been evaluated. To date, no organ dysfunction meeting the protocol-defined dose-limiting toxicity (DLT) criteria has been reported in any of these studies, and the maximum tolerated dose has not been defined.

In phase I or II studies of single-agent PF-3512676, the minimum dose level at which objective response was reported was 0.16 mg/kg weekly in patients with cutaneous T-cell lymphoma or RCC and 6 mg (approximately 0.10 mg/kg) weekly in patients with advanced melanoma.[14–16] A subsequent, randomized phase II study in Western patients with chemotherapy-naive NSCLC investigated PF-3512676 (0.2 mg/kg) in combination with standard taxane/platinum doublet chemotherapy (n = 74) and chemotherapy alone (n = 37).[17] The PF-3512676 dose of 0.2 mg/kg was selected to be above the minimum dose associated with antitumor activity in phase I and II studies of single-agent PF-3512676 and below the dose level (0.24 mg/kg) that, in the same single-agent studies, had been well tolerated for up to 6 months by the majority of patients. In the randomized phase II NSCLC study, the response rate in the PF-3512676 plus chemotherapy arm was higher than that in the chemotherapy-alone arm (30% *vs* 19% confirmed response rate, respectively). In addition, there was a trend toward improved median overall survival with addition of PF-3512676 to chemotherapy (12.3 months compared with 6.8 months for chemotherapy alone, P = 0.188). One-year survival was 50% and 33% for PF-3512676 plus chemotherapy and chemotherapy alone, respectively. Common adverse events (AEs) considered related to treatment with PF-3512676 and not to chemotherapy were injection-site reactions and flu-like symptoms. Other, less-common AEs considered related to treatment with PF-3512676 were febrile neutropenia, anemia, and thrombocytopenia. Overall, a 0.2 mg/kg dose of PF-3512676 in combination with taxane/platinum doublet chemotherapy appeared to have promising antitumor activity as well as a favorable safety profile and was recommended for further study in patients with advanced NSCLC.[17]

The present phase I study was conducted to investigate the safety and pharmacokinetics of PF-3512676 both as monotherapy and in combination with carboplatin and paclitaxel as first-line therapy for Japanese patients with advanced NSCLC.

Patients and Methods

Patients. Patients aged 20 to 75 years with histopathologically or cytologically diagnosed, previously untreated stage IIIB or IV NSCLC were eligible. To enroll in the study, patients were required to have a life expectancy ≥3 months, an Eastern Cooperative Oncology Group performance status (ECOG PS) of ≤1, and at least one measurable lesion of ≥20 mm according to Response Evaluation Criteria in Solid Tumors (RECIST).[18] Patients were also required to have adequate renal, liver, and bone marrow function (serum creatinine <1.5 × upper limit of normal [ULN], total bilirubin <1.5 × ULN, aspartate aminotransferase and alanine aminotransferase <2.5 × ULN, absolute neutrophil count ≥2000/mm^3, platelets ≥100 000/mm^3, and hemoglobin ≥10 g/dL).

Patients were excluded if they had brain or central nervous system metastases that were symptomatic or requiring treatment; any other malignancies within the past 5 years (except non-melanoma skin cancer or adequately treated *in situ* cervical cancer, gastric cancer, or colorectal cancer); autoimmune or antibody-mediated diseases; possible hypersensitivity to ODNs or castor oil; or hepatitis B or C infection. In addition, patients were excluded if they had participated in any other clinical trials; had received other investigational drugs within the previous 3 months; were pregnant or lactating; had uncontrolled infections or hypertension; had certain cardiac abnormalities; or required chronic treatment with therapeutic doses of systemic corticosteroids.

This study was conducted according to the Declaration of Helsinki and its amendments, Japanese Good Clinical Practice guidelines, and in agreement with the Institutional Review Board at the National Cancer Center Hospital (Tokyo, Japan). All patients provided written informed consent prior to study procedures.

Study design and treatments. This was an open-label phase I study in patients with advanced NSCLC. Patients received single-agent PF-3512676 subcutaneously on day 1, followed by 7 days of observation. If safety was confirmed, the patient immediately proceeded to the combination therapy phase. During combination therapy, carboplatin (area under the curve [AUC] 6 mg × min/mL) and paclitaxel (200 mg/m^2) were administered by intravenous (i.v.) infusion on day 1 and PF-3512676 was administered subcutaneously on days 8 and 15 of a 3-week cycle. Treatments were administered for a maximum of six cycles. Dexamethasone (20 mg) and chlorpheniramine maleate (10 mg) were administered by i.v. infusion 1 h before and ranitidine (50 mg) by i.v. infusion at least 30 min before each administration of paclitaxel.

During the monotherapy phase, patients in dose levels 1 and 2 were to be administered 0.1 mg/kg and 0.2 mg/kg PF-3512676, respectively. These doses were to be maintained during the combination therapy phase. Patients in dose level 3 were to receive 0.4 mg/kg PF-3512676 in the monotherapy phase and 0.2 mg/kg during the combination therapy phase. The three treatment arms with a maximum dose of 0.4 mg/kg PF-3512676 during the monotherapy phase were designed to establish one of the primary endpoints of this study: the pharmacokinetic (PK) profile of PF-3512676 in Japanese patients. Another study objective was to determine whether the same dose (0.2 mg/kg) of PF-3512676 that was used in combination with chemotherapy in the phase II and III studies of this agent in Western patients with NSCLC would also be recommended in Japanese patients. Therefore, PF-3512676 in dose level 3 was reduced from 0.4 mg/kg to 0.2 mg/kg when patients moved from the monotherapy to the combination phase. Patients with no DLT in the monotherapy phase could move immediately into the combination phase. For patients in level 3 only, any DLT observed during the monotherapy phase would have led to extension of the duration of this phase of the study by 1 week; if severity of toxicity decreased to ≤grade 1, patients would then continue into the combination therapy phase. A DLT was defined as any of the following: ≥grade 3 febrile neutropenia accompanied by infection; ≥grade 3 non-hematologic toxicity; ≥grade 3 injection site reaction; ≥grade 3 thrombocytopenia requiring transfusion; grade 4 flu-like symptoms; grade 4 neutropenia lasting 7 days; or grade 4 thrombocytopenia. DLT evaluation took place during monotherapy and the first cycle of combination therapy. PF-3512676 activates the immune system, and commonly associated AEs include flu-like symptoms and mild neutropenia believed to be the result of transient migration of neutrophils into peripheral tissues. This is distinct from bone-marrow suppression and may not necessarily be an indication of an increased risk of infection. Therefore, in this study, ≥grade 3 neutropenia was not considered a DLT unless it was accompanied by infection. If, following a DLT, continuation of study was judged to be possible with dose reduction of chemotherapeutic agents, and if study protocol dose-reduction criteria were satisfied, treatment could be continued. The dose of carboplatin could also be reduced to AUC 4.5 mg × min/mL and/or paclitaxel to 150 mg/m^2 if, in the absence of a DLT, patients had specific, predesignated hematologic or non-hematologic adverse events. These dose modifications were based on those reported for the Four-Arm Comparative Study.[19] The planned sample size for dose levels 1 and 3 was three patients each. If one DLT was observed in dose level 1 or 3, three additional patients were to be enrolled. The planned number of patients in dose level 2 in

this study was predefined to be six patients. If >1 DLT was observed in dose levels 1 or 2, the study would not have progressed to the next level. Dose level 2 in this study was the same dose used in preceding clinical studies in Western patients.

Primary endpoints were evaluation of safety and PK of PF-3512676 during the monotherapy and combination therapy phases. Secondary endpoints included evaluation of patient immune function and objective tumor response according to RECIST.

Pretreatment assessment and follow-up studies. History, physical examination (including temperature, blood pressure, heart rate, and weight) ECOG PS, and routine laboratory studies were performed at baseline, before each treatment cycle, and at end of the study. Routine laboratory studies included serum electrolytes, renal and liver function tests, complete blood count and differential white blood cell counts, coagulation studies, and urinalysis. Physical examination and complete blood count were also performed on days 2, 3, and 4 of the monotherapy phase and on days 1, 8, 9, 10, 11, and 15 of the first cycle of combination therapy. After patients completed one cycle of monotherapy and one cycle of combination therapy, these tests were performed on days 1, 8, and 15 of all other cycles of combination therapy. An electrocardiogram was performed at baseline as well as at 3 and 24 h after administration of PF-3512676 monotherapy. Severity of AEs and other symptoms were evaluated according to Common Terminology Criteria for AEs (CTCAE) version 3.0. Relevant radiologic studies to assess measurable and evaluable disease were repeated after every other cycle, and responses were scored according to RECIST.

Pharmacokinetics. To compare the PK of PF-3512676 in the monotherapy phase with its PK during the combination therapy phase, blood samples were collected predose and at 1, 2, 3, 5, 7, 10, 24, 48, 72, and 96 h postdose in the monotherapy phase as well as predose and at 1, 2, 3, 5, 7, 10, 24, 48, 72, and 96 h postdose on day 8 of the first cycle of the combination therapy phase. For each sample, 4 mL of whole blood was collected in a tube containing EDTA-2K dipotassium salt. Collected samples were centrifuged at 1000g for 10 min, and resultant plasma was stored in aliquots at or below −70°C until analysis. Concentrations of PF-3512676 were determined by Pharmaceutical Product Development (Richmond, VA, USA) using a validated hybridization assay with capture and detection probes complementary to either the 3′ or 5′ portions of the molecule. Pharmacokinetic parameters were calculated and summarized using descriptive statistics.

Pharmacodynamics. To evaluate patient immune function, blood samples were collected to measure the serum concentrations of IP-10, IL-6, IFN-α, IL-12p40, monocyte chemotactic protein-1 (MCP-1), and C-reactive protein (CRP). Serum samples were collected predose and at 1, 3, 7, 24, 48, 72, 96, and 168 h postdose of PF-3512676 in the monotherapy phase. During the combination therapy phase, samples were collected on day 8 of the first cycle of combination therapy predose and at 1, 3, 7, 24, 48, 72, 96, and 168 h postdose. For each sample, ≥3 mL of whole blood was collected, stored at room temperature for 30 min, and then centrifuged at 1000g for 10 min. Resultant serum was stored in aliquots at or below −70°C until analysis. Serum levels of IFN-α, IL-12p40, and MCP-1 were determined by the Human Custom Three-Plex Beads Kit (Invitrogen/Biosource, Carlsbad, CA, USA). Multianalyte profiling was performed on the BioPlex® Suspension Array System, and acquired fluorescence data were analyzed by the BioPlex Manager software versions 4.1 (BioRad Laboratories, Hercules, CA, USA). The levels of CRP, IP-10, and IL-6 were determined by ELISA (enzyme-linked immunosorbent assay). C-reactive protein was quantified with the C-reactive Protein (hsCRP) EIA kit (ALPCO Diagnostics, Salem, NH, USA). Interleukin-6 and IP-10 were detected using the Quantikine® HS Human IL-6 Immunoassay kit and Quantikine® Human CXCL10/IP-10 Immunoassay kit (R&D systems, Minneapolis, MN, USA), respectively. The levels of IFN-α, MCP-1, IL12-p40, and CRP were determined at Mitsubishi Chemical Medicine (Tokyo, Japan). The levels of IP-10 and IL-6 were determined at Quest Pharmaceutical Services (Newark, DE, USA).

Results

Patient characteristics. From June 2006 to March 2007, a total of 12 patients were enrolled, and all patients were treated with PF-3512676 monotherapy and at least one cycle of combination therapy. There were seven male and five female patients in this study, and median age was 60 (range, 41–69) years (Table 1). Most patients had stage IV disease (8/12, 67%) and adenocarcinoma (9/12, 75%). Forty-two total cycles of combination therapy were administered, and the median number of combination therapy cycles per patient was four (range, 1–6).

Safety. A list of any-grade AEs with incidence of 30% or more in either the monotherapy phase or the entire study (both monotherapy and combination therapy phases) is presented in Table 2. Many treatment-related AEs observed during the combination therapy phase were likely to be at least in part related to PF-3512676, as they also developed in patients during the monotherapy phase. Treatment-related AEs that occurred in >30% of patients during monotherapy included injection-site reactions (n = 12, 100%), flu-like symptoms (n = 11, 91.7%), lymphocytopenia (n = 6, 50.0%), leukopenia (n = 4, 33.3%), and anemia (n = 4, 33.3%). Neutropenia was also observed (n = 3, 25.0%). Through the entire study period the most common treatment-related AEs of any grade were injection-site reactions, neutropenia, and leukopenia (n = 12, 100% for each); anemia, flu-like symptoms, and lymphocytopenia (n = 11, 91.7% each) were also very common.

Only injection-site reactions and flu-like symptoms occurred with similar frequency in both monotherapy and combination therapy phases, suggesting these AEs were most closely related to treatment with PF-3512676. Certain AEs such as thrombocytopenia, monocytopenia, and malaise that were observed during the combination therapy phase were not observed at all during monotherapy phase, suggesting they were most closely related to chemotherapy.

Seven patients discontinued study therapy; one patient in dose level 1 discontinued as the result of progressive disease, while the remaining six patients (85.7%) discontinued as a result of

Table 1. Characteristics of patients

Enrolled patients, n	12
Age (years), median (range)	60 (41–69)
Gender, n (%)	
Men	7 (58)
Women	5 (42)
Baseline ECOG performance status	
0	7
1	5
Histologic classification of NSCLC, n (%)	
Adenocarcinoma	9 (75)
Squamous cell carcinoma	2 (17)
Other	1 (8)
Clinical stage, n (%)	
IIIB	4 (33)
IV	8 (67)

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small-cell lung cancer.

doi: 10.1111/j.1349-7006.2009.01361.x

Table 2. Treatment-related adverse events occurring in >30% of patients in either the PF-3512676 monotherapy phase or entire study (both monotherapy and combination therapy phases)

Level† (evaluable patients, *n*)	Patients, *n* (%)															
	Entire study (monotherapy phase + combination therapy phase)								Monotherapy phase							
	Level 1 (*n* = 3)		Level 2 (*n* = 6)		Level 3 (*n* = 3)		All levels (*n* = 12)		Level 1 (*n* = 3)		Level 2 (*n* = 6)		Level 3 (*n* = 3)		All levels (*n* = 12)	
	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3	All grades	≥Grade 3
Adverse events, hematologic																
Leukopenia	3	2	6	3	3	2	12 (100)	7 (58.3)	2	0	1	0	1	0	4 (33.3)	0
Neutropenia	3	3	6	5	3	3	12 (100)	11 (91.7)	1	0	1	0	1	0	3 (25.0)	0
Lymphocytopenia	2	2	6	1	3	1	11 (91.7)	4 (33.3)	2	0	3	0	1	1	6 (50.0)	1 (8.3)
Anemia	3	1	5	1	3	1	11 (91.7)	3 (25.0)	1	0	2	0	1	0	4 (33.3)	0
Thrombocytopenia	2	0	2	2	3	0	7 (58.3)	2 (16.7)	0	0	0	0	0	0	0	0
Monocytopenia	1	0	1	0	3	0	5 (41.7)	0	0	0	0	0	0	0	0	0
Adverse events, non-hematologic																
Injection-site reactions	3	0	6	0	3	1	12 (100)	1 (8.3)	3	0	6	0	3	0	12 (100)	0
Flu-like symptoms	2	0	6	0	3	1	11 (91.7)	1 (8.3)	2	0	6	0	3	0	11 (91.7)	0
Anorexia	1	1	4	0	2	1	7 (58.3)	2 (16.7)	0	0	2	0	0	0	2 (16.7)	0
Malaise	2	0	3	0	2	0	7 (58.3)	0	0	0	0	0	0	0	0	0
ALT increased	1	0	3	0	2	0	6 (50.0)	0	0	0	0	0	0	0	0	0
Constipation	0	0	3	0	2	0	5 (41.7)	0	0	0	0	0	1	0	1 (8.3)	0
Diarrhea	1	0	4	0	0	0	5 (41.7)	0	0	0	2	0	0	0	2 (16.7)	0
AST increased	0	0	2	0	2	0	4 (33.3)	0	0	0	0	0	0	0	0	0
Nausea	1	0	3	0	0	0	4 (33.3)	0	0	0	1	0	0	0	1 (8.3)	0

†Level 1: (Mono) PF-3512676 0.1 mg/kg → (Combo) PF-3512676 0.1 mg/kg + carboplatin AUC 6 + paclitaxel 200 mg/m^2; Level 2: (Mono) PF-3512676 0.2 mg/kg → (Combo) PF-3512676 0.2 mg/kg + carboplatin AUC 6 + paclitaxel 200 mg/m^2; Level 3: (Mono) PF-3512676 0.4 mg/kg → (Combo) PF-3512676 0.2 mg/kg + carboplatin AUC 6 + paclitaxel 200 mg/m^2.
ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; Combo, combination therapy; Mono, monotherapy.

AEs or laboratory abnormalities (one patient in dose level 1, three patients in dose level 2, and two patients in dose level 3). All of the discontinuations resulting from AEs or laboratory abnormalities occurred during combination therapy, and the AEs that led to discontinuation varied. The patient in dose level 1 discontinued as a result of grade 2 nausea and grade 2 vomiting that were related to both PF-3512676 and chemotherapy. One patient in dose level 2 discontinued after having multiple hematologic AEs that were related to both PF-3512676 and chemotherapy: grade 4 anemia, and grade 2 neutropenia and leukopenia. Another patient in dose level 2 discontinued after having PF-3512676-related, grade 2 flu-like symptoms (this event was considered unrelated to chemotherapy). The third discontinuation in dose level 2 was the result of grade 3 increase in γ-glutamyltransferase that was considered related to PF-3512676 and chemotherapy and a grade 3 rash considered related to chemotherapy, but not to PF-3512676. One discontinuation in dose level 3 was the result of grade 2 peripheral neuropathy that was considered to be related to paclitaxel. The other was a patient who developed PF-3512676-related grade 3 anorexia and flu-like symptoms (these events were considered unrelated to chemotherapy).

Although all patients reported treatment-related AEs of ≥grade 3, no serious AEs were reported. No DLTs occurred during the monotherapy phase. One patient in level 2 experienced a DLT in the combination therapy phase. This patient developed grade 3 rash and grade 3 increase in γ-glutamyltransferase on days 9 and 10 of the first cycle of combination therapy, respectively. Both events decreased to grade 2 by day 13 of the same cycle and to grade 1 after completion of the DLT observation period. The patient discontinued study therapy as a result of these AEs. No further DLTs were observed. Therefore, the study progressed to the highest planned dose level.

Efficacy. Of 12 patients treated with PF-3512676 and chemotherapy, one patient (8%) achieved a complete response (CR) and three patients (25%) had partial responses (PRs). All objective responses were among patients treated in dose levels 1 and 2. In addition, seven patients (58%) had stable disease (SD).

Pharmacokinetics. The plasma concentration profiles of PF-3512676 were similar in the monotherapy and combination therapy phases (Fig. 1), and overall pharmacokinetic parameters of PF-3512676 were not different with addition of chemotherapy (Table 3). Median time to highest plasma concentration ranged from 2–3 h and mean peak plasma concentration (C_{max}) of PF-3512676 appeared to be dose dependent. Furthermore, mean half-life ($t_{1/2}$) of PF-3512676 varied with dose, ranging from 4.8 to 21.6 h during the monotherapy phase and from 7.9 to 9.5 h in combination therapy phase, with longer $t_{1/2}$ for higher doses of PF-3512676. Based on these PK data, accumulation of PF-3512676 was not observed in this study.

Pharmacodynamics. IFN-α, IL-12p40, IL-6, IP-10, CRP, and MCP-1 were evaluated following treatment with PF-3512676 during both monotherapy and combination therapy phases for all dose levels. For each assayed cytokine or protein, detected levels began to escalate at approximately 3 h postdose, but time to peak concentration varied from approximately 24 to 96 h (Fig. 2). Levels returned to predose concentrations by ~168 h postdose. Pharmacodynamic profiles of the cytokines and proteins during the combination therapy phase were similar to their corresponding profiles in the monotherapy phase, although there was a trend toward lower peak cytokine and protein levels in the combination therapy phase. However, it must be noted that there was considerable variation in individual predose and maximum concentrations. Cytokine and protein profiles of patients who achieved objective responses were not different from those of patients without evidence of antitumor activity.

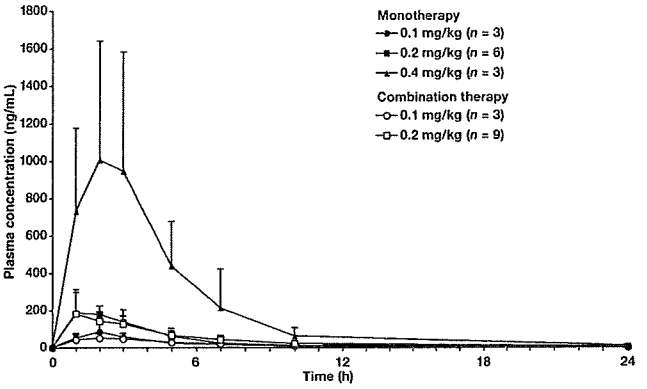

Fig. 1. Pharmacokinetics (PK) of PF-3512676 were similar during monotherapy and combination therapy phases. To compare the PK of PF-3512676 in the monotherapy phase with the PK of the combination therapy phase, blood samples were collected predose and at 1, 2, 3, 5, 7, 10, 24, 48, 72, and 96 h postdose in the monotherapy phase as well as predose and at 1, 2, 3, 5, 7, 10, 24, 48, 72, and 96 h postdose on day 8 in the first cycle of the combination therapy phase. A custom-designed hybridization enzyme-linked immunosorbent assay was used. Mean plasma concentration ± SD of each time point for each group is shown.

Discussion

This phase I study was conducted to examine the safety and PK of PF-3512676 as a single agent and in combination with carboplatin/paclitaxel therapy in Japanese patients with previously untreated NSCLC. Treatment with carboplatin and paclitaxel is a standard approach for patients with advanced NSCLC in Japan.[19] American Society of Clinical Oncology guidelines for treatment of previously untreated stage IV NSCLC recommend combination chemotherapy, but suggest stopping this

Table 3. Pharmacokinetics of PF-3512676

Dose level	*n*	Mean C_{max}, ng/mL (SD)	Mean $AUC_{(0-\infty)}$, ng × h/mL (SD)	Median t_{max}, hours (range)	Mean $t_{1/2}$, hours (SD)
Monotherapy					
0.1 mg/kg	3	90 (36)	376 (73)	2 (2–3)	4.8 (3.4)
0.2 mg/kg	6	217 (90)	856 (127)	2 (1–3)	12.8 (14.0)
0.4 mg/kg	3	1010 (633)	5270 (2450)	2 (2–2)†	21.6 (16.4)
Combination therapy					
0.1 mg/kg	3	55 (19)	379 (55)	3 (2–3)	7.9 (6.2)
0.2 mg/kg	9	226 (124)	1340 (775)	2 (1–3)	9.5 (6.9)

†All patients had reached maximum concentration of PF-3512676 by 2 h postdose. AUC, area under the curve; C_{max}, peak plasma concentration; SD, standard deviation; $t_{½}$, half-life; t_{max}, time to maximum plasma concentration.

doi: 10.1111/j.1349-7006.2009.01361.x

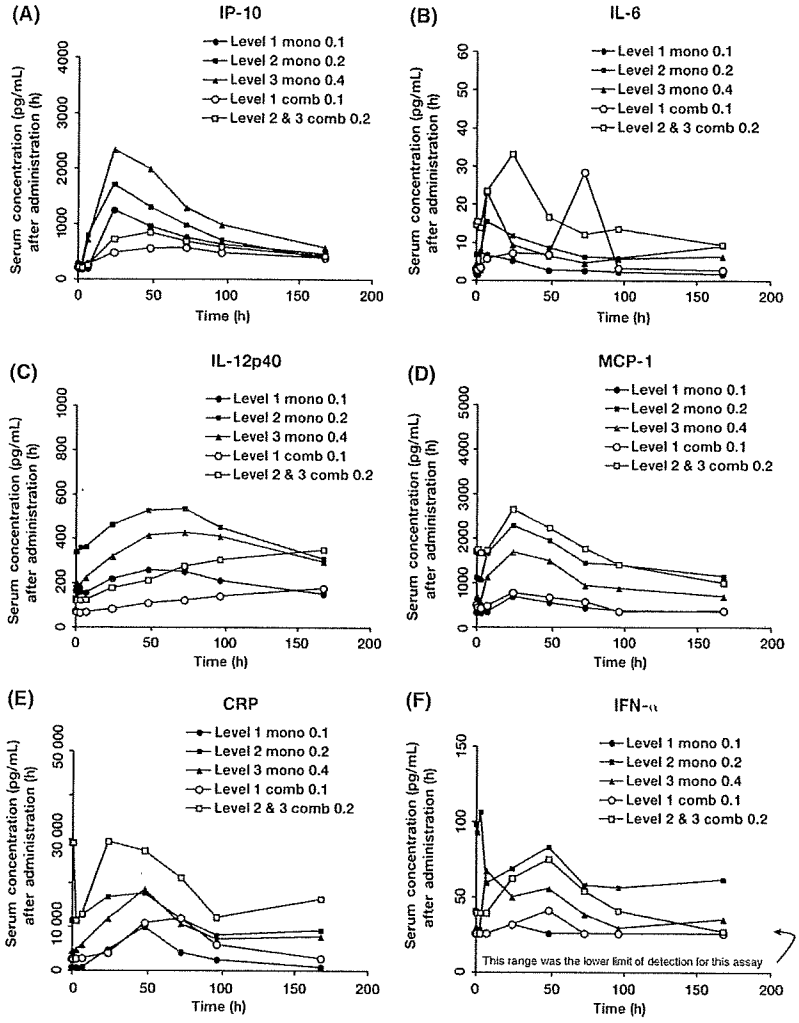

Fig. 2. Pharmacodynamics of cytokines, monocyte chemotactic protein-1 (MCP-1), and C-reactive protein (CRP) were similar during monotherapy and combination therapy phases of the study. The levels of (A) interferon-γ-inducible protein 10 (IP-10), (B) interleukin (IL)-6, (C) IL-12p40, (D) MCP-1, (E) CRP, and (F) interferon-alpha (IFN-α) were evaluated in patient sera at predose (0 h) and at 1, 3, 7, 24, 48, 72, 96, 168 h postdose of PF-3512676 during the monotherapy and combination therapy phases. Sample collection for the combination therapy phase occurred during the first cycle of treatment, and predose was on day 8 before treatment. In each case, the level of the assayed protein began to escalate after 3 h. The time at which highest expression was achieved varied but generally returned to baseline by 168 h postdose. comb, combination therapy; h, hour; mono, monotherapy.

treatment if patients do not respond after three or four cycles.[4] Furthermore, in a recent phase III trial in Japanese patients, the median number of cycles of first-line platinum-based chemotherapy was three.[20] In this phase I study, patients received a median of four cycles of chemotherapy combined with PF-3512676. Therefore, SC delivery of PF-3512676 was considered tolerable either as monotherapy or in combination therapy at the highest doses tested in this study (0.4 mg/kg and 0.2 mg/kg, respectively).

Through the entire study period, the most common treatment-related AEs of any grade were injection-site reactions, neutropenia, leukopenia, anemia, flu-like symptoms, and lymphocytopenia. Injection-site reactions and flu-like symptoms were likely related to treatment with PF-3512676 alone, as they occurred with similar frequency in both the monotherapy and the combination therapy phases. There was no clear dose relationship for these AEs during PF-3512676 monotherapy. Other AEs (eg, leukopenia, neutropenia, lymphocytopenia, anemia, and anorexia) observed during both phases of the study were probably related to treatment with both PF-3512676 and chemotherapy, because they occurred more frequently during the combination therapy phase than the monotherapy phase. There was no indication of cumulative toxicity. These safety results are similar to those from a previous phase II study in Western patients.[17] In that study, the most common AEs related to PF-3512676 and not to chemotherapy were mild to moderate injection-site reactions and flu-like symptoms. Other less common AEs considered related to treatment with PF-3512676 were neutropenia, anemia, and thrombocytopenia.

Across this study, the most frequently occurring AEs of ≥grade 3 were hematologic (e.g. neutropenia, leukopenia, or lymphocytopenia). Hematologic AEs were observed at all dose levels and were qualitatively similar to those reported with carboplatin and paclitaxel doublet chemotherapy.[21] When evaluating safety in studies of doublet chemotherapy, it is important to note that the incidence of ≥grade 3 neutropenia after doublet chemotherapy may be higher in Japanese patients[19] than in Western patients.[5,20,22] Although the small number of patients included in this study precludes a definitive comparison, 11 patients (91.7%) in the present study had ≥grade 3 neutropenia, and this is similar to the frequency reported (84%) in Japanese patients with NSCLC receiving doublet chemotherapy alone.[19]

Because the administration and observation periods were brief in this phase I study, patient blood samples were not analyzed for immunopathological changes that could potentially be indicative of autoimmune events. However, no symptoms suggestive of autoimmune disease were observed. Some patients in other PF-3512676 clinical trials developed positive tests for anti-DNA antibodies. The potential significance of these serologic results is not yet clear.

The PK profiles of PF-3512676 observed during the monotherapy and combination therapy phases were similar. The effect

of PF-3512676 on the PK of carboplatin and paclitaxel was not evaluated in this study. Median time required to achieve maximum plasma concentration (2–3 h) was consistent across all PF-3512676 doses with or without the addition of chemotherapy. The C_{max} increased with the dose administered and was highest in dose level 3 monotherapy in which patients received 0.4 mg/kg PF-3512676. The time required to clear drug from the body appeared to be dose dependent; shortest $t_{1/2}$ (4.8 h) was found in the 0.1 mg/kg dose level monotherapy phase, and longest $t_{1/2}$ (21.6 h) was observed in the 0.4 mg/kg monotherapy phase. However, these data may be confounded by the small number of patients per group and resultant high SD as well as the assay sensitivity level at the lowest dose level. Therefore, it is unclear whether clearance is truly dose dependent. Linearity was also not clearly defined because of the small sample size and the large variation in PK parameters.

The objective response rate (33%) in this study was similar to the rate of confirmed responses (30%) found in the previous phase II study.[17]

Treatment with PF-3512676 alone or in combination with chemotherapy, regardless of dose, modulated several cytokines and other proteins. Immunomodulation was transient, and all increases had dissipated by ~168 h postdose. The most robust responses observed were increases in the levels of IP-10 and IL-6, and this was consistent with the T_H1-like pattern of activation of the innate immune system previously observed in normal human volunteers subcutaneously injected with PF-3512676.[10] IP-10 is a potent chemokine for activated T lymphocytes and regulates cell proliferation, apoptosis, and adhesion molecule expression.[23] Its elevation is indicative of TLR9 activation. There appeared to be a trend toward reduced stimulation of cytokine and chemokine production in the combination therapy phases compared with monotherapy. Although the relevance of this finding is unclear, it should be noted that in this study design, patients who received monotherapy were treatment-naive, while patients who received combination therapy had already received monotherapy with PF-3512676. Increasing the single-agent dose to 0.4 mg/kg seemed to result in a similar pattern of cytokine and chemokine production to that observed with lower doses. Cytokine and chemokine profiles from patients who achieved CR or PR were similar to those from patients without evidence of antitumor activity. However, the small sample size in this study may have confounded these results, and further investigation in future, larger studies would be required for confirmation.

In addition to the present study, PF-3512676 has been investigated in two phase III clinical studies in which combination with platinum-based doublet chemotherapy was compared with platinum-based doublet chemotherapy alone in Western patients with previously untreated advanced NSCLC.[24,25] In those studies, addition of PF-3512676 to doublet chemotherapy did not produce an improvement in overall survival and was associated with increased toxicity. After completion of the study described in this manuscript and based on results from these phase III studies, all clinical trials of PF-3512676 in combination with cytotoxic chemotherapy agents for treatment of NSCLC were discontinued. However, clinical trials in other settings and in combination with targeted or immunotherapeutic agents are ongoing or planned.

In conclusion, PF-3512676 as a single agent and in combination with carboplatin and paclitaxel had an acceptable safety profile in Japanese patients with treatment-naive NSCLC, and PF-3512676 showed evidence of immune activation in the study. It is, therefore, still considered to have potential utility as an anticancer agent.

Acknowledgments

The authors thank Fumiaki Koizumi, MD, PhD; Kazuto Nishio, MD, PhD; and Koji Kono, MD, PhD, for their immunologic advice. The authors also thank Tamara Fink, PhD, ProEd Communications Inc.,[16] for her medical editorial assistance with this manuscript.

Disclosure Statement

Financial support for this study was provided by Pfizer, Inc. Junichi Hashimoto and Emiko Ohki are employed by and hold stock in Pfizer Japan. Yuichiro Ohe receives speaker's bureau honoraria from Pfizer Japan. None of the other authors has a conflict to disclose. Financial support for medical editorial assistance was provided by Pfizer, Inc.

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Drug Metab. Pharmacokinet. 24 (6): 565-574 (2009).

SNP Communication

Genetic Polymorphisms of Copper- and Platinum Drug-efflux Transporters *ATP7A* and *ATP7B* in Japanese Cancer Patients

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

Summary: ATP7A and ATP7B are involved in cellular resistance to platinum compounds such as cisplatin. By sequencing *ATP7A*, 38 genetic variations, including 30 novel ones were detected from 203 Japanese cancer patients. Of these, seven nonsynonymous variations were found: novel 1030A>G (R344G), 2111A>G (Q704R), 2200C>A (Q734K), 2948C>T (T983M) and 3112G>A (V1038I) at 0.004 frequencies and known 2299G>C (V767L) and 4390A>G (I1464V) at 0.351 and 0.075 frequencies, respectively. Regarding *ATP7B*, 28 novel and 33 known genetic variations were detected including 13 nonsynonymous ones: novel 1258A>G (M420V), 1426G>A (A476T), and 2401A>C (T801P) were found at 0.002, 0.005, and 0.002, respectively and known 1216G>T (A406S), 1366G>C (V456L), 2495A>G (K832R), 2785A>G (I929V), 2855G>A (R952K), 2871delC (P957PfsX9), 3419T>C (V1140A), 3836A>G (D1279G), 3886G>A (D1296N) and 3889G>A (V1297I) at 0.483, 0.463, 0.387, 0.005, 0.384, 0.005, 0.387, 0.002, 0.012, and 0.015 frequencies, respectively. Linkage disequilibrium between detected variations was also analyzed. Our results would provide fundamental and useful information for genotyping *ATP7A* and *ATP7B* in the Japanese and probably other Asian populations.

Keywords: ATP7A; ATP7B; genetic variation; amino acid alteration; linkage disequilibrium

Received; August 19, 2009, Accepted; October 15, 2009
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On Aug. 19, 2009, these variations were not found on the homepage of the Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB (http://www.pharmgkb.org/do/) database.
This study was supported in part by the program for the Promotion of Fundamental Studies in Health Sciences from National Institute of Biomedical Innovation, by a Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare in Japan.

Introduction

ATP7A and ATP7B are copper transporters that sequester copper from the cytosol into the trans-Golgi network for loading onto copper-requiring enzymes.[1] ATP7A is expressed in the majority of tissues except for the liver, while ATP7B expression is found mainly in the liver, but also in the kidney and placenta.[1-4] Under elevated copper levels in polarized cells, ATP7A relocates toward the basolateral plasma membranes, while ATP7B travels to the apical side of the membrane to export the metal from the cell. Both proteins are predicted to have 8 transmembrane domains (TMD).[1,4,5] Several functionally important motifs facing the cytoplasm have been found: 6 repeated metal binding motifs (GMxCxxCxxIE) in the N-terminal domain; the transduction motif (TGExxP) in the loop between TMDs 4 and 5; ATP binding (GDGxNDxD) and phosphorylation motifs (DKTGTLT) in the loop between TMDs 6 and 7 and the endocytic signal LL in the C-terminal.[5] Certain mutations in *ATP7A* and *ATP7B* abrogate protein function and cause Menkes and Wilson diseases, respectively.[1-3,5] The *ATP7A* gene located on q13.2-q13.3 of the X chromosome consists of 23 exons spanning approximately 140 kb. The *ATP7B* gene spanning *ca.*79 kb is comprised of 21 exons and located on chromosome 13q14.3. The two transporter proteins share ~65% amino acid sequence similarity.

Recent studies demonstrate that ATP7A and ATP7B are involved in cellular resistance to platinum compounds such as cisplatin.[5,6] Regarding ATP7A, the resistance to cisplatin, carboplatin and oxaliplatin has been observed through sequestration of the drugs into intracellular vesicles in an ATP7A-transfected cell line.[7] Oxaliplatin exposure to HT29 cells enhances ATP7A expression.[8] As for ATP7B, Komatsu *et al.* showed that overexpression of ATP7B conferred cisplatin resistance to a human epidermal carcinoma cell line through ATP-dependent decrease of drug accumulation.[9] Similar resistance to carboplatin due to increased expression of ATP7B has been reported,[10] while oxaliplatin resistance is controversial depending on the cell line used.[11] It has been reported that tumor tissues show higher expression levels of ATP7A[12] and ATP7B[13,14] proteins than corresponding normal tissues and that this higher expression is associated with shorter survival times in cisplatin or carboplatin-based chemotherapy. Higher ATP7B expression levels in tumors are also associated with shorter time to progression in colorectal cancer patients treated with oxaliplatin-based chemotherapy.[15] The polymorphisms of *ATP7A* and *ATP7B* may thus possibly affect the efficacy or toxicity of platinum drugs. In this study, we sequenced the *ATP7A* and *ATP7B* genes of 203 Japanese subjects to survey novel variations of these genes.

Materials and Methods

Human genomic DNA samples: A total of 203 Japanese cancer patients administered paclitaxel/carboplatin (90 non-small cell lung and 6 other cancer patients) or oxaliplatin/5-fluorouracil/leucovorin (107 colorectal cancer patients) participated in this study. The ethical review boards of the National Cancer Center, the Aichi 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 was extracted from blood leukocytes.

PCR conditions for sequencing *ATP7A* The reference sequences (GenBank), NT_011651.17 (genomic) and NM_000052.4 (mRNA) were used for assignment of nucleotide positions and primer design. For sequencing *ATP7A*, two sets of long-range PCRs were made to amplify all 23 exons from 50 ng of genomic DNA using multiple primers (1 μM) and 0.02 units/μl of Z-Taq (Takara Bio Inc., Shiga, Japan). In the first set, 5 pairs of primers amplified the regions from the promoter region to exon 2 and from exons 7 to 18; in the second set, 2 pairs of primers amplified from exons 3 to 6 and from exons 19 to 23. The primers were designed in the promoter or intronic regions as listed in "1st PCR" of **Table 1**. The conditions for the 1st round PCR were 30 cycles of 98°C for 5 sec, 55°C for 10 sec and 72°C for 190 sec. Next, in the 2nd round PCR, the promoter region and exonic regions, except for exon 1, were separately amplified using the 1st PCR products as templates by Ex-Taq (0.02 units/μl, Takara Bio Inc.) with the primers (0.2 μM) listed in "2nd PCR" of **Table 1**. Because of a high GC content, exon 1 was amplified using 0.05 units/μl of LA-Taq (Takara Bio Inc.) in GC buffer I with 0.5 μM of the primers shown in **Table 1**. The 2nd round 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. 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 version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the primers listed in "Sequencing" of **Table 1**. 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 detected rare variations were confirmed by repeating the PCR from the genomic DNA and sequencing newly generated PCR products.

PCR conditions for sequencing *ATP7B*: The following sequences obtained from GenBank were used as reference sequences of *ATP7B*: NT_024524.14 (genomic) and NM_000053.2 (mRNA). First, multiplex long-range PCR was performed to amplify the promoter region and

Table 1. Primers used for sequencing *ATP7A*

		Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified length (bp)
1st PCR	Mix 1	Promoter to Exon 1	GAGCCTCTCCCTCTTTTTACTGTTA	GTGTCAAAGATAAGATGCCACAGGG	1,755
		Exon 2	TCTTGGAAGTCACACCTTGTCGCTT	TAGTGAGACCCCCATCGCTACAAAA	2,373
		Exon 7 to Exon 12	ATTTGTGGTATGCCCTTTGGTCAAT	GCGGTTTCCCCTATGCTGTTGTCAT	8,077
		Exon 13 to Exon 14	TTTTCCTGTCTTTTTCTGAGCCCTC	CACAGTCCAGTTCTGCTTTACCACT	3,073
		Exon 15 to Exon 18	CCTCCTGCCTTAGCCTCCAAAAGTA	GAGAGAGACAAAATGGGCACTTTAT	11,619
	Mix 2	Exon 3 to Exon 6	TAAATCTTCTGACTCCCAACCCAGT	GAGCCACCACACCCAGCCTACATTT	17,069
		Exon 19 to Exon 23	ACGGAGTTTCTCTCTTGTTGCCCAA	AAACCTCACCTTCAAAAGCCTTGCC	11,876
2nd PCR		Promoter	AGAGACTGTAACACTTTTGC	CCACGGGAAAGAGAGCGACT	774
		Exon 1[a]	ACACAGTCTACGGGAAGCAAGTTA	TCACTAAGCAAAGACCCCAGTCCA	1,116
		Exon 2	CAGGAAGAATGCTTACCATA	GTTCAGTATGAGATTCAGAG	615
		Exon 3	CCATTAGATTGAGTTGTCTC	ACCTCAATGATACAGCAAGC	727
		Exon 4	TGATGACAAGAATGAGAGAG	CCACGAGTTATTGTTTCCAG	1,055
		Exon 5	TGCGGAGGAAAGTGTAGAGA	GGTTGTCCCACACATTACTG	509
		Exon 6	GTTTGGGGTCAAGACTGGTA	GCTTGAAGAGTACCATTAGA	488
		Exon 7	AAGAATCACTTGAACCTGGA	CCTTTGCCTAACTTTTCCTG	541
		Exon 8 to Exon 9	GTATTCCCCAGAGTGACTTG	TGAACTCTTTCTTAGGGGTT	825
		Exon 10	TCTCCCTTTAGTGTTTATGG	AGCAAACTGATGTGACAGACTTAG	864
		Exon 11	TTGTGTACTTCGTCTTTCTG	CTGGGAGACAGATTATGTGA	425
		Exon 12	GTTCACTAACAGTAAGCAAG	AGCCACAAAGTAAATCTGAG	461
		Exon 13	GGTTTTTCCAGTTCAAGGTT	GAACTTAGGAGGTCAAGGGT	564
		Exon 14	TTTATAGAAACAGGGTCTCC	TTGACAGTAAATGACAGAGC	709
		Exon 15	TTCTGGAATCTCAGTATGTC	CCTACCTCAAATCTCTGGAT	544
		Exon 16	TCCCGAAGACCATCAGTTTT	AGTCTTTTTAGCCTCATACC	459
		Exon 17	CAAAATCCACTGTCAAGTAG	CATAGGGTATTGACTTGAGG	487
		Exon 18	CACTGTTGGAGGCTATGTTC	GAATAACCCTCATAGTTCAG	376
		Exon 19	AAGTCTGTGTGGGCTTAGAG	AGGAACCAGATAGGACTACT	421
		Exon 20	CCACATCCTTGCTATCACTA	ATGACTTCCCATAATCCCAC	503
		Exon 21	AAAGTGTTTTCAGAACCCTG	CACCATACCAGTAGGCTACA	444
		Exon 22	ATACCCCACAGAAACTCTCA	TAGTAGACATAGGGTTTCAC	576
		Exon 23	ACTAAGTGTGGATGAGCAAA	AAAGATGGGAGGCAGGGAAC	1,134
			GTGCTTTTTTAGATGCTCCA	CTGGTAATGGGAACAAAATG	1,182
			AGTTAGTGTGGTTGGCAAAT	GCAGTATTTTTGATTCCCTC	1,070
			ACAGGAGAAAGAGGTGATTA	GTGCTCTATCTGGTTACTCA	960
Sequencing[b]		Promoter	AGAGACTGTAACACTTTTGC	CCACGGGAAAGAGAGCGACT	
		Exon 1	GGACTCGTACCCTAACAAAG	GTTAGGGGAGGTAAAACATA	
		Exon 4	TGATGACAAGAATGAGAGAG	GAAACTACTATGCTGCTTAC	
			GTAAGCAGCATAGTAGTTTC	CCACGAGTTATTGTTTCCAG	
		Exon 5	GAGGAAAGTGTAGAGATAAC	GAGAACAAAAAAGATGGAGC	
		Exon 7	AAAAAAAGTGGTAACTCAT	GAAGTGTTCAAAGGAGTTAG	
		Exon 8 to Exon 9	GTATTCCCCAGAGTGACTTG	CATTGTGACCATTTCATCCA	
			CTGGATGAAATGGTCACAAT	TGAACTCTTTCTTAGGGGTT	
		Exon 10	TCTCCCTTTAGTGTTTATGG	AGACATACTGTACTATCTAC	
				TATTTCTCATTTGTCTCTCT	
		Exon 14	AAAGTGTTGGGATTACAGGT	CTCTCCCACTCCAAACCTTT	
		Exon 22	TCTACCACCAAGAGGATAAA	ATGGTTTGGGCTTATCATTG	
		Exon 23	ACTAAGTGTGGATGAGCAAA	GCAGCAGTTCAGCAATCTCT	
			GCCCAAGAAGAAGAAAATGA	CAATGAAAAACCACCTAAAC	
			GTGCTTTTTTAGATGCTCCA	CGAAACCCCGTCTCTACTGA	
			TATTTTTCAGTAGAGACGGG	CTGGTAATGGGAACAAAATG	
			AGTTAGTGTGGTTGGCAAAT	CATTGGTCTAAAAAAAGGGC	
			AAGGCAAACCCATTTCACTG	GCAGTATTTTTGATTCCCTC	
			ACAGGAGAAAGAGGTGATTA	ATGACACACCATACATCTTG	
			GTAGTCTCAAGATGTATGGT	GTGCTCTATCTGGTTACTCA	

[a] LA-Taq with GC buffer I was used for amplification because of its high GC content.
[b] Exons not listed were sequenced using 2nd PCR primers.

Table 2. Primers used for sequencing *ATP7B*

	Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified length (bp)
1st PCR	Promoter to Exon 1	GGTAGCATTCCTGGGGTTTTTTCCT	ACCAGGCTCTGAGTAACTTCTCCAG	2,148
	Exon 2 to Exon 4	GTGTGTAAGTGACTCTATGATGGTC	ATGAACAATGTCACCTGTACTCGGA	9,526
	Exon 5 to Exon 9	TCCCACTCCTGATGCTGAACCAATG	CTAACCCCAAGGAAATACAGAAGCC	10,283
	Exon 10 to Exon 16	TCACCAGTATTTCCCCCTTGTCTGT	TGTACTCTGTGCGACACCAGTCTGT	11,551
	Exon 17 to Exon 21	GCTCAGATTCTATCCTGGGCTTTAC	TCGTAAGTGGGAGATGAACAATGAG	8,565
2nd PCR	Promoter to Exon 1[a]	GCCTTCCAGCCAATAGAATA	TTTCTCCCACGCCAAGACAT	1,145
	Exon 2	GTTGTGTGAGAACGACATTT	AGAAGGCTCTCACCAGATGT	1,825
	Exon 3	GAGGGACAAGGTAGTTACTG	AATGCCAGTTATACAAGGAC	573
	Exon 4	GAGACCAGACATCGTGATTG	CATTGTTGTCGGCTTCAAAG	517
	Exon 5	AGGGAAAGGCTCTTGGCTGC	CTTTCTCTTACCCATTCACT	480
	Exon 6	GAGGCACTTTTAGATTCACT	GAGGGTTCACATTACAAGGG	334
	Exon 7	ATGTGACAAAGGCAGGTCTT	GCCCTTAGTAGTCCCCCACA	496
	Exon 8	CATAAACGCCCATCACAGAG	TAAGTCTGTCTCTATGCTGT	492
	Exon 9	AGAGCCTTTTATCGTGCCGT	TGCCCACACTCACAAGGTCT	335
	Exon 10 to Exon 12	AACAGTGCCTGGTATTCAGC	GGCTTAGATTTTGCTGTCAA	1,061
	Exon 13	ATGGCAGAGCAGTGTGGAAT	TCAGGCTTTTCTCTCAATGT	428
	Exon 14	AACCCTGAGATTGAACGACA	CTTTGTGATAACCTGGAACT	532
	Exon 15	AGTTCCCGCTTTCCGCTGCT	CCCAAGAACATAAGAGAAAC	458
	Exon 16	AGAGGTGCTTACAAGGTTAC	ACAATCTTCTGGAAAACAGG	419
	Exon 17	TGCTTCCAGACTTTTGTGTA	AGAGAAAAGCATCCAGCAAG	460
	Exon 18 to Exon 19	CAACATCACTGACTGGACCC	AAACAGCCTTTCTAAAACGC	644
	Exon 20	TGGGAACATCAGGGCGAGTGGAA	TTGAGGAGCAGAGTAAGGGC	574
	Exon 21	CTCTTGAGGTTTTGATACTG	AGCAAAGACCACAAGGACAT	1,010
		TGTGCTTGTCAGTGGGGACC	AGTGAAACTAACCATCCAAG	1,162
		GCACTTGATTCAGGAGGTCA	ATCCTCCTCTGCCCCCTAAA	550
Sequencing[b]	Promoter to Exon 1	GCCTTCCAGCCAATAGAATA	TGAGAGCGTGAGGGGAGAGT	
		ACTCTCCCCTCACGCTCTCA	TTTCTCCCACGCCAAGACAT	
	Exon 2	GTTGTGTGAGAACGACATTT	GGACCTTGCCTTCAATGGAG	
		TGCCATCGGTTGTGTGCCTG	ACTGGGCTGGTACAAGAAGG	
		CTTGGAGAACAAAACTGCCC	AGAAGGCTCTCACCAGATGT	
	Exon 10 to Exon 12	AACAGTGCCTGGTATTCAGC	CCCAGAACTCTTCACATAAT	
		TAACTTCATCTTTCTCGTTTTAG	GGCTTAGATTTTGCTGTCAA	
	Exon 20	TCAGGGCGAGTGGAAGAGAG	GTGAATGGGCAGCAGTGAAT	
	Exon 21	TAGAATGGCTCAGATGCTGT	GGGCAGGATGACTGGACATA	
		TATGTCCAGTCATCCTGCCC	AGCAAAGACCACAAGGACAT	
		TGTGCTTGTCAGTGGGGACC	CTCCTTTTCTGAAGCCCCTG	
		TGTGTGGCTTGGAGGAAATG	AGTGAAACTAACCATCCAAG	
		GCACTTGATTCAGGAGGTCA	ATCCTCCTCTGCCCCCTAAA	

[a] LA-Taq with GC buffer I was used for amplification because of its high GC content.
[b] Exons not listed were sequenced using 2nd PCR primers.

all 21 exons of *ATP7B* from 50 ng of genomic DNA with 0.025 units/μl of Z-Taq and five sets of primers (in "1st PCR" of **Table 2**, 1 μM) designed in the promoter or intronic regions. The 1st round PCR conditions were 30 cycles of 98°C for 5 sec, 55°C for 10 sec, and 72°C for 190 sec. Next, exonic regions, except for promoter to exon 1 region, were amplified separately in the 2nd round PCR using the 1st PCR products as templates by Ex-Taq (0.02 units/μl) with the primers (0.2 μM) listed in "2nd PCR" of **Table 2**. Because of its high GC content the promoter to exon 1 region was amplified using 0.05 units/μl of LA-Taq in GC buffer I with 0.5 μM of the primers listed in **Table 2**. The 2nd round PCR conditions, purification of the PCR products and sequencing with the primers listed in "Sequencing" of **Table 2** were performed as described in the above *ATP7A* section. All rare variations were confirmed by repeating PCR from the genomic DNA and sequencing newly generated PCR

Table 3. Summary of *ATP7A* variations detected in this study

SNP ID		Location	Position		Nucleotide change	Amino acid change	Frequency	
This Study	dbSNP (NCBI)		NT_011651.17	From the translational initiation site or from the end of the nearest exon				95% Confidence interval
MPJ6_A7A001[a]		5′-Flanking	462076_462077	−61371_−61370 (−586_−585)[b]	TTACATCTTGGC/ins 98bp/AGTTAACACAGT		0.004	0.000–0.010
MPJ6_A7A002	rs17174131	5′-Flanking	462154	−61293 (−508)[b]	GACTTATAAGGAT>GCTTTTATGTTAC		0.086	0.059–0.113
MPJ6_A7A003[a]		5′-Flanking	462472	−60975 (−190)[b]	GCCGCCGCGGCGG>TGGGGTGGGAAAA		0.004	0.000–0.010
MPJ6_A7A004[a]		5′-UTR, Exon 1	462520	−60927 (−142)[b]	GCTGCCGCCGCCG>ACAGCCGCAGCTA		0.004	0.000–0.010
MPJ6_A7A005[a]		Intron 2	523760	IVS2+194	GATATATTTTCAA>GTTTAAAAACATC		0.183	0.145–0.220
MPJ6_A7A006[a]		Intron 2	523829	IVS2+263	TATTTTATAAGTA>GTATGAGTATTTA		0.004	0.000–0.010
MPJ6_A7A007[a]		Intron 3	541000	IVS3−37	AAGTAGCCCAGGA>GATAACTGAATTA		0.004	0.000–0.010
MPJ6_A7A008[a]		Exon 4	541456	1030[c]	CCGGGGCTATATA>GGAGTTAGTATCA	Arg344Gly	0.004	0.000–0.010
MPJ6_A7A009[a]		Intron 4	541816	IVS4+54	CTTCCATTTTGCT>CGCTTCTTTTGGC		0.037	0.019–0.056
MPJ6_A7A010[a]		Intron 5	550575_550576	IVS5+86_87	TGTAACTATGTT/insT/ATGATTCTTGGT		0.343	0.297–0.389
MPJ6_A7A011[a]		Exon 9	563418	2111[c]	TCCTGGAGCGCCA>GGATTCTTCCAGG	Gln704Arg	0.004	0.000–0.010
MPJ6_A7A012[a]		Intron 9	563491_563492	IVS9+12_+13	GCAAGTGAATTG/insAATTG/CAAATATATTTG		0.019	0.005–0.032
MPJ6_A7A013[a]		Exon 10	564711	2200[c]	TACTTCTACATTC>AAGGCTTATAAAG	Gln734Lys	0.004	0.000–0.010
MPJ6_A7A014	rs2227291	Exon 10	564810	2299[c]	ATTATTCTTCTAG>CTTGCAATGTATG	Val767Leu	0.351	0.304–0.397
MPJ6_A7A015[a]		Intron 10	565122	IVS10+205	ATAGTACAGTATG>ATCTGTTTATTTT		0.004	0.000–0.010
MPJ6_A7A016	rs5959964	Intron 10	566283	IVS10−184	AAACATTTTCTAG>TTGAAACATTTTG		0.295	0.250–0.339
MPJ6_A7A017	rs7053543	Intron 13	572344	IVS13+141	TTTTGAGATAGGG>ATCTCACTCTGTT		0.351	0.304–0.397
MPJ6_A7A018[a]		Intron 13	572721	IVS13−29	ATGCTTCTTCTTC>ATTATTATTGTTG		0.351	0.304–0.397
MPJ6_A7A019[a]		Exon 15	581086	2948[c]	CCCGAACAGAAAC>TGATAATACGATT	Thr983Met	0.004	0.000–0.010
MPJ6_A7A020[a]		Exon 16	583206	3112[c]	ATTTTTTTACAGG>ATAAAGGTAGTGG	Val1038Ile	0.004	0.000–0.010
MPJ6_A7A021[a]		Intron 18	590825	IVS18+37	TAACTCAATGTTT>GTGTTATTGTTTT		0.004	0.000–0.010
MPJ6_A7A022[a]		Intron 21	597158	IVS21−117	AATCTCTACCAC/delC/AAGAGGATAAAT		0.004	0.000–0.010
MPJ6_A7A023	rs2234938	Exon 23	598262	4390[c]	AGCAGAGCCTCTA>GTAAACTCACTAC	Ile1464Val	0.075	0.049–0.100
MPJ6_A7A024[a]		3′-UTR	598480	4608[c] (*105)[d]	TTTTCTCATGCTC>TTTATATTAGGGA		0.004	0.000–0.010
MPJ6_A7A025[a]		3′-UTR	598705	4833[c] (*330)[d]	CAAAAAAAAAAAG>CGCCCAAGAAGAA		0.004	0.000–0.010
MPJ6_A7A026[a]		3′-UTR	598947	5075[c] (*572)[d]	CTGCATCCTTGTC>TCTTGCAGGTGCT		0.004	0.000–0.010
MPJ6_A7A027[a]		3′-UTR	599056	5184[c] (*681)[d]	CTGACAACTGTTC>GTAATATTTTGCT		0.004	0.000–0.010
MPJ6_A7A028[a]		3′-UTR	599309	5437[c] (*934)[d]	CAAAGATTAAAAC>TTATTATACATAT		0.056	0.034–0.078
MPJ6_A7A029[a]		3′-UTR	599390_599392	5518_5520[c] (*1015_*1017)[d]	TTGTTGTTGTTG/delTTG/AGACAGAGTCTT		0.011	0.001–0.021
MPJ6_A7A030[a]		3′-UTR	599466	5594[c] (*1091)[d]	ACCTCTGCCTACC>TGGATTCAAGGAA		0.004	0.000–0.010
MPJ6_A7A031[a]		3′-UTR	599855	5983[c] (*1480)[d]	ACTAAAATTTCCC>TTAGGTTATGACG		0.343	0.297–0.389
MPJ6_A7A032	rs1062471	3′-UTR	600286	6414[c] (*1911)[d]	GTAGGGGATGGAG>CTTCTTCCTTTCC		0.325	0.279–0.370
MPJ6_A7A033	rs1062472	3′-UTR	600335	6463[c] (*1960)[d]	CATATATACACAT>CGCAAAGTTTACA		0.422	0.374–0.470
MPJ6_A7A034[a]		3′-UTR	600567	6695[c] (*2192)[d]	TATTTATTATTTT>AAATTCCAGTGGC		0.004	0.000–0.010
MPJ6_A7A035	rs17139614	3′-UTR	600616	6744[c] (*2241)[d]	TTCTAGAAGACAG>CAGCTGATAGGGT		0.078	0.052–0.104
MPJ6_A7A036[a]		3′-UTR	600837	6965[c] (*2462)[d]	ACAGAAAACATGC>ATAATTAGAAAAA		0.004	0.000–0.010
MPJ6_A7A037[a]		3′-UTR	600904	7032[c] (*2529)[d]	CACAAGTCTTTTT>CTGCAATCTTGAA		0.004	0.000–0.010
MPJ6_A7A038[a]		3′-UTR	601497	7625[c] (*3122)[d]	TTTTTTAAAAAGT>CATTCTTTATTCA		0.004	0.000–0.010

[a] Novel variations detected in this study.
[b] Positions in parenthesis are calculated by skipping the intron 1.
[c] Positions in cDNA (NM_000052.4).
[d] Numbered from termination codon TAA.

products.

Linkage disequilibrium (LD) analysis: Hardy-Weinberg equilibrium and LD analysis were performed by SNPAlyze software (Dynacom Co., Chiba, Japan) and pairwise LD between variations with minor allele frequency (MAF) greater than 0.03 was analyzed using r^2 values.

Results and Discussion

For *ATP7A*, the 5′-flanking region (up to 872 bases upstream of exon 1), all 23 exons and their flanking introns were sequenced for 203 Japanese subjects. Thirty-eight genetic variations, including 30 novel ones were detected (see **Table 3**): 3 were in the 5′-flanking region, 1 in the 5′-untranslated region (UTR), 7 in the coding exons (7 nonsynonymous variations), 12 in the introns and 15 in the 3′-UTR. Since we did not find any significant differences in the frequencies of these variations between the 96 patients with carboplatin- and 107 patients with oxaliplatin-based chemotherapies (by Fisher's exact test, $P > 0.13$), the data for all subjects were analyzed as one group. Since this gene resides on the X-chromosome, allele frequencies were also compared between 138 males and 65 females and no significant differences were found (by Fisher's exact test, $P > 0.24$). In the female patients (with two X chromosomes), detected variations were in Hardy-Weinberg equilibrium ($P \geqq 0.10$). Five novel nonsynonymous variations, 1030A>G (R344G), 2111A>G (Q704R), 2200C>A (Q734K), 2948C>T (T983M) and 3112G>A (V1038I), were found as heterozygotes in single patients at 0.004 frequencies (**Table 3**). Among these, Q734 is presumed to be the first amino acid following TMD2 and is conserved between ATP7A and ATP7B.[4] Using the PolyPhen program (http://genetics.bwh.harvard.edu/pph/) to predict functional effects of amino acid substitutions, Q734K was expected to probably alter the protein function based on the PSIC (position specific independent count) profile score differences derived from multiple alignments. R344G and Q704R substitutions were predicted to have possible functional alterations. The effects of T983M and V1038I were predicted as benign. Functional analysis for these variations is warranted. Moreover, it is necessary to evaluate real frequencies of very rare variations found in only one subject (frequency: 0.004). We also detected the previously published variations 2299G>C (V767L) and 4390A>G (I1464V) at 0.351 and 0.075 frequencies, respectively.

Regarding *ATP7B*, 61 genetic variations including 28 novel ones, were detected by sequencing the 5′-flanking regions (up to 768 bases upstream of exon 1), all 21 exons and their flanking introns of 203 Japanese subjects: 9 were in the 5′-flanking region, 2 in the 5′-UTR, 19 in the coding exons (13 nonsynonymous and 6 synonymous ones), 25 in the introns, 5 in the 3′-UTR and 1 in the 3′-flanking region (see **Table 4**). Just as with *ATP7A*, no significant differences were found in the frequencies of these variations between patients with carboplatin- and patients with oxaliplatin-based chemotherapies (by Fisher's exact test, $P > 0.20$) and the data for all subjects were analyzed as one group. Detected variations were in Hardy-Weinberg equilibrium ($P > 0.05$), except for −408T>C and IVS13−129C>T. The deviations were probably caused by an unexpected occurrence of one extra homozygote in these low-frequency variations. Three novel nonsynonymous variations, 1258A>G (M420V), 1426G>A (A476T) and 2401A>C (T801P) were found at 0.002, 0.005 and 0.002, respectively. The PolyPhen program predicted that M420V and T801P, located within conserved regions between ATP7A and ATP7B, probably had damaging effects on protein function. Functional analysis should be conducted for these variations. Moreover, it is necessary to evaluate real frequencies of very rare variations found in only one subject (frequency: 0.002). We also detected 10 known nonsynonymous variations, 1216G>T (A406S), 1366G>C (V456L), 2495A>G (K832R), 2785A>G (I929V), 2855G>A (R952K), 2871delC (P957PfsX9), 3419T>C (V1140A), 3836A>G (D1279G), 3886G>A (D1296N) and 3889G>A (V1297I) at 0.483, 0.463, 0.387, 0.005, 0.384, 0.005, 0.387, 0.002, 0.012 and 0.015 frequencies, respectively. Of these, 2871delC (P957PfsX9), the most frequent causative variation for Wilson disease in Japanese,[16] causes a frame-shift downstream of codon 957, resulting in an early stop codon at codon 966. This variation most probably results in a non-functional protein without 34% of the protein at the C-terminus, including TMDs 6–8 and the large cytoplasmic loop containing the ATP binding site.[3] Compared to Chinese healthy individuals, MAFs in this study are lower for V456L (0.463 in Japanese *vs.* 0.609 in Chinese) and comparable for K832R and V1140A (0.387 *vs.* 0.42 for both variations), respectively.[17] Functional changes were not observed for K832R, I929V and R952K when assessed by growth of recombinant yeast in the presence of copper cations.[18]. Known variations −119_−118insCGCCG and −75A>C were detected at 0.488 and 0.468 frequencies, these values being higher than those in Chinese volunteers (0.218 for −119_−118insCGCCG and 0.372 for −75A>C).[17]

Using the detected variations at >0.03 frequencies, linkage disequilibrium (LD) was analyzed. For *ATP7A*, using 14 variations, strong linkages ($r^2 > 0.8$) were observed between −61293T>G and 6744 (*2241) G>C, and among IVS5+86_87insT, 2299G>C (V767L), IVS13+141G>A, IVS13−29C>A, and 5983 (*1480)C>T.

As for the 22 common variations (MAF>0.03) of *ATP7B*, strong linkages ($r^2 > 0.8$) were observed among −520C>T, −119_−118insCGCCG and −75A>C; between 1216G>T (A406S) and IVS2+287A>G;

Table 4. Summary of *ATP7B* variations detected in this study

SNP ID		Reference	Location	Position		Nucleotide change	Amino acid change	Frequency	
This Study	dbSNP (NCBI)			NT_024524.14	From the translational initiation site or from the end of the nearest exon				95% Confidence interval
MPJ6_A7B001[a]			5′-Flanking	33566377	−904	GTAGACTAGTGTT>ACGGCGTGGCGCA		0.005	0.000–0.012
MPJ6_A7B002[a]			5′-Flanking	33566130	−657	TCTTGCCGCGGT/delT/GCTTCCTTTGGG		0.002	0.000–0.007
MPJ6_A7B003	rs28362533		5′-Flanking	33566061	−588	AGCGCAGAGCGGA>CCCCGACGCGGCG		0.017	0.005–0.030
MPJ6_A7B004[a]			5′-Flanking	33566055	−582	GAGCGGACCCGAC>TGCGGCGCCGCCG		0.005	0.000–0.012
MPJ6_A7B005	rs9563084		5′-Flanking	33565993	−520	CTGAGTCTGCGGC>TCCGGCTCTGCGC		0.488	0.439–0.536
MPJ6_A7B006	rs28362532		5′-Flanking	33565881	−408	GGAGGACAGGCCT>CCCGCCCTGCGGC		0.039	0.020–0.058
MPJ6_A7B007[a]			5′-Flanking	33565841	−368	GACATTGTGGCAC>GTGGCACGGCAGA		0.002	0.000–0.007
MPJ6_A7B008[a]			5′-Flanking	33565835	−362	GTGGCACTGGCAC>GGGCAGAGAACAC		0.002	0.000–0.007
MPJ6_A7B009[a]			5′-Flanking	33565751	−278	GCGAGGGTCCGAG>TGCCCACTCTCCC		0.002	0.000–0.007
MPJ6_A7B010	rs28362531	19)	5′-UTR	33565592_33565591	−119_−118	CGAGCCGCGCCG/insCGCCG/ATGCCCTCACAC		0.488	0.439–0.536
MPJ6_A7B011	rs2277448	19)	5′-UTR	33565548	−75	GACTTTAACACCA>CCGCTCTCCTCCA		0.468	0.419–0.517
MPJ6_A7B012[a]			Exon 2	33528876	480[b]	CTGTGTCAGCTCC>AATTGAAGGCAAG	Ser160Ser	0.002	0.000–0.007
MPJ6_A7B013		16)	Exon 2	33528234	1122[b]	TGCATCCTGTGTC>GCATTCCATTGAA	Val374Val	0.002	0.000–0.007
MPJ6_A7B014	rs1801243	19)	Exon 2	33528140	1216[b]	CTTTATAATCCCG>TCTGTAATTAGCC	Ala406Ser	0.483	0.434–0.532
MPJ6_A7B015[a]			Exon 2	33528098	1258[b]	GCTATAGAAGACA>GTGGGATTTGAGG	Met420Val	0.002	0.000–0.007
MPJ6_A7B016	rs1951922		Intron 2	33527784	IVS2+287	GATATGGAATTTA>GTTTCTTATAGTT		0.483	0.434–0.532
MPJ6_A7B017	rs3742288		Intron 2	33524978	IVS2−93	GGGAGCCGGGACA>CATGAACCCTCAC		0.463	0.415–0.512
MPJ6_A7B018	rs1801244	19)	Exon 3	33524805	1366[b]	ACACCTACATCTG>CTGCAGGAAGTGG	Val456Leu	0.463	0.415–0.512
MPJ6_A7B019[a]			Exon 3	33524745	1426[b]	CCGGACATCTTGG>ACAAAGTCCCCAC	Ala476Thr	0.005	0.000–0.012
MPJ6_A7B020[a]			Intron 3	33524588	IVS3+40	TAGGAATGCTGCG>ATATAGACCTCGT		0.002	0.000–0.007
MPJ6_A7B021[a]			Intron 3	33522913	IVS3−170	ATCGTGATTGTCG>AAAGGCTTTCCAA		0.025	0.010–0.040
MPJ6_A7B022	rs2147363		Intron 3	33522796	IVS3−53	TTGACTGTGTCAA>CCCTAGAGGCCCT		0.463	0.415–0.512
MPJ6_A7B023	rs9535809		Intron 5	33516114	IVS5−65	AAAGTGCTTTCTG>ACCAATGCATATT		0.037	0.019–0.055
MPJ6_A7B024[a]			Exon 6	33516023	1896[b]	TGCTTCCCTGGCC>ACAGAGAAACCCC	Ala632Ala	0.002	0.000–0.007
MPJ6_A7B025[a]			Intron 6	33515876	IVS6+97	TTCCCATGGTGCC>TTTCCTCCTGGAT		0.002	0.000–0.007
MPJ6_A7B026[a]			Intron 6	33514462	IVS6−4	TGCATTTGCTTTC>TCAGGTGGAAGAA		0.020	0.006–0.033
MPJ6_A7B027[a]			Exon 9	33511698	2401[b]	TCTCTCCAAGCCA>CCAGAAGCCACCG	Thr801Pro	0.002	0.000–0.007
MPJ6_A7B028[a]			Intron 9	33511612	IVS9+40	TGGTTGGTATCTA>GTCAATCTGTGTG		0.005	0.000–0.012
MPJ6_A7B029	rs9526811		Intron 9	33504560	IVS9−25	GAGTGGCCATGTG>AAGTGATAAGTGG		0.350	0.303–0.396
MPJ6_A7B030	rs1061472	19)	Exon 10	33504488	2495[b]	GCGATATCGTCAA>GGGTGGTCCCTGG	Lys832Arg	0.387	0.339–0.434
MPJ6_A7B031	rs2281814		Intron 10	33504327	IVS10−30	ATGGGGCTGAGCA>GAGTGACAGTTGT		0.010	0.000–0.019
MPJ6_A7B032		18)	Exon 12	33503878	2785[b]	GTCCCATTTATCA>GTCATCATGTCAA	Ile929Val	0.005	0.000–0.012
MPJ6_A7B033	rs732774	19)	Exon 12	33503808	2855[b]	GTGTTGTTCAGAG>AATACTTTCCTGT	Arg952Lys	0.384	0.337–0.431
MPJ6_A7B034	rs2296246		Intron 12	33500704	IVS12−90	ACGTTGTGTCCAG>TTGCCCCCCTGAA		0.345	0.299–0.391
MPJ6_A7B035	rs7325983		Intron 12	33500627	IVS12−13	GCCTCTGACTCTG>CTCCTGTTTTCAG		0.030	0.013–0.046

Table 4. (Continued)

SNP ID		Reference	Location	Position		Nucleotide change	Amino acid change	Frequency	
This Study	dbSNP (NCBI)			NT_024524.14	From the translational initiation site or from the end of the nearest exon				95% Confidence interval
MPJ6_A7B036		16)	Exon 13	33500609	2871[b]	TTTTCAGAACCC/ delC /AACAAGCACATC	Pro957ProfsX9	0.005	0.000–0.012
MPJ6_A7B037	rs1801247	19)	Exon 13	33500471	3009[b]	CGGGGTGGCCGC G>A CAGAACGGCATC	Ala1003Ala	0.007	0.000–0.016
MPJ6_A7B038	rs17076121		Intron 13	33498556	IVS13–129	GACAGAGGATCA C>T GTTAGGAAGCTG		0.017	0.005–0.030
MPJ6_A7B039	rs17076116		Intron 14	33498207	IVS14+38	CCCTCCCCGCCA A>G TGCTCTTTTATT		0.002	0.000–0.007
MPJ6_A7B040[a]			Intron 14	33498125	IVS14+120	AAAACCACTTAG A>G GGGCCCTTCTGC		0.005	0.000–0.012
MPJ6_A7B041[a]			Intron 14	33498080	IVS14+165	TCACAGTCAGCC/ delC /TTGCCACAGTTC		0.007	0.000–0.016
MPJ6_A7B042[a]			Intron 15	33496515	IVS15+7	AAAAAGGTATTG C>T TGGCTTTTGTCT		0.002	0.000–0.007
MPJ6_A7B043	rs1801249	19)	Exon 16	33495354	3419[b]	GAATAGATGCAG T>C CCCCCAGACCTT	Val1140Ala	0.387	0.339–0.434
MPJ6_A7B044[a]			Intron 16	33495135	IVS16+82	GTCCTCCTTTAT A>G AAAGAAAAGAAG		0.002	0.000–0.007
MPJ6_A7B045[a]			Exon 17	33493319	3567[b]	AGGTGTGCTCTG T>C GGGATGATCGCA	Cys1189Cys	0.002	0.000–0.007
MPJ6_A7B046		21)	Exon 18	33491679	3836[b]	TGGCCCAGGCAG A>G CATGGGTGTGGC	Asp1279Gly	0.002	0.000–0.007
MPJ6_A7B047		22)	Exon 18	33491629	3886[b]	ATCGAGGCAGCC G>A ACGTCGTCCTTA	Asp1296Asn	0.012	0.002–0.023
MPJ6_A7B048		20)	Exon 18	33491626	3889[b]	GAGGCAGCCGAC G>A TCGTCCTTATCA	Val1297Ile	0.015	0.003–0.027
MPJ6_A7B049	rs2282057	19)	Intron 18	33491606	IVS18+6	TATCAGAGTGAG C>T GTGGCTGCAGCC		0.397	0.349–0.444
MPJ6_A7B050[a]			Exon 19	33491443	3990[b]	CCTGGCACTGAT T>C TATAACCTGGTT	Ile1330Ile	0.002	0.000–0.007
MPJ6_A7B051	rs9535795		Intron 19	33491362	IVS19+50	AGAAAGGCTTCT G>C TCTCCCAGGTTC		0.394	0.347–0.442
MPJ6_A7B052[a]			Intron 19	33490036	IVS19–205	GAGAGCCAGGCC C>T ACTCAACAGCAT		0.007	0.000–0.016
MPJ6_A7B053	rs2282059		Intron 19	33489990	IVS19–159	AGCCTCACTTTG G>C GGGGGGCCTGTG		0.037	0.019–0.055
MPJ6_A7B054[a]			Intron 19	33489990	IVS19–159	AGCCTCACTTTG G>T GGGGGGCCTGTG		0.002	0.000–0.007
MPJ6_A7B055[a]			Intron 20	33489547	IVS20+182	CATGAGCAGGCA A>G TTCACTGCTGCC		0.002	0.000–0.007
MPJ6_A7B056[a]			3′-UTR	33487929	5361b (*963)[c]	AGCCTCCCTGCA C>T GGCCCAAGGGGC		0.005	0.000–0.012
MPJ6_A7B057[a]			3′-UTR	33487764	5526b (*1128)[c]	ACGCTGCCCAGG G>A GCTTCAGAAAAG		0.002	0.000–0.007
MPJ6_A7B058	rs1051332		3′-UTR	33487720	5570b (*1172)[c]	AAGGGAGCATCT G>A TTTACCTGGCAG		0.350	0.303–0.396
MPJ6_A7B059[a]			3′-UTR	33487483	5807b (*1409)[c]	CAACCAACCAGC A>C GGGTAGCTATTA		0.007	0.000–0.016
MPJ6_A7B060	rs928169		3′-UTR	33487110	6180b (*1782)[c]	TTTCAGCCCCCC C>G ACTCCAGCCCGC		0.384	0.337–0.432
MPJ6_A7B061	rs9535793		3′-Flanking	33486762	6485+43[d] (*2087+43)[c]	GCCAGTGCCGTC T>C TGTCTTCACGAG		0.384	0.337–0.432

[a] Novel variations detected in this study.

[b] Positions in cDNA (NM_000053.2).

[c] Positions are shown as * and bases from the translational termination codon TGA.

[d] Positions are shown as 6485 (*2087) (final base of exon 21) + bases from the end of exon 21.

among IVS2−93A>C, 1366G>C (V456L) and IVS3−53A>C; between IVS5−65G>A and IVS19−159G>C; and among IVS9−25G>A, 2495A>G (K832R), 2855G>A (R952K), IVS12−90G>T, 3419T>C (V1140A), IVS18+6C>T, IVS19+50G>C, 5570 (*1172)G>A, 6180 (*1782)C>G and 6485+43 (*2087+43)T>C.

We analyzed colorectal and mostly non-small cell lung cancer patients treated with oxaliplatin/5-fluorouracil/leucovorin and paclitaxel/carboplatin, respectively. In these tissues in normal, ATP7A but not ATP7B is reported to be expressed mainly. However, ATP7B levels are up-regulated in colorectal and lung cancer tissues with varying degrees.[15,23] In addition to *ATP7A* polymorphisms, some *ATP7B* polymorphisms found in the promoter region may affect the expression levels of ATP7B in the tumor tissues to thus possibly influence the efficacy of oxaliplatin and carboplatin treatment by changing the drug concentrations within tumor cells. As for adverse effects of these platinum drugs, bone marrow toxicities and neuropathies (especially in oxaliplatin-administered patients) were frequently observed in our patients. Since ATP7A is expressed in the majority of normal tissues except for liver, the detected polymorphisms in the *ATP7A* possibly influence the onset of these toxicities. We are planning to conduct association analysis between the polymorphisms of both genes and efficacy and adverse reactions caused by these drugs after increase in patient number.

In conclusion, 38 and 61 genetic variations, including 30 and 28 novel ones, were detected in *ATP7A* and *ATP7B*, respectively, in a Japanese population. Our results would provide fundamental and useful information for genotyping the platinum drug transporters *ATP7A* and *ATP7B* in the Japanese and probably other Asian populations.

Acknowledgments: Hiromi Fukushima-Uesaka and Yoshiro Saito both contributed equally to this work. We thank Chie Sudo for secretarial assistance.

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Carcinogenesis Advance Access published January 8, 2010

Individuals susceptible to lung adenocarcinoma defined by combined *HLA-DQA1* and *TERT* genotypes

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Keywords: lung adenocarcinoma, SNP, GWAS, susceptibility, HLA-DQA1

Adenocarcinoma (ADC) is the commonest histological type of lung cancer, and its weak association with smoking indicates the necessity to identify high risk individuals for targeted screening and/or prevention. By a genome-wide association study (GWAS), we identified an association of polymorphisms in the 6p21.31 locus containing four HLA (human leukocyte antigen)-class II genes with lung ADC risk. DQA1*03 of the *HLA-DQA1* gene was defined as a risk allele with odds ratio (OR) of 1.36 (95%CI=1.21–1.54, P=5.3x10^{-7}) by analysis of 1,656 ADC cases and 1,173 controls. DQA1*03 and the minor allele for a polymorphism, rs2736100, in *TERT*, another lung cancer susceptibility locus identified in recent GWASs on Europeans and Americans, were indicated to independently contribute to ADC risk with per allele OR of 1.43 (95%CI=1.31–1.56, P=7.8x10^{-16}). Individuals homozygous both for the *DQA1*03* and minor *TERT* alleles were defined as high-risk individuals with an OR of 4.76 (95%CI=2.53–9.47, P=4.2x10^{-7}). The present results indicated that individuals susceptible to lung ADC can be defined by combined genotypes of *HLA-DQA1* and *TERT*.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world. Adenocarcinoma (ADC) is the commonest histological type comprising ~40% of lung cancer cases among European, North American and Asian countries, and is increasing in incidence [1]. Development of ADC is more weakly associated with smoking than those of two other major histological types of lung cancer, squamous (SQC) and small (SCC) cell lung carcinomas [1-3]. Therefore, identification of high-risk individuals for lung ADC and targeted screening and/or prevention for these individuals will be a powerful way to reduce the number of lung cancer deaths in the world.

Recent GWASs with single nucleotide polymorphism (SNP) array methodology have led to the identification of three loci associated with lung cancer risk, *CHRNA3/5* at chromosome 15q25.1, *TERT* and *CLPTM1L* at 5p15.33, and *BAT3-MSH5* at 6p21.33 [4-10]. Among these loci, 5p15.33 was revealed as being a locus specifically associated with risk of ADC among major histological types of lung cancer [11]. However, loci associated with lung ADC risk in Asians remain obscure. Here, we performed a GWAS on the risk of lung ADC in a Japanese population for 23,010 polymorphic microsatellite loci and identified *HLA-DQA1* at 6p21.31 as a novel locus associated with lung ADC risk. We further examined whether or not individuals susceptible to ADC can be defined by combined genotypes of *HLA-DQA1* and other lung cancer susceptibility loci described above.