCCCA	TGGGTTTGGTTTGGGAGATG	581
	Exon 5	CCCTCCGTGTTGTTACTTCT	AGTCGTCCAGCCAGACCTTT	565
	Exon 6	GTCAACCAGATGAAGCCTCT	TCTGTGTTGGAGGTGCGTGT	432
	Exon 7	CCTGATGCTCTGGGTTTATG	ACCCTATGACGGAGTGTCTT	361
	Exon 8	CCCTGCTTCTTGTCGTATGT	ATGAGCCCTTCTGCCAAAGA	538
	Exons 9–10	CTGAGATTCCCTGTGCTTTG	ACTATGACAGTGACGGGGTA	662
	Exons 11–12	TGTGTCAGACCGTGTAGTGT	GCTGGACAGATGCTGAGAAT	921
	Exons 13–14	TCGGGCTGGCTTGTGAGATT	TCTCACCTTGTGGGACTGCT	709
	Exons 14–16	GACGCTGCTGTACTACGGCT	CCAGAGGAGTCTTTGTCACT	675
	Exon 16	GTGGACTACATCAAGAAACT	GGTCTCTTCTATTCTCCCTT	657
Sequencing	5′-Flanking	AAAAGATGGAGAAGGGGCTCTG	GTCTCGCTATGATGCCCAGGTT	
		AACCTGGGCATCATAGCGAGAC	CAGAGAAATGAAAGTGCGT	
	Exon 1	CCACGCACTTTCATTTCTCT	GTGGAAAAGTCGACCCTCAG	
	Exon 2	TGAGTGAGCCCCTTCTCCTA	CCCAAGAGTCACCCCAAAAT	
	Exon 3	AGCCCTGGTGTTGGATTAGA	GTTAGGCAAGAATGACTCCC	
	Exon 4	ACAGTGAGAAGCAAGTCCCA	TGGGTTTGGTTTGGGAGATG	
		AGAGGAACTTAGAAGGGACT	TTGGTTTGGGAGATGTGGCG	
	Exon 5	CCCTCCGTGTTGTTACTTCT	AGCCAGACCTTTCTTGCCT	
	Exon 6	GTCAACCAGATGAAGCCTCT	TCTGTGTTGGAGGTGCGTGT	
	Exon 7	CCTGATGCTCTGGGTTTATG	ACCCTATGACGGAGTGTCTT	
	Exon 8	CCCTGCTTCTTGTCGTATGT	ATGAGCCCTTCTGCCAAAGA	
	Exons 9–10	CTGAGATTCCCTGTGCTTTG	ACTATGACAGTGACGGGGTA	
	Exons 11–12	TGTGTCAGACCGTGTAGTGT	TGCAGGATGGCCAGGATGTG	
		CCCAATCAGCCCCATCTCAC	GCTGGACAGATGCTGAGAAT	
	Exons 13–14	TCGGGCTGGCTTGTGAGATT	TCTCACCTTGTGGGACTGCT	
	Exons 14–16	GACGCTGCTGTACTACGGCT	CCAGAGGAGTCTTTGTCACT	
	Exon 16	GTGGACTACATCAAGAAACT	GGTCTCTTCTATTCTCCCTT	

*LA-Taq with GC buffer I was used for amplification in the 2nd PCR because of high GC contents.

Linkage disequilibrium (LD) and haplotype analysis: Hardy-Weinberg equilibrium, LD analysis and diplotype configurations (a combination of haplotypes) were analyzed with SNPAlyze software ver. 7 (Dynacom Co., Chiba, Japan). Pairwise LD between variations with minor allele frequencies (MAF) of greater than 0.03 was analyzed using r^2 and $|D'|$ values. Nomenclature for the haplotypes was based on the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, and these haplotypes were tentatively named as numbers (Arabic numbers for known and novel unambiguously determined haplotypes as defined by the Committee, and Roman numbers for ambiguously estimated ones with nonsynonymous variations) plus lower-case alphabetical letters in this study. The haplotypes inferred in single subjects (ambiguous ones) are described with haplotype names and a question mark, since the predictability for these very rare haplotypes is known to be low in some cases. Of these ambiguous haplotypes, the ones without amino acid changes were put together into "**1 others*" and with 1508C>T (A503V) into "**28 others*".

Table 2. Summary of *POR* variations detected in this study

SNP ID		Reference	Location	Position		Nucleotide change	Amino acid change	Allele frequency (n = 235)	
This Study	dbSNP (NCBI)			NT_079595.2	From the translational initiation site or from the end of the nearest exon				95% Confidence interval
MPJ6_POR_001[a]			5′-Flanking	777673	−39914	cagctagtaataA/Gtagtagtagtag		0.011	0.001–0.020
MPJ6_POR_002[a]				778455	−39132	ccggcccggtgaC/Ttgcagggtccga		0.011	0.001–0.020
MPJ6_POR_003	rs72553984	9)		778485	−39102	agaagccgcagcC>Ggccgtctccagg		0.006	0.000–0.014
MPJ6_POR_004	rs12537282	9)		778488	−39099	agccgcagccgcC>Ggtctccaggcga		0.004	0.000–0.010
MPJ6_POR_005[a]				778512	−39075	actccgccacccC>Gcggaaccacgca		0.006	0.000–0.014
MPJ6_POR_006[a]				778540	−39047	tcatttctctgcC>Ggggcgacccagc		0.002	0.000–0.006
MPJ6_POR_007	rs72553972	9)		778544	−39043	ttctctgccgggC>Agacccagccgag		0.019	0.007–0.032
MPJ6_POR_008[a]			5′-UTR (Exon 1)	778710	−38877	aggcggtggtagC>Tgcctcagtggtg		0.002	0.000–0.006
MPJ6_POR_009	rs3823884	9)		778731	−38856	ggtgtgggcctgA>Cgccctgcccagg		0.255	0.216–0.295
MPJ6_POR_010[a]			Intron 1	778825	−38762	gtcgggccccaaG>Aactcgggtttg		0.002	0.000–0.006
MPJ6_POR_011	rs3735508			778910	−38677	tgggcactgcgcG>Atctggcccgacg		0.009	0.000–0.017
MPJ6_POR_012[a]				779010	−38577	gcctgagggtcgA>Gcttttccacagc		0.002	0.000–0.006
MPJ6_POR_013[a]				779011	−38576	cctgagggtcgaC>Ttttccacagct		0.006	0.000–0.014
MPJ6_POR_014	rs10262966	6, 8, 9)	Exon 2	817601	15	gatcaacatgggA>Ggactcccacgtg	Gly5Gly	0.011	0.001–0.020
MPJ6_POR_015[a]				817672	86	ttttcagcatgaC>Tggacatgattct	Thr29Met	0.002	0.000–0.006
MPJ6_POR_016	rs10225188	6)	Intron 3	842950	IVS3−95	gggggcggcctgG>Tagggccccgcc		0.028	0.013–0.042
MPJ6_POR_017	rs10239977	6, 9)	Intron 4	843262	IVS4+89	gcaggggggaggcC>Tggcagggagtgg		0.028	0.013–0.042
MPJ6_POR_018	rs72554000	9)		843420	IVS4+247	ccccgagtgccG>Agctgccctcctg		0.013	0.003–0.023
MPJ6_POR_019[a]				843818	IVS4−115	cacgacactcagA>Ccatccctggcct		0.002	0.000–0.006
MPJ6_POR_020	rs1135612	6, 8, 9)	Exon 5	843953	387	gagcagcctgccA>Ggagatcgacaac	Pro129Pro	0.440	0.396–0.485
MPJ6_POR_021[a]			Intron 5	844115	IVS5+33	tatgggctcccgG>Atggcctgcggtg		0.013	0.003–0.023
MPJ6_POR_022	rs72555505	9)		844124	IVS5+42	ccggtggcctgcG>Agtgcctccctgg		0.013	0.003–0.023
MPJ6_POR_023[a]			Intron 6	845032	IVS6−79	gatggggtgggT>Acggggcgtgcct		0.002	0.000–0.006
MPJ6_POR_024	rs2286819	8)		845039	IVS6−72	tgggtcgggcA>Gtgcctggcacca		0.140	0.109–0.172
MPJ6_POR_025	rs2286820	6, 8, 9)		845084	IVS6−27	ctcccctgagccG>Actccccctctcc		0.009	0.000–0.017
MPJ6_POR_026	rs17853284	6, 9)	Exon 7	845152	683	agcagttctggcC>Tggccgtgtgtga	Pro228Leu	0.002	0.000–0.006
MPJ6_POR_027	rs10954732	6, 9)	Intron 7	845425	IVS7+225	aggacacatcgcG>Atcgggctctgtg		0.417	0.372–0.462
MPJ6_POR_028[a]				845427	IVS7+227	gacacatcgcgtC>Tgggctctgtggc		0.002	0.000–0.006
MPJ6_POR_029	rs2286821	6)		845631	IVS7−187	gcagctccacgcC>Tgcctcctcctt		0.419	0.375–0.464
MPJ6_POR_030[a]				845736	IVS7−82	aaggccatgcacG>Cgtctcccctgta		0.002	0.000–0.006
MPJ6_POR_031[a]				845736	IVS7−82	aaggccatgcacG>Agtctcccctgta		0.004	0.000–0.010
MPJ6_POR_032	rs3815455	6, 8, 9)	Intron 8	846032	IVS8+116	ccagacccgtgC>Tcccgagtgggtg		0.434	0.389–0.479
MPJ6_POR_033	rs72557926	9)		846055	IVS8+139	tgtgagtgtccaC>Tgacctgtccacg		0.013	0.003–0.023
MPJ6_POR_034[a]				846211	IVS8+295	gatcctctttggC>Aagaagggctcat		0.013	0.003–0.023
MPJ6_POR_035	rs13223707	8)		847046	IVS8−68	tgcaaccagaagG>Cgtccttggagac		0.134	0.103–0.165
MPJ6_POR_036	rs13240147	8)		847059	IVS8−55	gtccttggagacA>Ggagactcagatc		0.134	0.103–0.165
MPJ6_POR_037	rs41301394	6, 8)		847079	IVS8−35	agatcaaagcccC>Tggccgctcactg		0.434	0.389–0.479
MPJ6_POR_038[a]			Intron 9	847260	IVS9+30	cacccccgaacC>Gctcactctgggc		0.004	0.000–0.010
MPJ6_POR_039[a]			Intron 10	847471	IVS10+21	cacagtcagggcG>Acccgccgggct		0.002	0.000–0.006
MPJ6_POR_040	rs41301400	9)		847551	IVS10+101	gcctgaagccccG>Cgtgcctgggagg		0.098	0.071–0.125
MPJ6_POR_041	rs4732514	8)		848274	IVS10−97	ggcacctgttgcC>Tgcagagctggcc		0.419	0.375–0.464
MPJ6_POR_042[a]				848301	IVS10−70	aggtgtcaccccC>Ttcctgccgcagc		0.002	0.000–0.006

Continued on next page.

Continued.

SNP ID		Reference	Location	Position		Nucleotide change	Amino acid change	Allele frequency (n = 235)	
This Study	dbSNP (NCBI)			NT_079595.2	From the translational initiation site or from the end of the nearest exon				95% Confidence interval
MPJ6_POR_043	rs4732515	8)		848305	IVS10−66	gtcaccccctccC>Tgccgcagccacc		0.134	0.103–0.165
MPJ6_POR_044	rs4732516	8, 9)		848358	IVS10−13	agtcctgcctgtG>Ctcttccctgcag		0.132	0.101–0.163
MPJ6_POR_045	rs41301424	6, 9)	Exon 11	848540	1236	ggcctcctcctcC>Tggcgagggcaag	Ser412Ser	0.002	0.000–0.006
MPJ6_POR_046		3, 6)		848541	1237	gcctcctcctccG>Agcgagggcaagg	Gly413Ser	0.009	0.000–0.017
MPJ6_POR_047	rs72557931	9)	Intron 11	848563_848571	IVS11+11_19	aggtgcgccccTCAGCCCCC/ - gcaacctccgcc		0.006	0.000–0.014
MPJ6_POR_048	rs2286822	6, 8, 9)		848564	IVS11+12	ggtgcgcccctC>Tagccccgcaac		0.389	0.345–0.433
MPJ6_POR_049	rs2286823	6, 8, 9)		848572	IVS11+20	cctcagcccccG>Acaacctccgccc		0.389	0.345–0.433
MPJ6_POR_050	rs41301427	8, 9)	Intron 12	848833	IVS12+32	tgccagccacacG>Actggaggcccag		0.011	0.001–0.020
MPJ6_POR_051[a]				848993	IVS12−180	tcagcatctgtcC>Tagccccggtccc		0.002	0.000–0.006
MPJ6_POR_052	rs6961174			849000	IVS12−173	ctgtccagccccA>Ggtccccagaacc		0.134	0.103–0.165
MPJ6_POR_053	rs2302429	6)		849053	IVS12−120	tctgaggttttggG>Atgccaggtgggc		0.323	0.281–0.366
MPJ6_POR_054	rs2302430			849065	IVS12−108	gtgccaggtgggC>Gtggaagaggccc		0.100	0.073–0.127
MPJ6_POR_055	rs2302431	8, 9)		849139	IVS12−34	caagggcctcggC>Tgtggcggtggag		0.134	0.103–0.165
MPJ6_POR_056	rs2302432	8, 9)		849140	IVS12−33	aagggcctcggtT>Gtggcggtggagc		0.134	0.103–0.165
MPJ6_POR_057	rs72557947	9)	Exon 13	849227	1453	tacgagaccaagG>Actggccgcatca	Ala485Thr	0.002	0.000–0.006
MPJ6_POR_058	rs2228104	6, 8)		849229	1455	cgagaccaaggcC>Tggccgcatcaac	Ala485Ala	0.134	0.103–0.165
MPJ6_POR_059	rs1057868	3, 6, 8, 9)		849282	1508	ccaaggagcctgC>Tcggggagaacgg	Ala503Val	0.434	0.389–0.479
MPJ6_POR_060		3, 6)		849284	1510	aaggagcctgccG>Agggagaacggcg	Gly504Arg	0.002	0.000–0.006
MPJ6_POR_061[a]				849422	1648	ttcatccaggagC>Tgggcctggctgc	Arg550Trp	0.002	0.000–0.006
MPJ6_POR_062	rs2302433	9)	Intron 13	849476	IVS13+33	gagaggggggtgaC>Tgactgggagccc		0.091	0.065–0.118
MPJ6_POR_063[a]			Exon 14	849555	1708	tactacggctgcC>Tgccgctcggatg	Arg570Cys	0.002	0.000–0.006
MPJ6_POR_064	rs1057870	6, 8, 9)		849563	1716	ctgccgccgctcG>Agatgaggactac	Ser572Ser	0.028	0.013–0.042
MPJ6_POR_065		4)		849585	1738	tacctgtaccggG>Caggagctggcgc	Glu580Gln	0.002	0.000–0.006
MPJ6_POR_066	rs72557949	9)		849611	1764	gttccacagggaC>Tggtgcgctcacc	Asp588Asp	0.002	0.000–0.006
MPJ6_POR_067	rs72557952	9)	Intron 14	849670	IVS14+8	acaaggtgagacG>Aggcgggcaccac		0.004	0.000–0.010
MPJ6_POR_068[a]				849708	IVS14+46	gaggctggcaggG/- ccacagccacag		0.002	0.000–0.006
MPJ6_POR_069	rs72557953	9)		849712	IVS14+50	ctggcagggccaC>Gagccacagtgcc		0.028	0.013–0.042
MPJ6_POR_070[a]			Exon 16	850007	1975	gccatggagcacG>Acgcaggcggtgg	Ala659Thr	0.002	0.000–0.006
MPJ6_POR_071[a]			3′-UTR	850225_850227	2193_2195 (*150_*152)[b]	gggtgcatcctcCTC/ - agccccaggcc		0.006	0.000–0.014
MPJ6_POR_072	rs41302348	6, 8)		850282	2250 (*207)[b]	gcccagggcctgC>Gatgggggcaccg		0.045	0.026–0.063
MPJ6_POR_073	rs17685	6, 8)		850381	2349 (*306)[b]	cagcccctccacG>Atgatttccagtg		0.385	0.341–0.429
MPJ6_POR_074	rs2286824	6)		850447	2415 (*372)[b]	gttctgttttctG>Atatttgcctggt		0.045	0.026–0.063
MPJ6_POR_075[a]			3′-Flanking	850614	2417+165[c] (*374+165)[b]	agggcactgggcC>Tcaggtcttcctt		0.002	0.000–0.006

[a]Novel variations detected in this study.
[b]Positions are shown as * and bases from the translational termination codon TAG.
[c]Position was shown as the last base of exon 16 (2417) and bases downstream of this base.

Results and Discussion

***POR* genetic variations found in a Japanese population:** The 5′-flanking regions (up to 39968 bases upstream of the translational start site, 1077 bases upstream of the transcriptional start site), all the 16 exons and their flanking introns of *POR* were sequenced in 235 Japanese subjects. Seventy-five genetic variations, including 26 novel ones, were detected: 7 were in the 5′-flanking region, 2 in the 5′-untranslated region (5′-UTR, non-coding exon 1), 16 in the coding exons (10 nonsynonymous and 6 synonymous), 45 in the introns, 4 in the 3′-UTR and 1 in the 3′-flanking

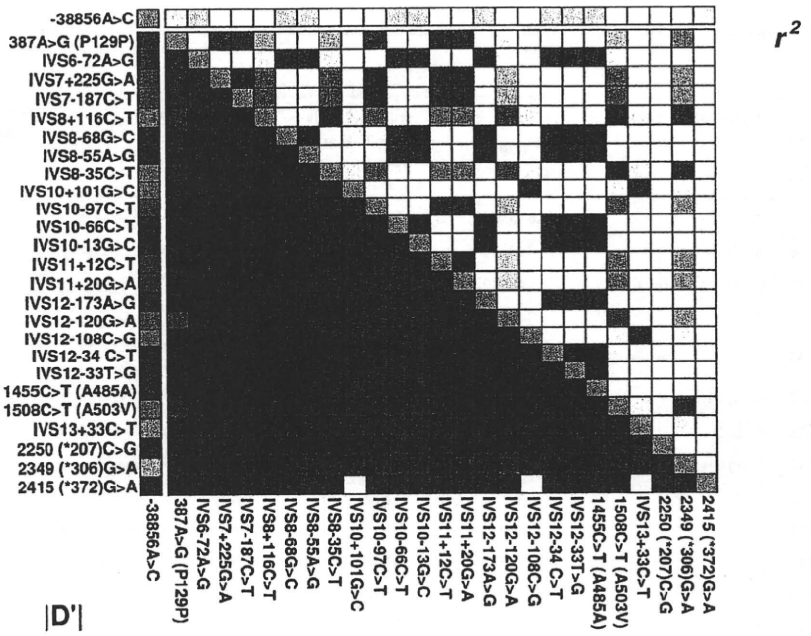

Fig. 1. Linkage disequilibrium (LD) analysis of *POR*
Pairwise LD is expressed as r^2 (upper right) and |D'| (lower left) values (from 0 to 1) by 10-graded blue colors. A denser color represents closer linkage.

region (see **Table 2**). All of the detected variations were found in Hardy-Weinberg equilibrium ($P \geq 0.11$).

All of the four novel nonsynonymous variations, 86C>T (T29M), 1648C>T (R550W), 1708C>T (R570C) and 1975G>A (A659T), were found as individual heterozygotes at 0.002 frequencies (**Table 2**). The T29 is located in the transmembrane anchoring domain, and the latter three substitutions are within the NADP(H)-binding domain, based on the crystal structure of rat POR which has 92% homology to human POR.[11] Note that the corresponding position of human A659 is Thr in rat POR. The other three substitution positions are conserved between both species. Using PolyPhen program (http://genetics.bwh.harvard.edu/pph/) to predict the functional effect by the amino acid substitution, two substitutions, R550W and R570C seem to cause probably damaging on protein function based on the PSIC (position-specific independent count) profile score differences derived from multiple alignment (the predictability of this program was checked by 12 known *CYP2C9* alleles with reduced enzymatic activities *in vitro*, and it was found that the successful predictability was 0.583 [7/12].). The effects of T29M and A659T were predicted to be benign. Functional significance of these 4 novel variations should be clarified in the future. We also detected six known nonsynonymous variations, 683C>T (P228L), 1237G>A (G413S), 1453G>A (A485T), 1508C>T (A503V), 1510G>A (G504R) and 1738G>C (E580Q) with the frequencies of 0.002, 0.009, 0.002, 0.434, 0.002 and 0.002, respectively. Of these, the P228L variant enzyme was shown to render over 60% reduced activities (V_{max}/K_m values) of CYP1A2 and CYP2C19.[10] In contrast, the effects of G413S, A485T and G504R substitutions were minimal for both CYP enzymes.[10] The frequency (0.434) of the most frequent nonsynonymous variation 1508C>T (A503V) was higher than those in Chinese-Americans (0.367), Mexican-Americans (0.310), European-Americans (0.264) and African-Americans (0.191) although its functional effects were reported to be very mild.[9,10]

In addition to nonsynonymous variations, three intronic variations, IVS3+88G>A, IVS4+89C>T and IVS11+20G>A, were reported to be significantly associated with altered CYP activities, although their mechanisms were not clarified.[6] IVS3+88G>A was not detected in our Japanese population. IVS4+89C>T was found at 0.028 frequency in Japanese, which is much lower than those in European-Americans (0.363), Chinese-Americans (0.138), African-Americans (0.135) and Mexican-Americans (0.129).[9] The last one, IVS11+20G>A was detected at 0.389 frequency, similar to those in Chinese-Americans (0.360) and Mexican-Americans (0.407), but slightly and much lower frequencies were reported in European-Americans (0.317) and African-Americans (0.194), respectively.[9]

Linkage disequilibrium (LD) analysis: Using the 26 genetic variations detected with ≥0.03 frequencies, LD analysis was performed by the r^2 and |D'| statistics, and the pairwise values of both are shown with a 10-graded blue scale in **Figure 1**. |D'| is used to assess the probability for past recombinations, and r^2 is used as a parameter for the linkage between a pair of variations.

For r^2 values, perfect linkage ($r^2 = 1$) was detected among IVS8+116C>T, IVS8−35C>T and 1508C>T (A503V), among IVS8−68G>C, IVS8−55A>G, IVS10−66C>T, IVS12−173A>G, IVS12−34C>T, IVS12−33T>G and 1455C>T (A485A), and between IVS11+12C>T and IVS11+20G>A. Relatively strong linkages were observed among 387A>G (P129P), IVS7+225G>A, IVS7−187C>T, IVS10−97C>T, IVS11+12C>T and IVS11+20G>A ($r^2 \geq 0.69$), among IVS6−72A>G, IVS10−13G>C and the above perfect linkages of IVS8−68G>C ~ 1455C>T (A485A) ($r^2 \geq 0.93$), among IVS10+101G>C, IVS12−108C>G and IVS13+33C>T ($r^2 \geq 0.91$). In addition 2349 (*306)G>A was strongly linked with the above perfect linkages of IVS8+116C>T ~ 1508C>T (A503V) ($r^2 = 0.80$). On the other hand, only weak linkages ($r^2 \leq 0.31$) were observed between −38856A>C and rest of the variations. For |D′| values, strong LD (|D′| ≥ 0.8) was observed in 97.3% (292/300) of combinations of 25 variations from 387A>G (P129P) to 2415 (*372)G>A. Between −38856A>C and 387A>G (P129P), only 40% (10/25) of combinations showed strong LD (|D′| ≥ 0.8).

Based on the above results, we divided the analyzed region of *POR* into two LD blocks as indicated in **Figure 1**. Block 1, spanning at least 1.3 kb, included 13 variations from −39914A>G in the 5′-flanking region to −38576C>T in intron 1. Block 2, which includes the 62 variations from 15A>G (G5G) to 2417+165 (*374+165)C>T was ranging approximately 33.0 kb. Six variations from exon 2 to intron 4 were tentatively included in Block 2, since the distance (26.4 kb) from 387A>G (P129P) to 15A>G (G5G) is less than that (38.6 kb) from −38576C>T to 15A>G (G5G). The 2417+165 (*374+165)C>T was also included into Block 2.

Haplotype estimation: We then analyzed haplotype structures of *POR* for each block. The haplotypes in each block inferred by SNPAlyze software and their frequencies are shown in **Table 3A** and **3B**. Using all the 13 and 62 variations, 14 and 46 haplotypes were inferred in Block 1 and 2, respectively. The diplotype configurations were obtained at probabilities over 0.99 for 99% and 91% of the 235 subjects in Blocks 1 to 2, respectively. Of all the estimated haplotypes, 2 in Block 1 and 23 in Block 2 were detected in only one chromosome ambiguously. Common haplotypes were defined as ones with more than 0.03 frequencies in this study.

Block 1 includes only variations detected in the 5′-transcriptional regulatory region, non-coding exon 1 and intron 1. The most dominant haplotype was **1a** at a frequency of 0.717, which was followed by **1b** at 0.202 frequency. The frequencies of the other haplotypes were less than 0.03. Although many haplotypes were predicted, these 2 common haplotypes (**1a** and **1b**) accounted for 92% of all the inferred haplotypes.

Block 2 covers all the coding exons. In Block 2, 11 haplotype groups (**1*, **28*, **35*, **36*, **42*, **43*, **I*, **II*, **III*,

Table 3A. *POR* Block 1 haplotypes

Region	5′-flanking							Exon 1 (5′-UTR)			Intron 1	Number	Frequency
Nucleotide change[a]	−39914 A>G	−39132 C>T	−39102 C>G	−39099 C>G	−39075 C>G	−39047 C>G	−39043 C>A	−38877 C>T	−38856 A>C	−38677 G>A	−38576 C>T		
Amino acid change													
Haplotypes[b,c] *1 *1a												337	0.717
*1b												95	0.202
*1c												9	0.019
*1d												5	0.011
*1e												5	0.011
*1f												4	0.009
*1g												3	0.006
*1h												3	0.006
*1i												3	0.006
*1j												2	0.004
*1k												1	0.002
*1l												1	0.002
*1 others[d]												2	0.004
												470	1.000

[a]A of the translational start codon of *POR* is numbered 1. NT_079595.2 was used as the reference sequence.
[b]Major allele, white; minor allele, gray
[c]The haplotypes are described as numbers plus lowercase alphabetical letters.
[d]The haplotypes inferred in only one chromosome ambiguously are grouped together in "others" and variations found only in these ambiguous haplotypes were not included in this table.

Table 3B. *POR* Block 2 haplotypes

Region		Exon 2		Intron 3	Intron 4		Exon 5	Intron 5		Intron 6			Exon 7	Intron 7			Intron 8						Intron 9	Intron 10				Exon 11
Nucleotide change[a]		15 A>G	86 C>T	IVS3 −95 G>T	IVS4 +89 C>T	IVS4 +247 G>A	387 A>G	IVS5 +33 G>A	IVS5 +42 G>A	IVS6 −79 T>A	IVS6 −72 A>G	IVS6 −27 G>A	683 C>T	IVS7 +225 G>A	IVS7 −187 C>T	IVS7 −82 G>A	IVS8 +116 C>T	IVS8 +139 C>T	IVS8 +295 C>A	IVS8 −68 G>C	IVS8 −55 A>G	IVS8 −35 C>T	IVS9 +30 C>G	IVS10 +101 G>C	IVS10 −97 C>T	IVS10 −66 C>T	IVS10 −13 G>C	1237 G>A
Amino Acid Change		G5G	T29M				P129P						P228L															G413S
Haplotypes[b,c]	*1	*1a																										
		*1b																										
		*1c																										
		*1d																										
		*1e																										
		*1f																										
		*1g																										
		*1h																										
		*1i																										
		*1 others[d]																										
	*28	*28a																										
		*28b																										
		*28c																										
		*28d																										
		*28e																										
		*28f																										
		*28g																										
		*28h																										
		*28i																										
		*28j																										
		*28k																										
		*28 others[d]																										
	*35	*35a																										
	*36	*36a?[e]																										
	*42	*42a																										
		*42b?[e]																										
	*43	*43a																										
	*I	*Ia?[e]																										
	*II	*IIa?[e]																										
	*III	*IIIa?[e]																										
	*IV	*IVa?[e]																										
	*V	*Va?[e]																										

[a]A of the translational start codon of *POR* is numbered 1. NT_079595.2 was used as the reference sequence.
[b]Major allele, white; minor allele, gray
[c]The haplotypes are described as numbers plus lowercase alphabetical letters.
[d]The haplotypes inferred in only one chromosome ambiguously are grouped together in "others" and variations found only in these ambiguous haplotypes were not included in this table.
[e]The haplotypes inferred in only one subject are described with haplotype names and a question mark.

Continued on next page.