Table 4 Plasma pharmacokinetic parameters (mean ± SD) of figitumumab given in combination with carboplatin and paclitaxel

Figitumumab dose level (mg/kg)	Cycle 1				Cycle 4			Accumulation ratio
	n	C_{max} (mg/L)	$AUC_{(0-day22)}$ (mg·h/L)	$t_{½}$ (h)	*n*	C_{max} (mg/L)	AUC_{tau} (mg·h/L)	
6	6	113±16	22,400±4,050	264[a]	4	178±35	39,000, 66,000[b]	1.7, 2.6[b]
10	6	197±33	36,700±10,400	301[a]	4	294±61	96,100, 96,800[b]	2.2, 2.2[b]
20	6	485±59	82,700±11,200	248[a]	5	550±89	116,000, 190,000[b]	1.6, 2.1[b]

$AUC_{(0-day22)}$ area under the plasma concentration–time curve from time zero to day 22, AUC_{tau} AUC from time zero to tau (the actual time of the pre-dose sample for the next cycle), C_{max} maximum observed plasma concentration after the end of figitumumab infusion, *SD* standard deviation, $t_{1/2}$ apparent disposition half-life

[a] n=4 at 6 mg/kg, n=1 at 10 mg/kg, and n=4 at 20 mg/kg (sampling was not sufficient to capture terminal disposition phase in other patients)

[b] n=2

No cases of grade 3 or 4 hyperglycemia (treatment-related or all-causality) were reported in the present study (grade 2 hyperglycemia was reported in one patient). Hyperglycemia has been reported in other studies of figitumumab and in studies of other IGF-1R-targeted mAbs [8–11, 13, 15–17]. Hyperglycemia may be a characteristic of the anti-IGF-1R class of compounds; however, its mechanism is unknown.

Incidences of grade 3 and 4 treatment-related neutropenia and thrombocytopenia in the figitumumab arm of the larger Western phase II randomized study of figitumumab in combination with paclitaxel and carboplatin in chemotherapy-naïve NSCLC were 28% and 7%, respectively, compared with 84% and 21% in the current trial [13]. Similar ethnic differences in the incidence of neutropenia have also been observed in a Japanese–US common-arm analysis of carboplatin plus paclitaxel in advanced NSCLC, and were suggested to be related to differences in allelic distribution of genes associated with DNA repair and paclitaxel disposition [18]. However, such an ethnic difference was not observed for thrombocytopenia [18]. Further studies would be required to determine whether there are pharmacogenetic or other reasons for ethnic differences in the incidence of thrombocytopenia in patients treated with figitumumab.

In line with previous phase I studies [8–11], figitumumab plasma exposure increased in an approximately dose-proportional manner and concentrations declined in a multi-exponential manner when figitumumab 6–20 mg/kg was given in combination with carboplatin and paclitaxel in the present study. The approximate 2-fold accumulation following repeated administration supported the 21-day regimen as appropriate for figitumumab administration in Japanese patients; similar accumulation was reported in Western studies [8–11]. No relationship between ethnicity and pharmacokinetics was expected, since figitumumab (as a mAb) extravasates mainly by convection and is eliminated by catabolism and/or target-mediated clearance.

High serum total IGF-1 and low IGFBP3 levels have been associated with higher incidence of NSCLC [19]. Hepatic IGF-1 production is stimulated by hGH, and hGH production is regulated by IGF-1 through negative feedback [20]. A previous phase I study demonstrated that single-agent figitumumab (20 mg/kg) altered the endocrine feedback mechanisms regulating hGH [8]. In the present study, both hGH and serum total IGF-1 concentrations appeared to increase following dosing with study medication, and this suggests blockade of IGF-1R with loss of IGF-1 regulatory feedback at the pituitary. In a phase I

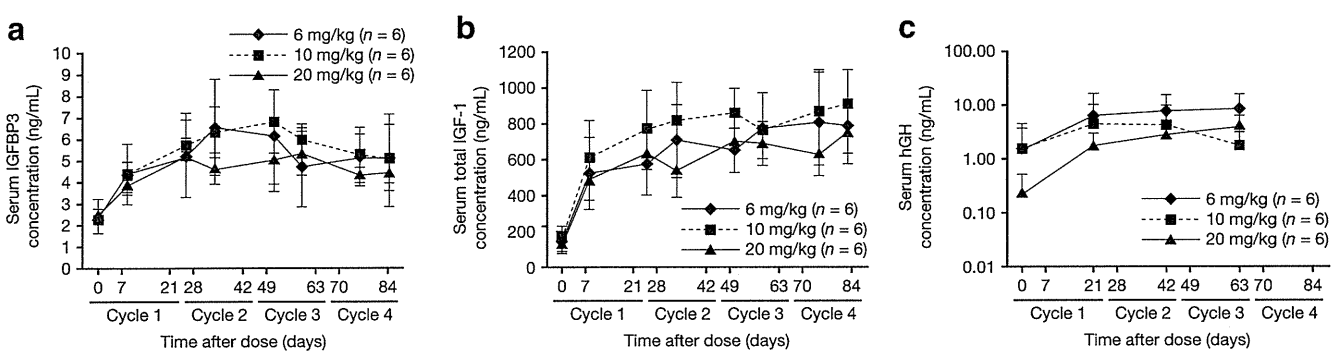

Fig. 2 Circulating biomarker concentrations during study treatment: concentration–time profile of serum IGFBP3 (panel **a**), serum total IGF-1 (panel **b**), and serum hGH (panel **c**). Data shown are mean ± standard deviation (panel **a** and panel **b**) and mean + standard deviation (panel **c**)

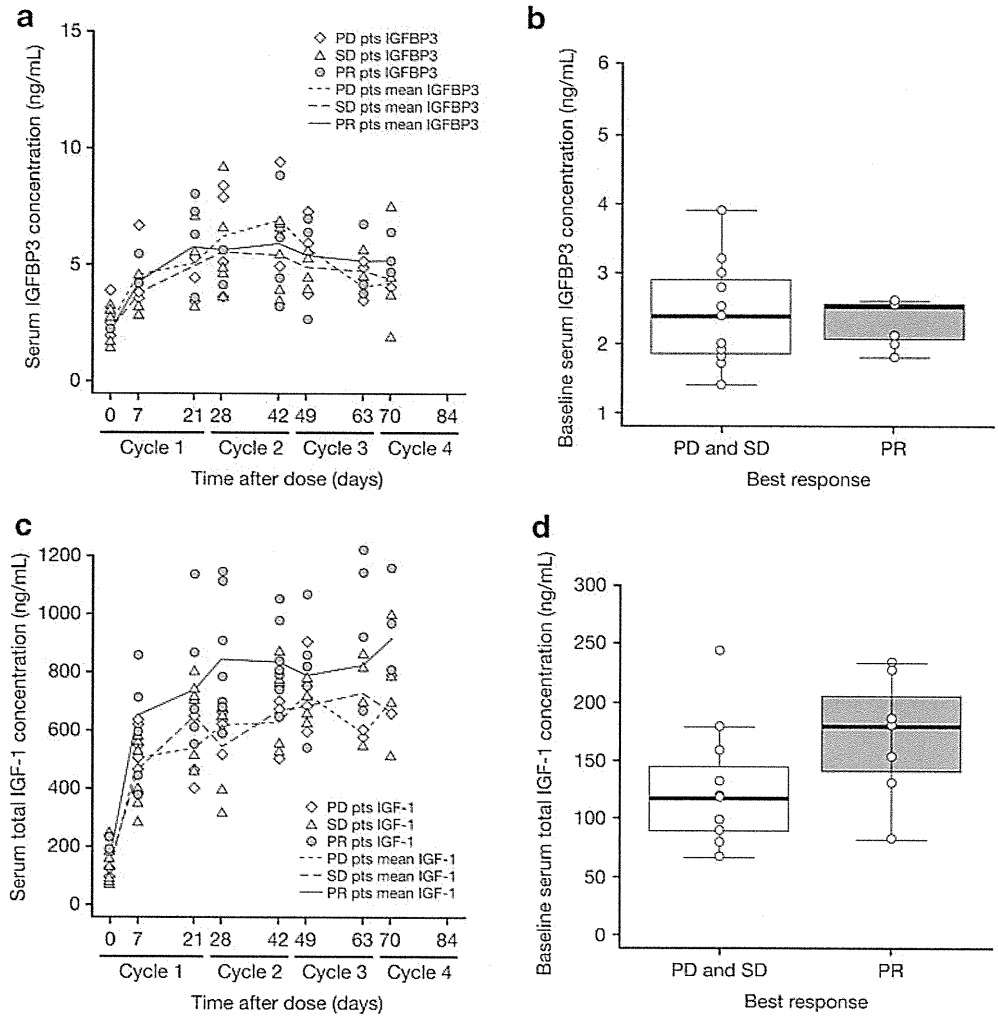

Fig. 3 Relationship between biomarkers and clinical response: serum IGFBP3 concentration–time profiles by best response (panel **a**); baseline serum IGFBP3 concentrations by best response—boxes represent median, 25%, and 75% percentiles (panel **b**); serum total IGF-1 concentration–time profiles by best response (panel **c**); baseline serum total IGF-1 concentrations by best response—boxes represent median, 25%, and 75% percentiles (panel **d**). *Pts* patients

study in myeloma patients and in the Western phase II trial in NSCLC patients described above, dose-dependent sustained elevations of serum IGF-1 and IGFBP3 concentrations were observed following administration of figitumumab [10, 21], indicating dose-dependent blockade of IGF-1R by figitumumab. However, a similar dose-dependent relationship between figitumumab and circulating IGF-1 and IGFBP3 concentrations was not clearly demonstrated in the current phase I study. Large inter-individual variability in serum total IGF-1 and IGFBP3 concentrations is known to occur naturally, and the differences between the studies may reflect the small number of patients included in each dose cohort level in the current study. Alternatively, the lack of a dose-dependent elevation of IGF-1 noted in this study may be related to the Japanese patients in this study having lower body mass index (BMI) compared with patients in other figitumumab studies. This possibility is supported by a report which suggests a relationship between BMI and IGF-1 levels [22].

Closure of the phase III studies of figitumumab in NSCLC (ADVIGO [ADVancing IGF-IR in Oncology] 1016 and 1018) due to potential futility of the combination regimens (figitumumab with paclitaxel plus carboplatin, and with erlotinib, respectively) has underscored the need to identify patients most likely to benefit from anti-IGF-1R therapy [23]. Studies have indicated that baseline levels of circulating free IGF-1 may be a positive biomarker for clinical response to figitumumab [13, 21, 23]. In the present study, serum total IGFBP3 and IGF-1 concentration–time profiles were stratified by best response as part of an exploratory analysis of the relationship between biomarker levels and antitumor activity. No clear differences were observed in the IGFBP3 concentration–time profiles according to clinical response, or when baseline IGFBP3 concentration was stratified by best response. However, the serum total IGF-1 concentration–time profile in patients with PR as their best response was higher than the profiles in both SD and PD patients. Additionally, baseline serum total IGF-1 concentration appeared higher in patients with PR compared with patients having SD/PD as their best response. Although the relationship between outcome and biomarkers was not examined statistically due to the exploratory nature of these investigations and the small number of patients, these observations suggest that serum

total IGF-1 concentrations prior to the start of treatment may also be a positive biomarker for response.

In summary, figitumumab 20 mg/kg in combination with carboplatin and paclitaxel was well tolerated in chemotherapy-naïve Japanese patients with advanced NSCLC. Serum total IGF-1 is a potential biomarker for clinical response to figitumumab and requires further investigation.

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Quantity of supporting information None.

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Genetic Variations of Orosomucoid Genes Associated with Serum Alpha-1-Acid Glycoprotein Level and the Pharmacokinetics of Paclitaxel in Japanese Cancer Patients

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ABSTRACT: Alpha-1-acid glycoprotein (AGP) encoded by orosomucoid genes (*ORM1* and *ORM2*) is an acute-phase response protein and functions as a drug-binding protein that affects pharmacokinetics (PK)/pharmacodynamics of binding drugs. To explore the effects of genetic variations of *ORMs* and a role of AGP on paclitaxel (PTX) therapy, we analyzed the duplication and genetic variations/haplotypes of *ORMs* in 165 Japanese cancer patients and then investigated their associations with serum AGP levels and the PK parameters of PTX. No effects of *ORM* duplications on serum AGP levels at baseline or PK of PTX were observed, but close associations of *ORM1* −559T > A with the increases of AGP levels and area under the curve (AUC) of PTX metabolites were detected. In addition, a significant correlation between the serum AGP level and the AUCs of PTX metabolites was observed, suggesting that AGP may function as a carrier of PTX from the blood into the liver via putative receptors. This study provided useful information on the possible clinical importance of *ORM* genetic polymorphisms and a novel role of AGP in PTX therapy. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4546–4559, 2011

Keywords: alpha-1-acid glycoprotein; *ORM1*; *ORM2*; paclitaxel; pharmacogenomics; pharmacokinetics; glycoproteins/glycoprotein receptors; hepatic metabolism; cancer chemotherapy

INTRODUCTION

Human alpha-1-acid glycoprotein (AGP) is an approximately 44-kDa plasma protein consisting of 183 amino acids with high-carbohydrate content (45%). AGP is an acute-phase protein, and its plasma levels are increased by inflammation, stress, and several types of cancers.[1] On the contrary, AGP functions as a binding protein for many basic and hydrophobic drugs such as paclitaxel (PTX).[2] Therefore, there is a possibility that the pharmacokinetics (PK)/pharmacodynamics (PD) of such drugs are influenced by plasma levels of AGP.[3,4] There are large interindividual and intraindividual variations in AGP levels compared with other plasma proteins such as albumin. Inflammatory cytokines and some types of drugs, such as phenobarbital, rifampicin, and clarithromycin, are known to be AGP inducers.[5] However, details of its expression and induction mechanisms remain unclear.

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Human AGP is encoded by two genes, *ORM1* and *ORM2*, located in tandem on chromosome 9 and separated by approximately 3.3 kb. Both *ORM* genes consist of six exons, and their coding sequences have 94% homology.[1,2,6,7] AGP is highly polymorphic, and more than 70 AGP variations in the serum have been identified by isoelectric focusing methods.[8] *ORM* genetic variations, mainly focusing on the exon regions, have been investigated in several ethnic populations, and ethnic differences were recognized.[9–13] Duplications or null variations of *ORM* genes have been also detected in several populations, and the frequency of *ORM1* duplication in Japanese subjects was reported to be approximately 20%, although null variations of *ORM* genes were very rare.[8,14,15] Although knowledge of the gene structures of *ORM*s has been accumulating in some ethnic populations,[10,11,14] detailed information is still limited regarding *ORM* genetic variations and the haplotypes covering *ORM1* and *ORM2*. In addition, the clinical relevance of *ORM* genotypes to AGP expression or the PK/PD of drugs binding to AGP remains to be elucidated.

In the current study, to screen *ORM* genetic variations in Japanese subjects and their functional significance, we first analyzed the copy number and genetic variations/haplotypes of *ORMs* including all six of their exons and the 5′-flanking regions. Next, we examined the associations of *ORM* genotypes with serum AGP levels and the PK parameters of PTX and consider a clinical role of AGP in PTX therapy.

MATERIALS AND METHODS

Patients

We previously obtained DNA samples and PK and clinical data from Japanese cancer patients who received PTX therapy at the National Cancer Center, Tokyo, Japan.[16,17] In this study, an association study was performed using data from 165 patients with non-small cell lung cancer who received PTX at doses of 175–210 mg/m^2 (high-dose group in the previous study). Population characteristics of the patients are shown in Table 1. Eligibility criteria for PTX therapy and PTX regimens were as previously described.[16] This study was approved by the ethics committees of the National Institute of Health Sciences and the National Cancer Center, and written informed consent was obtained from all the participants.

Analysis of *ORM* Gene Variations

For determination of *ORM1* and *ORM2* gene duplications, long-range polymerase chain reaction (PCR) was performed using four primers that amplify the intergenic regions from exon 4 (upstream) to exon 1 (downstream) of *ORMs* (6.5 kb) according to the method by Nakamura et al.[14] Duplications of *ORM1* and *ORM2* were discriminated by the PCR products amplified by the primer sets AGP1-E4K/AGP1-L4R and AGP2-E4K/AGP2-L4R, respectively, and the single tandem type of *ORM* genes (*ORM1–ORM2*) was amplified by only the primer set AGP1-E4K and AGP2-L4R (Fig. 1; Table 2).

Homozygous deletions of *ORM* genes were assessed by the absence of PCR products amplified from the promoter region (from −1 kb upstream of translation initiation site) to exon 6 by using primer sets ORM1-ZF1/ORM1-ZR1 for *ORM1* and ORM2-ZF1/ORM2-ZR2 for *ORM2* [Table 2 (B) and (C), first PCR]. These homozygous deletions were confirmed by the PCR products amplified from exon 1 to exon 3 using primer sets ORM1Ex1F1/ORM1Ex2.3R2 for *ORM1* and ORM2Ex1F1/ORM2Ex2.3R1 for *ORM2* [Table 2 (B) and (C), second PCR].

Table 1. Population Characteristics of 165 Japanese Paclitaxel-Administered Patients

Backgrounds and Dosing	Mean ± SD (range)
Number of subjects	165
Sex (male/female)	115/50
PS (0/1/2)	47/115/3
Stage (II/III/IV / postsurgical recurrence)	3/54/60/30
Drinking (yes/no)	62/103
Smoking (yes/no)	15/150
Age (years)	61.2 ± 10.2 (33–80)
Body weight (kg)	59.3 ± 11.7 (38.3–137.0)
BSA (m^2)	1.63 ± 0.18 (1.31–2.55)
Paclitaxel dose (mg/m^2)	199.3 ± 4.2 (175–210)
Infusion time (h)	3.15 ± 0.18 (2.75–3.92)

BSA, body surface area.

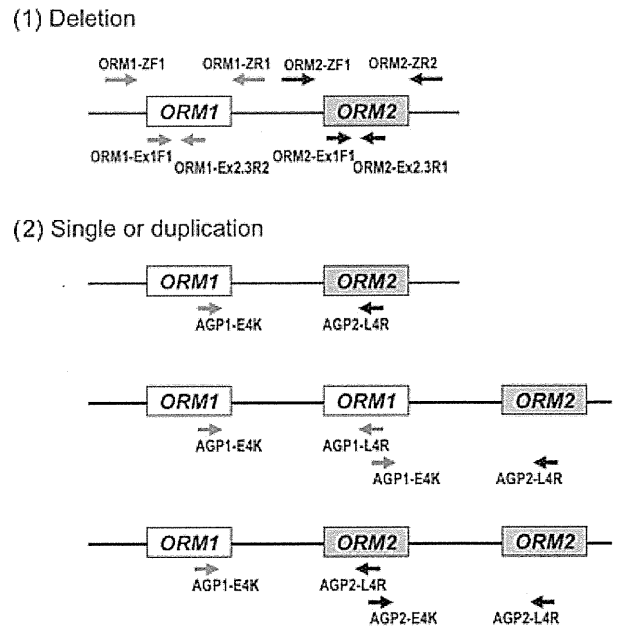

Figure 1. The PCR primer sets for the determination of deletion/duplication of *ORM* genes. Primer sequences are shown in Table 2.

Table 2. A List of Primers Used in This Study

Region	Primer Name	Primer Sequence	Reference
(A) Duplication check for *ORMs*	AGP1-E4K	5′-GACCTACATGCTTGCTTTTG-3′	
	AGP2-E4K	5′-GACCTTGATGTTTGGTTCCT-3′	14
	AGP1-L4R	5′-GGATCAGCAAGTGAGCGAA-3′	
	AGP2-L4R	5′-GGAACAGCAGGTGAGCAAC-3′	
(B) *ORM1* (deletion check and sequencing)			This study
Frist PCR			
−1.2 kb ~ exon 6	ORM1-ZF1	5′-CAAGCAATCTTCCAGCCTCAGAGTC-3′	
	ORM1-ZR1	5′-GCACATACGGAATAGATGGAACAAC-3′	
Second PCR			
−1.2 kb ~ exon 1	ORM1-1kF1	5′-GAATACTTTCTGAGTAATCCCAGCA-3′	
	ORM1Ex1R1	5′-TGATGCTGAATCTTTGACTG-3′	
Exon 1 ~ exon 3	ORM1Ex1F1	5′-GGGCTTTTGTAACCTCTCCA-3′	
	ORM1Ex2.3R2	5′-TAGAGTTGTTTCTGAGCACA-3′	
Exon 4 ~ exon 5	ORM1Ex4.5F3	5′-AGTGCATCTATAACACCACC-3′	
	ORM1Ex4.5R1	5′-GGTGGTAACTCCCCGCATTA-3′	
Exon 5 ~ exon 6	ORM1Ex5.6F1	5′-ACATGCTTGCTTTTGACGTG-3′	
	ORM1Ex6R1	5′-GAACAGCAGGTTGTATTATG-3′	
Sequencing			
−1.2kb ~ −900	ORM1-1kF1	5′-GAATACTTTCTGAGTAATCCCAGCA-3′	
	ORM1-1kR2s	5′-AGTTTCACTCCCGTCCCCCA-3′	
−860 ~ −90	ORM1-1kF2s	5′-ACAAATAAAGACTTGACCCG-3′	
	ORM1-1kR1	5′-GCCCAGCAAAAGTTTACTCTGT-3′	
Exon 1	ORM1Ex1F1	5′-GGGCTTTTGTAACCTCTCCA-3′	
	ORM1Ex1R1	5′-TGATGCTGAATCTTTGACTG-3′	
Exon 2 ~ exon 3	ORM1Ex2.3F1	5′-GACAGTCAAAGATTCAGCAT-3′	
	ORM1Ex2.3R1	5′-GTTTCTGAGCACATCGTTAG-3′	
Exon 4 ~ exon 5	ORM1Ex4.5F1	5′-GCCTCCTCACCTGTAAGACA-3′	
	ORM1Ex4.5R1	5′-GGTGGTAACTCCCCGCATTA-3′	
Exon 6 (IVS5-470~)	ORM1Ex6F2	5′-TGAACTACCTCCTGCCCCCATC-3′	
(IVS5-180~)	ORM1Ex6F1	5′-CAGAGACAGAAAATGACTTG-3′	
	ORM1Ex6R1	5′-GAACAGCAGGTTGTATTATG-3′	
(C) *ORM2* (deletion check and sequencing)			
Frist PCR			This study
−1.2kb ~ exon 6	ORM2-ZF1	5′-GTAGACAATGCCCAGTGTTTAGCGT-3′	
	ORM2-ZR2	5′-TTTGGGAGAGAACCTGGATTTA-3′	
Second PCR			
−1.2kb ~ exon 1	ORM2-ZF1	5′-GTAGACAATGCCCAGTGTTTAGCGT-3′	
	ORM2Ex1R1	5′-GCAGTGAGGCTTCTGTGTTT-3′	
Exon 1 ~ exon 3	ORM2Ex1F1	5′-TTCATCATTAGGTTTGTGGC-3′	
	ORM2Ex2.3R1	5′-AGGGATTTTTTCTGAGCACG-3′	
Exon 4 ~ exon 5	ORM2Ex4.5F3	5′-AGTGCTTCTATAACTCCAGT-3′	
	ORM1Ex4.5R1[a]	5′-GGTGGTAACTCCCCGCATTA-3′	
Exon 5 ~ exon 6	ORM2Ex5.6F1	5′-TGATGTTTGGTTCCTACCTG-3′	
	ORM1Ex6R1[a]	5′-GAACAGCAGGTTGTATTATG-3′	
Sequencing			
−1.2kb ~ −900	ORM2-1kF1	5′-AGCGTAAGAATCAGGGTCCA-3′	
	ORM2-1kR2s	5′-GAGTTTCGCCATTTTGCTCT-3′	
−850 ~ −360	ORM2-1kF2s	5′-TAAAGACTTGACCCATAAGG-3′	
	ORM2-1kR1	5′-CCTATCCCTCTGAGTGCCAA-3′	
Exon 1	ORM2Ex1F1	5′-TTCATCATTAGGTTTGTGGC-3′	
	ORM2Ex1R1	5′-GCAGTGAGGCTTCTGTGTTT-3′	
Exon 2 ~ exon 3	ORM1Ex2.3F1[a]	5′-GACAGTCAAAGATTCAGCAT-3′	
	ORM2Ex2.3R1	5′-AGGGATTTTTTCTGAGCACG-3′	
Exon 4 ~ exon 5	ORM2Ex4.5F1	5′-GACTCCTCACCTGTAAGACA-3′	
	ORM1Ex4.5R1[a]	5′-GGTGGTAACTCCCCGCATTA-3′	
Exon 6 (IVS5-470~)	ORM2Ex6F2	5′-GTGAACTACCTCCTGCCCCATC-3′	
(IVS5-180~)	ORM1Ex6F1[a]	5′-CAGAGACAGAAAATGACTTG-3′	
	ORM1Ex6R1[a]	5′-GAACAGCAGGTTGTATTATG-3′	

NT_008470.19 was used as the reference sequence for *ORM1* and *ORM2*.
[a]The same primer was used for *ORM1* analysis.

For the analysis of *ORM* genetic variations, 5′-flanking region (approximately 1.2 kb), all six exons and their adjacent intronic regions of both *ORM* genes were directly sequenced using the DNA samples from 113 subjects homozygous for the single tandem type of *ORMs*. The first PCR was performed using 25 ng of genomic DNA with 2.5 units of LA-taq (Takara Bio Inc., Shiga, Japan) and 0.4 μM of primers [Table 2 (B) and (C), first PCR]. PCR conditions were as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. The second PCR was performed using the first PCR products with 1 unit of Ex-taq (Takara Bio Inc.) and 0.4 μM of primers [Table 2 (B) and (C), second PCR] under the same reaction conditions described above. The PCR products were treated with a PCR product Pre-Sequencing Kit (USB Company, Cleveland, Ohio) and directly sequenced on both strands by using an ABI BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Foster City, California) with the sequencing primers shown in Table 2 [(B) and (C), sequencing]. Excess dye was removed using a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3730 DNA analyzer (Applied Biosystems). All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. Genbank NT_008470.19 was used as the reference sequence. The translational initiation site was designated as +1 to describe the polymorphism positions.

As of January 18, 2011, the novel variations reported here are not found in the database of Japanese Single Nucleotide Variations (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).

Linkage Disequilibrium and Haplotype Analyses

Hardy–Weinberg equilibrium and linkage disequilibrium (LD) analyses of *ORM1/ORM2* genetic variations detected at not less than 3% frequency were performed using SNPAlyze 3.1 software (Dynacom Company, Yokohama, Japan). Pairwise LDs were shown as rho square (r^2) and |D′| values in Figure 2. Diplotype configurations (haplotype combinations) of *ORM1/ORM2* (single tandem type) were inferred using SNPAlyze software (Dynacom Company) with the *ORM1/ORM2* variations detected in four or more chromosomes.

Analysis of Serum AGP Level and PK Data

The serum AGP levels at baseline (pretreatment with PTX) in the patients were determined by nephelometry and assessed for their associations with *ORM* genotypes. The PK data of PTX have been obtained previously.[16] For the association analysis, the area under the concentration–time curve (AUC) values for PTX and its metabolites, C3′-p-hydroxypaclitaxel (3′-*p*-OH-PTX), 6α-hydroxypaclitaxel (6α-OH–PTX), and 6α-C3′-*p*-dihydroxypaclitaxel (*di* OH–PTX), were used.

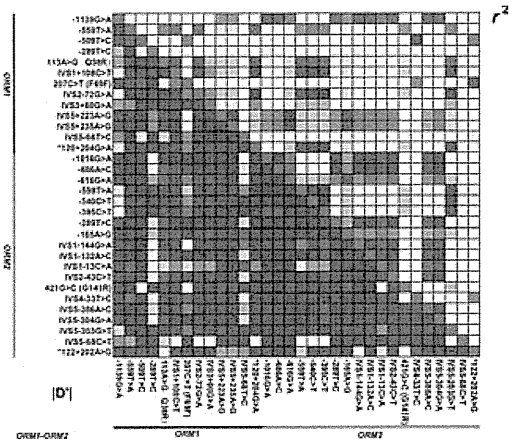

Figure 2. Linkage disequilibrium (LD) analysis of single tandem *ORM* (*ORM1–ORM2*) in 113 Japanese subjects. Pairwise LD (r^2 values and |D′|) of variations detected in no less than 3% of allele frequencies is shown as a 10-graded blue color.

Statistical Analysis

Statistical significance (two sided, $p < 0.05$) for associations of the serum AGP levels or PK data with *ORM* genotypes (duplication, SNPs and haplotypes) and with the categorical data [age, sex, history of smoking or drinking, performance status (PS), and tumor stage] was determined using the Mann–Whitney *U*-test or Kruskal–Wallis test. Correlations among the serum AGP levels, PK parameters, and patient background factors [body surface area, serum biochemistry of glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), and creatinine] were analyzed by Spearman's rank correlation test. Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to AGP levels were further determined by multiple regression analysis after logarithmic transformation, using the independent variables selected by the above bivariate analysis ($p < 0.05$ or the absolute value of Spearman's $r > 0.5$). These analyses were conducted using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, California) or JMP version 8.02 (SAS Institute Inc., North Carolina).

RESULTS

Genetic Variations and Haplotypes of *ORM* Genes

We first determined the duplication of *ORM* genes among the 165 Japanese subjects by the long-range

PCR method reported by Nakamura et al.[14] The frequency of patients homozygous of the single tandem type of *ORMs* (*ORM1–ORM2*) was 0.685, and those with duplication of *ORM1* (*ORM1–ORM1–ORM2*) or *ORM2* (*ORM1–ORM2–ORM2*) at least in one chromosome were 0.309 and 0.006, respectively. No patients had duplications of both *ORM1* and *ORM2*, homozygous deletion of any of *ORM* genes, or a reported nonsense mutation in exon 4 of *ORM1*.[15]

Using the DNA samples from 113 patients who were homozygous for the single tandem *ORM*, analysis of *ORM* variations was performed. With specific primers for *ORM1* and *ORM2*, direct sequencing was conducted for the 5′-flanking regions, all six exons and their adjacent intronic regions in both *ORMs*. Detected variations of *ORM1* and *ORM2* are shown in Tables 3 and 4, respectively. All these variations were in Hardy–Weinberg equilibrium ($p > 0.1$).

For *ORM1*, 43 genetic variations including seven novel ones were detected at frequencies ranging from 0.004 to 0.399 (Table 3). Novel variations included two in the 5′-flanking region, four in the intronic regions, and one in the 3′-untranslated region at frequencies ranging from 0.004 to 0.057. Among intronic novel variations, an 18-base substitution of *ORM2* for *ORM1* was found in intron 2 (IVS2-42_-25) at a frequency of 0.009. We also detected the reported *ORM1* variation in the 5′-flanking region, −686A >C, which was detected in Africans,[11] and the intronic variations (intron 5) IVS5-259G>T, IVS5-252C>A, IVS5-248A>T, IVS5-243A>T, and IVS5-227A>G, which were detected in Africans, including Ghanaians,[10,11] at a frequency of 0.004. The nonsynonymous *ORM1*113G>A (Q38R), which was detected in a variety of ethnic populations and has been designated as *ORM1*S*,[8] was also found in our Japanese population at a frequency of 0.171. Another nonsynonymous *ORM1* variation, 509G>A (R170K), previously designated as *ORM1*B9*,[8] was also found in our study, but its frequency was low (0.009). The frequencies of the other reported *ORM1* variations 207C>T (F69F) and 430G>T (V144F) were 0.039 and 0.004, respectively. Although the nonsynonymous *ORM1* SNP 520G>A (V174M), designated as *ORM1*F2*,[8] was reported in Mexicans, Africans, and Europeans at frequencies of 4%–17%,[13] this SNP was not found in our Japanese population.

Regarding *ORM2*, 40 variations including seven novel ones were found at frequencies ranging from 0.004 to 0.013 (Table 4). Novel variations included one in the 5′-flanking region, one nonsynonymous variation, four in the intronic regions, and one in the 3′-flanking region. The novel nonsynonymous variation was 328G>A (E110R) at exon 3, and its frequency was 0.013. The reported *ORM2* variations in intron 5, IVS5+76A>G, and IVS5-375T>C, which were detected in Africans including Ghanaians,[10,11] were found at frequencies of 0.022 and 0.004, respectively, in this Japanese population. The nonsynonymous *ORM2* 421G>C (G141R), which was designated as *ORM2*H19*,[8] was found at a frequency of 0.044. The synonymous *ORM2* SNP 531C>T (D177D), which was detected in the Africans at the frequency of 0.47,[11] was also detected in our population, but the frequency was relatively low (0.013). Another reported synonymous *ORM2* variation, 207T>C (F69F), was also detected at a frequency of 0.004.

LD and Haplotypes Analysis

The *ORM1/ORM2* haplotype structures were analyzed using 32 common variations that were detected in four and more chromosomes in 113 patients (more than 3% allele frequency). The LD profile of the *ORM1/ORM2* variations in the single tandem *ORM* is shown in Figure 2. As assessed by r^2 (Fig. 2, upper right), close linkages were observed among *ORM1*-1139G>A and *ORM2* SNPs of −1016G>A, −686A>C, IVS1-144G>A, IVS1-132C>A, and IVS5-386A>C ($r^2 \geqq 0.825$). Other close linkages were found among *ORM1* SNPs of 113A>G (Q38R), IVS1+108C>T, IVS2-72G>A, and *120+204G>A and *ORM2* SNPs of −559T>A, −540C>T, −395C>T, and IVS5-203G>T ($r^2 \geqq 0.882$). Complete linkages ($r^2 = 1$) were found between *ORM1* −509T>C and *ORM2* IVS4-33T>C, among IVS1+108C>T, IVS2-72G>A, and *120+204G>A in *ORM1*, between IVS5+223A>G and IVS5+235A>G in *ORM1*, and among −686A>C, IVS1-132A>C, IVS2-43C>T, and IVS5-304G>A in *ORM2*. Regarding |D′| values, strong LD was also observed throughout the region analyzed. Overall, because close associations between the variations were observed throughout the entire *ORM1/ORM2* genes, the sequenced region was analyzed as a single LD block for the haplotype inference.

A total of 41 haplotypes were identified/inferred, classified into 13 subgroups, and further categorized into four major groups (Fig. 3). Our haplotypes nomenclature system is based on the recommendation of Nebert.[18] Haplotypes without any amino acid substitutions were assigned as **1* and named with small alphabetical letters in descending frequency order (**1a* to **1w*). Haplotypes with nonsynonymous variations were assigned from **2* to **4*, and their subtypes were named with small alphabetical letters. Ambiguous rare haplotypes inferred from only one patient are shown with "?" in Figure 3.

The most frequent **1* haplotype **1a* (also classified as**IA* subgroup) harbors no genetic variations, and its frequency was 0.407. The second frequent **1* subgroup was **IB* (frequency: 0.226), which harbors IVS5+223A>G in *ORM1* with six other linked variations, −686A>C, −616G>A, −289T>C, −165A>G, IVS1-13C>A, and IVS5-304G>A in *ORM2*. The **1* haplotype harboring only one variation

Table 3. Summary of Genetic Variations of *ORM1* Detected in This Study

SNP Identification			Position				
This study	NCBI(dbSNP) / JSNP/Ref	Location	NT_008470.19	From the Translational Initiation Site or from the Nearest Exon	Nucleotide Change and Flanking Sequences (5′ to 3′)	Amino Acid Change	Allele Frequency ($N = 226$)[a]
MPJ6_ORM1_001	rs1017989	5′-flanking	46248807	−1139	gcactttgggaggccgagac**G/A** ggtgagtcacctgaggtcgg		0.246
MPJ6_ORM1_002	rs10982150	5′-flanking	46248882	−1064	cccatctctactgaaaataa**C/T** aacaacaaaaaaaattagct		0.004
MPJ6_ORM1_003[b]		5′-flanking	46249161	−785	actcattccacagaatatac**C/T** gagtgcccttgaagtgctgg		0.013
MPJ6_ORM1_004	rs116994374	5′-flanking	46249204	−742	actgctccaggattgggggc**G/A** tattggtgaaagagaagcaa		0.022
MPJ6_ORM1_005	rs117454680	5′-flanking	46249254	−692	tcagatggcagggaatgggg**G/A** aaaacagggagacagtttcc		0.013
MPJ6_ORM1_006		5′-flanking	46249260	−686	ggcagggaatgggggaaaac**A/C** gggagacagtttcctgtttg		0.004
MPJ6_ORM1_007[b]		5′-flanking	46249267	−679	aatgggggaaaacagggaga**C/T** agtttcctgtttgagatgtt		0.009
MPJ6_ORM1_008	rs4510934	5′-flanking	46249387	−559	agggctgggttggggccccca**T/A** tgaactttggctctgacaca		0.083
MPJ6_ORM1_009	rs1766076	5′-flanking	46249437	−509	acactcagtggaactgaatc**T/C** atgtttgtcttccccggcat		0.035
MPJ6_ORM1_010	rs10706198	5′-flanking	46249522	−424	cctggggaccctcaaaggtg**G/-** cttcatcattaggtttgtgg		0.013
MPJ6_ORM1_011	rs3827819 IMS-JST189260	5′-flanking	46249657	−289	tggactcacacgtttactaa**T/C** gttgctgcagccccgcaccc		0.132
MPJ6_ORM1_012	rs1107080	5′-flanking	46249718	−228	tactctagggcttttgtaac**C/G** tctccatgtgaggaactcaa		0.009
MPJ6_ORM1_013	rs4978580	5′-flanking	46249781	−165	gtgctccgagctggcctttg**G/A** gggaggttttgtgcgaggca		0.004
MPJ6_ORM1_017	rs17650	Exon1	46250058	113	caccaacgccaccctggacc**A/G** ggtgagtgcctgggctagcc	Q38R	0.171
MPJ6_ORM1_019	rs1687381	Intron1	46250167	IVS1+108	cccccactcccggctctgcc**C/T** ttttctcttctgggtcccca		0.136
MPJ6_ORM1_020	rs10909578	Intron1	46250331	IVS1-144 (+272)	ggcaatgactgatcctcagg**G/A** tgagctcctgcatgcgcact		0.004
MPJ6_ORM1_022	rs700126	Exon2	46250567	207	caagcaaccttcttttactt**C/T** acccccaacaagacagagga	F69F	0.039
MPJ6_ORM1_023	rs1111796 IMS-JST099907	Intron2	46250758	IVS2-72	ctccaccagactcttgcccc**G/A** ggactgtgatgggcgattgg		0.136
MPJ6_ORM1_024[b]		Intron2	46250772	IVS2-58	tgccccaggactgtgatggg**C/T** gattggccacttctcaataa		0.004
MPJ6_ORM1_025[b]		Intron2	46250788 _46250805	IVS2-42_-25[c]	tgggcgattggccacttctc**I/II** ttccgccttctgtttggctt		0.009
MPJ6_ORM1_027[b]		Intron3	46250960	IVS3+60	acagggcaggccagcataag**G/A** tgggggctggatgtagagcc		0.057
MPJ6_ORM1_034	rs1126746	Exon4	46251703	430	agaagaactgggggctgtct**G/T** tctatggtaggcatgcttag	V144F	0.004
MPJ6_ORM1_035	rs10982154	Intron4	46251744	IVS4+35	cagccccaaactcatgcccc**T/A** ctcaggcctcacccccatt		0.026
MPJ6_ORM1_036	rs10982155	Intron4	46251786	IVS4-75	acccacccctgggctggccc**C/T** tagaaccccagccctccctg		0.026
MPJ6_ORM1_040	rs3182041	Exon5	46251933	509	cgactgcttgcgcattccca**A/G** gtcagatgtcgtgtacaccg	K170R	0.009
MPJ6_ORM1_043		Intron5	46252155	IVS5+191	tggtggatgagagcccagga**G/A** ggagaagtccctgtgaaacc		0.013
MPJ6_ORM1_044	rs1687382	Intron5	46252187	IVS5+223	tgtgaaaccagggaggacct**A/G** aaagctaacaggagggaaca		0.399
MPJ6_ORM1_045	rs2070869 IMS-JST005917	Intron5	46252199	IVS5+235	gaggacctgaaagctaacag**A/G** agggaacagcgtgagccacg		0.399

Continued

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Table 3. Continued

SNP Identification			Position		Nucleotide Change and Flanking Sequences (5′ to 3′)	Amino Acid Change	Allele Frequency ($N = 226$)[a]
This study	NCBI(dbSNP) / JSNP/Ref	Location	NT_008470.19	From the Translational Initiation Site or from the Nearest Exon			
MPJ6_ORM1_046[b]		Intron5	46252215	IVS5+251	acaggagggaacagcgtgag**C/T** cacgggttgggggattggc		0.009
MPJ6_ORM1_047		Intron5	46252843	IVS5-261	aggagctgcagcataagggc**T/C** cggcaggtcctaagtgcaca		0.004
MPJ6_ORM1_048		Intron5	46252845	IVS5-259	ggagctgcagcataagggctc**G/T** gcaggtcctaagtgcacagt		0.004
MPJ6_ORM1_049		Intron5	46252852	IVS5-252	agcataagggctcggcaggt**C/A** ctaagtgcacagtaaatgcc		0.004
MPJ6_ORM1_050		Intron5	46252856	IVS5-248	taagggctcggcaggtcctaa**A/T** gtgcacagtaaatgccagtg		0.004
MPJ6_ORM1_051		Intron5	46252861	IVS5-243	gctcggcaggtcctaagtgc**A/T** cagtaaatgccagtgattct		0.004
MPJ6_ORM1_052		Intron5	46252877	IVS5-227	gtgcacagtaaatgccagtg**A/G** ttcttaagagtctgagctcc		0.004
MPJ6_ORM1_053	rs2787340	Intron5	46252886	IVS5-218	aaatgccagtgattcttaag**A/G** gtctgagctcccattgtaga		0.018
MPJ6_ORM1_054	rs28587868	Intron5	46252901	IVS5-203	ttaagagtctgagctcccat**T/G** gtagaggcaagtaagctgag		0.018
MPJ6_ORM1_055	rs10982161	Intron5	46252904	IVS5-200	agagtctgagctcccattgt**A/G** gaggcaagtaagctgaggtt		0.013
MPJ6_ORM1_056	rs1766097	Intron5	46253036	IVS5-68	agtcatctgccctctccc**T/C** ggaagacctcccaccctgtc		0.031
MPJ6_ORM1_057[b]		3′-UTR	46253210	*41	ggacagagacttgggggccca**T/A** cctgccctccaaccgaca		0.018
MPJ6_ORM1_058	rs1687387	3′-flanking	46253340	*120+51	tctgaagcctgccctgagtt**C/T** aaccctggctgggcactgga		0.026
MPJ6_ORM1_059	rs62559488	3′-flanking	46253493	*120+204	aaaatctgggaaggcttccct**G/A** gaagggcatctgaaccaat		0.136
MPJ6_ORM1_060	rs1766098	3′-flanking	46253519	*120+230	ggcatctgaaccaatctaga**A/G** agatgaggagcataatacaa		0.026

[a] Number of chromosomes.
[b] Novel variation detected in this study.
[c] IVS2-42_-25; I = aataatcttccctgttttc; II = gataacattactgttttt.

was classified as **IC* subgroup (frequency: 0.137). The **IE* subgroup (frequency: 0.035) harbors −509T>A in *ORM1* with two other linked variations, −165A>G and IVS4-33T>C in *ORM2*.

The **2* haplotype (**II* group) was defined by one nonsynonymous substitution, 113A>G (Q38R) in *ORM1*. The most frequent **2* subgroup, **IIA* (frequency: 0.049), harbors additional variations: IVS1+108C>T and IVS5+223A>G in *ORM1* and −616G>A, −559T>A, −540C>T, −395C>T, −165A>G, and IVS5-203G>T in *ORM2*. Subgroup **IIC* (frequency: 0.04) harbors an additional variation, −559T>A in *ORM1*. Subgroup **IIE* (frequency: 0.018) harbors additional variations including −686A>C, −289T>A, IVS1–13C>A, and IVS5-304G>A in *ORM2*. The **3* haplotype (**III* group) harbors two nonsynonymous substitutions, 113A>G (Q38R) in *ORM1* and 421G>C (G141K) in *ORM2* (frequency: 0.040), and the **4* haplotype (**IV* group) harbors one nonsynonymous substitution, 421G>C (G141K) in *ORM2* (frequency: 0.004).

Association of *ORM* Genotypes with Serum AGP Levels

The effects of duplications of *ORM1* or *ORM2* genes are summarized in Table 5. The median AGP levels of the wild-type (single tandem *ORM*) and *ORM1*-duplicated groups were 100.0 and 104.0 mg/dL, respectively, and no significant difference between two groups was observed. The AGP level for *ORM2* duplication observed in only one patient was 126.0 mg/dL, which fell within the range of the wild-type group. Regarding the effects of *ORM* variations, an insignificant but more than 40% increase in the median AGP level was observed in patients bearing *ORM1* −559T>A and *ORM2* 421 G>C (G141R) ($p = 0.07$ and $p = 0.19$, respectively, Mann–Whitney *U*-test) (Fig. 4). No other *ORM* variations exhibited any remarkable relationships with the AGP levels.

The relationship between diplotypes (combination of haplotypes) and AGP levels was investigated. The median AGP levels in the patients with **I/*I*, **II/(*I or *II)*, and **III/*I* were 97, 121, and 145 mg/dL, respectively. The patients with haplotype **III*, which harbors two nonsynonymous variations of 113A>G (Q38R) in *ORM1* and 421G>C (G141K) in *ORM2*, had a 50% higher AGP levels than those with haplotype **I*; however, this difference was not significant ($p = 0.088$, Mann–Whitney *U*-test).

Effects of *ORM* Genotypes on PTX PK

Next, we examined the effects of the *ORM* genotypes on the PK parameters of PTX and its metabolites. PTX is metabolized to 6α-OH–PTX and 3′-*p*-OH–PTX by CYP2C8 and CYP3A4, respectively, and both metabolites are further hydroxylated to *di* OH–PTX.[19–22] Regarding *ORM* duplications, no significant differences in the PK parameters were observed among the

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Table 4. Summary of Genetic Variations of *ORM2* Detected in This Study

SNP Identification			Position				
This study	NCBI (dbSNP)/ JSNP/Ref	Location	NT_008470.19	From the Translational Initiation Site or from the Nearest Exon	Nucleotide Change and Flanking Sequences (5′ to 3′)	Amino Acid Change	Allele Frequency ($N = 226$)[a]
MPJ6_ORM2_001	rs112164771	5′-flanking	46255534	−1183	aaggcgctctggaaggccaggC/T gcggtggctcatgcttgtaa		0.004
MPJ6_ORM2_002	rs10982163	5′-flanking	46255565	−1152	atgcttgtaatcccagcactG/T tgggaggccgagg		0.022
MPJ6_ORM2_003	rs10982164	5′-flanking	46255599	−1118	tgggcggatcacctgaggtaG/A ggagttcgagaccagcctga		0.018
MPJ6_ORM2_004	rs7040680	5′-flanking	46255701	−1016	acctgtaatcccagcgactcG/A ggaggctgaggcaagagaat		0.217
MPJ6_ORM2_005[b]		5′-flanking	46255975	−742	actgctccaggattgggggcA/G tattggtgaaagagaagcaa		0.004
MPJ6_ORM2_006	rs2993412	5′-flanking	46256031	−686	ggcagggaatggggaaaaacA/C gggagacagtttcctgtttg		0.243
MPJ6_ORM2_007	rs10817591	5′-flanking	46256101	−616	tttactggaaatagacattcG/A acttggatgtccttttttgg		0.385
MPJ6_ORM2_008	rs35609402	5′-flanking	46256158	−559	agggctgggttggggccccaT/A tgaactttggctctgacata		0.146
MPJ6_ORM2_009	rs10982165	5′-flanking	46256177	−540	aatgaactttggctctgacaC/T agctgttgccacactcagtg		0.133
MPJ6_ORM2_010	rs71503594	5′-flanking	46256208	−509	acactcagtggaactgaatcC/T atgtttgccttcacccggca		0.027
MPJ6_ORM2_011	rs10706198	5′-flanking	46256293_46256294	−424_ −423	cctggggaccctcaaaggtg-/G cttcatcattaggtttgtgg		0.013
MPJ6_ORM2_012	rs12340691 rs59332824	5′-flanking	46256322	−395	ttaggtttgtggctgggtccC/T actgaagtaagtcttggcac		0.142
MPJ6_ORM2_013	rs3827819	5′-flanking	46256428	−289	tggactcacacgtttactaaT/C gttgctgcagccccgcaccc		0.257
MPJ6_ORM2_014	rs4978580	5′-flanking	46256552	−165	gtgctccgagctggcctttgA/G gggaggttttgtgcgaggca		0.429
MPJ6_ORM2_019[b]		Intron1	46256835	IVS1+5	cgccaccctggaccgggtgaG/C tgcctgggctagccctgtcc		0.004
MPJ6_ORM2_021	rs2636887 rs115684630	Intron1	46256898	IVS1+68	ccttctctgggcttcccttA/C cctgctggctgtggtcgcac		0.018
MPJ6_ORM2_022	rs2787338	Intron1	46256916	IVS1+86	ttacctgctggctgtggtcgC/G acccccactcccagctctgc		0.004
MPJ6_ORM2_023	rs2636888	Intron1	46256929	IVS1+99	gtggtcgcacccccactcccA/G gctctgccttttttctcttct		0.018
MPJ6_ORM2_024	rs1687381	Intron1	46256938	IVS1+108	cccccactcccagctctgccT/C ttttctcttctgggtcccca		0.004
MPJ6_ORM2_025	rs10909578	Intron1	46257102	IVS1-144	ggcaatgactgatcctcaggG/A tgagctcctgcatgcgcact		0.230
MPJ6_ORM2_026	rs71505503	Intron1	46257114	IVS1-132	tcctcagggtgagctcctgcA/C tgcgcactgcccaccagggg		0.243
MPJ6_ORM2_027	rs2787339 rs117654479	Intron1	46257233	IVS1-13	agcccccatcaccagctcccC/A ccttctccccagatcactgg		0.288
MPJ6_ORM2_028	rs700126	Exon2	46257338	207	caagcaaccttcttttacttT/C acccccaacaagacagagga	F69F	0.004
MPJ6_ORM2_029[b]		Intron2	46257490	IVS2+102 (−114)	ggccttcccatgggtggaacC/T gggagggttggctttaatct		0.004
MPJ6_ORM2_030	rs17230081	Intron2	46257561	IVS2-43	ggcgattggccacttctcctC/T gataacattactgtttttct		0.243
MPJ6_ORM2_031[b]		Intron2	46257585	IVS2-19	aacattactgtttttcttccG/A ccttctggttgactttagcc		0.009
MPJ6_ORM2_033[b]		Exon3	46257674	328	atgggaccgtctccagatacG/A gtgagggccagccctcaggc	E110R	0.013
MPJ6_ORM2_034	rs12685968	Exon4	46258471	421	tggacgatgagaagaactggG/C ggctgtctttctatggtagg	G141R	0.044
MPJ6_ORM2_035	rs113974644	Intron4	46258605	IVS4-33	cctcccgccgggccccaccaT/C gtccccagtcagtctccttg		0.035
MPJ6_ORM2_039	rs1826232	Exon5	46258732	531	tcagatgtcatgtacaccgaC/T tggaaaaaggtaaacgcaag	D177D	0.013
MPJ6_ORM2_040	rs116296992	Intron5	46258817	IVS5+76	cccagaggcccagagcaggaA/G agctgccaggcaaggctgca		0.022
MPJ6_ORM2_041	rs118158426	Intron5	46259495	IVS5-386	agtcagctcacttgatccacA/C gcctggcacctccactgtct		0.199
MPJ6_ORM2_042	rs115413547	Intron5	46259506	IVS5-375	ttgatccacagcctggcaccT/C ccactgtctggctagggagc		0.004
MPJ6_ORM2_043	rs1976193 rs41465345	Intron5	46259577	IVS5-304	tcagttacatcatctgcataG/A tagtggtggttgtgaggaat		0.243
MPJ6_ORM2_044[b]		Intron5	46259585	IVS5-296	atcatctgcatagtagtggtG/C gttgtgaggaattcaggagc		0.004
MPJ6_ORM2_045	rs1687417	Intron5	46259678	IVS5-203	ttaagggtctgagctcccatG/T gtagaggcaagtaagctgag		0.142
MPJ6_ORM2_046	rs71238720	Intron5	46259813	IVS5-68	agctcattctgccctctcccC/T ggaagacctcccaccctg		0.031
MPJ6_ORM2_047[b]		3′-flanking	46260159	*122+91	gagtgctgtgggagcccagcA/G ctgtgggaagacattttctt		0.004
MPJ6_ORM2_048	rs62559488	3′-flanking	46260270	*122+202	aaaatctgggaaggcttcctA/G gaaggggcatctgaaccaat		0.040
MPJ6_ORM2_049	rs1766098	3′-flanking	46260296	*122+228	ggcatctgaaccaatctagaA/G agatgaggagcataatacaa		0.013

[a] Number of chromosomes.
[b] Novel variation detected in this study.

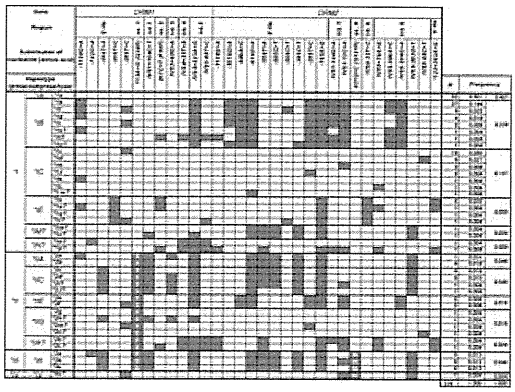

Figure 3. Haplotypes of single tandem *ORM (ORM1–ORM2)* in 113 Japanese subjects. Forty-one haplotypes were identified/inferred and categorized into four haplotypes groups: **I* (without nonsynonymous substitutions), **II* [harboring 113A>G (Q38R) in *ORM1*], **III* [harboring 113A>G (Q38R) in *ORM1* and 421G>C (G141K) in *ORM2*], and **IV* [harboring 421G>C (G141K) in *ORM2*]. The "?" symbol denotes ambiguous haplotypes ($n = 1$). Sites for nonsynonymous substitutions (tagging of II to IV groups) are indicated by their group-name numbers (II, III, and IV).

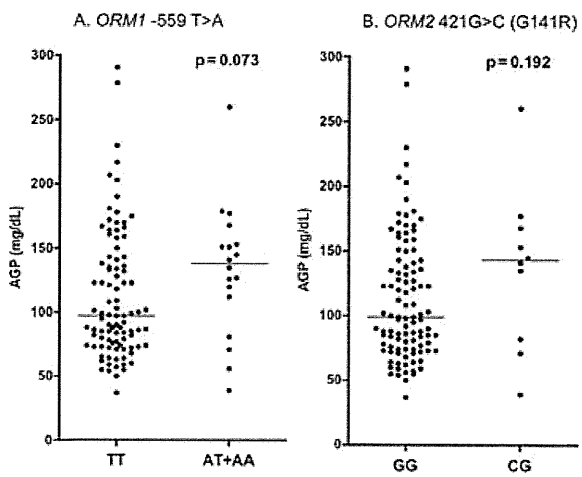

Figure 4. Effects of *ORM* genetic variations on serum AGP levels. The bars represent the medians. Dots show the individual AGP levels at baseline in the Japanese patients ($n = 113$) treated with PTX.

wild-type and duplicated groups (Table 5). Subsequently, we examined the associations of the genotype of *ORMs* and PK parameters of PTX in the 113 patients with no duplication. Among the *ORM1* and *ORM2* SNPs, significant increases in the AUC values of all of the metabolites were observed in the patients bearing *ORM1* −559T>A compared with those in patients without these variations (Fig. 5). *ORM2* 421 G>C (G141R) also showed a significant association with higher AUC values of *di* OH–PTX compared with those without this variation ($p = 0.033$, Mann–Whitney *U*-test). These two genotypes did not significantly affect the clearance of the parent compound PTX ($p = 0.17$ and 0.38 for *ORM1* −559T>A and *ORM2* 421 G>C, respectively).

The associations of haplotypes **II* and **III* with the PK parameters were also examined. Significant increases (50%) in the AUC values of *di* OH–PTX were observed in patients with haplotype **III* compared with **I*, ($p = 0.01$, Mann–Whitney *U*-test). The median AUC of *di* OH–PTX for diplotypes **I/*I*, **II/(*I or *II)*, and **III/*I* were 0.201, 0.273, and 0.580 h × (μg/mL), respectively. No significant effects of **II* or **III* on the clearance of PTX were observed (data not shown).

The Relationships Between AGP Levels and PK of PTX and Its Metabolites

The association of the AGP level with PK of PTX was examined using the clearance of PTX and AUCs of PTX and its metabolites. Although PTX clearance was not correlated with the serum AGP level, positive correlations were found between the AGP level and the AUC values of PTX metabolites (Fig. 6). In particular, AUC values of 6α-OH–PTX, 3′-*p*-OH–PTX, and *di*OH–PTX were highly correlated (Spearman's *r* were 0.33, 0.32, and 0.29, respectively, and $p < 0.01$ for all) with AGP levels.

The Relationship Between AGP Levels and Patients' Background Factors

The serum AGP levels in the 165 cancer patients varied from 34 to 395 mg/dL (median: 103 mg/dL). A significant correlation between AGP levels and

Table 5. Effects of *ORM* Duplications on the Serum AGP Levels and PK Parameters of 165 Japanese Paclitaxel-Administered Patients

		Median (Range)		
		Wild Type ($n = 113$ or 112)	*ORM1* Duplication ($n = 51$)	*ORM2* Duplication ($n = 1$)
AGP level	(mg/dL)	100.0 (37.0–291.0)	104.0 (34.0–395.0)	126.0
AUCt(PTX)	[h × (μg/mL)]	18.41 (10.26–38.37)	18.55 (6.29–36.29)	18.18
AUCt(6α-OH)	[h × (μg/mL)]	1.621 (0.538–8.455)	1.372 (0.416–13.12)	1.196
AUCt(3′p-OH)	[h × (μg/mL)]	0.441 (0.128–1.355)[a]	0.401 (0.159–1.881)	0.280
AUCt(*di*OH)	[h × (μg/mL)]	0.264 (0.000–2.551)[a]	0.319 (0.000–7.954)	0.061

[a]Number of patients was 112.

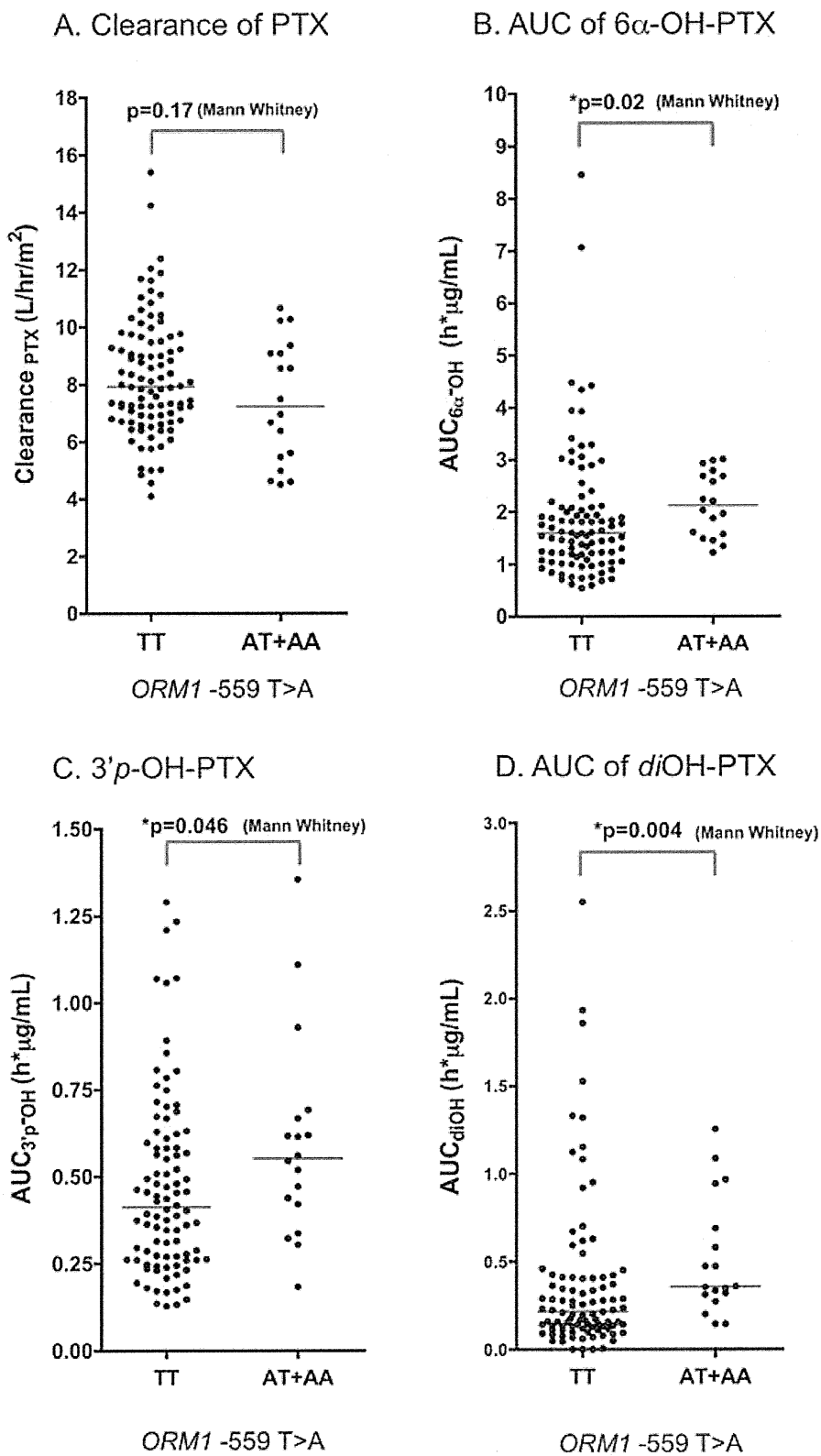

Figure 5. Effects of *ORM* genetic variations *ORM1* -559 T>A on the PK of PTX and its metabolites. The bars represent the medians. Dots show the individual AUC values in Japanese patients (n = 113) treated with PTX.

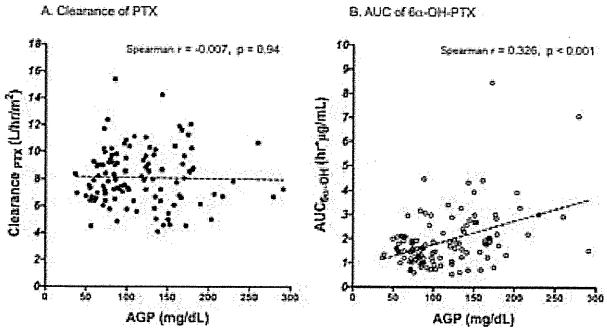

Figure 6. Correlations between serum AGP levels and the PK parameters of PTX. The broken lines represent the regression curves.

PS (0 vs. 1 and 2) was observed ($p = 0.0003$, Mann–Whitney *U*-test), and the median AGP levels for PS of 0, 1, and 2 were 84, 120, and 173 mg/dL, respectively. The serum AGP level also correlated with other serological parameters. The AGP level was positively correlated with CRP (Spearman's $r = 0.8405, p < 0.0001$), neutrophil counts ($r = 0.627, p < 0.0001$), and serum levels of ALP ($r = 0.365, p < 0.0001$) and γ-GTP ($r = 0.278, p = 0.006$). Consistent with previous reports,[1,2,23] a negative correlation between serum AGP and albumin levels was observed (Spearman's $r = -0.626, p < 0.0001$). The AGP level was also negatively correlated with serum levels of hemoglobin ($r = -0.4457, p < 0.0001$) and total bilirubin ($r = -0.450$, $p < 0.0001$). No significant correlation was found for age, sex, tumor stage, and serum levels of GOT, GPT, and creatinine.

Multiple Regression Analysis on AGP Level

As described in a previous section, the serum AGP level is associated with nongenetic factors such as inflammation, disease status, or hepatic functions. To further evaluate the effects of *ORM* variations on AGP levels, multiple regression analysis was conducted, including patient background factors such as PS, CRP levels, neutrophil counts, and serum albumin levels, which were highly correlated with AGP levels (absolute value of Spearman's $r > 0.5$). As shown in Table 6, the estimated coefficient of *ORM1* −559T>A after adjustment for the patient backgrounds were 0.056, meaning this SNP results in 1.13-fold higher AGP levels than the wild type. However, the p value for −559T>A was borderline significant (0.0605). The other *ORM* variations/haplotypes including 421G>C or haplotype **III* did not show any significance in the multivariate analysis. On the contrary, positive correlations were found for the following nongenetic factors: PS level (1 or 2) ($p = 0.002$), CRP level ($p < 0.0001$), neutrophil count ($p = 0.016$) at baseline, and the negative correlation of serum albumin level ($p = 0.0001$) (Table 6). These results suggested that nongenetic patient factors such as inflammation and liver function rather than *ORM* variations have a major impact on plasma AGP levels.

Table 6. Multiple Regression Analysis for Log-Transformed AGP Levels in Japanese Cancer Patients

Independent Variables	Coefficient	SE	p
Intercept	2.364	0.1357	< 0.0001
PS (0 vs. 1,2)	0.081	0.0252	0.0018
CRP (mg/dL)	0.024	0.0047	< 0.0001
Neutrophile count	1.0×10^5	$4.2. \times 10^6$	0.0164
Albumin (g/dL)	−0.136	0.0341	0.0001
ORM1 -559T>A	0.056	0.0298	0.0605

Summary of analysis: $r^2 = 0.628$, $n = 113$.

DISCUSSION

The primary purposes of this study were to elucidate the genomic variations of *ORM1* and *ORM2* in Japanese subjects and to identify the variations that are strongly related to plasma levels of AGP. The secondary purposes were to describe the relationship between AGP levels and the PK/PD of PTX and to consider the mechanisms linking between them.

ORM Variations and Haplotypes

The current analysis on the *ORM* gene structure revealed that the frequencies of patients with duplicated *ORM1* and *ORM2* (heterozygous or homozygous) were 31% and 0.6%, respectively, which does not conflict with previously reported findings.[14,24] In our preliminary study, we detected the homozygous deletions of both *ORMs* in a cancer cell line established from a Japanese patient, but in the current study analyzing Japanese patients, no homozygous deletions of any *ORMs* or no reported null variation in exon 4 of *ORM1*[15] were found. Although the gene deletion was postulated to form in the process of duplicated gene formation by unequal crossover, no *ORM1*-null homozygote was reported, and the frequency of *ORM2* null variation was estimated to be very low in Japanese (0.04).[25]

In the 113 patients without any duplicated *ORMs*, we found 14 novel *ORM* variations (seven in *ORM1* and seven in *ORM2*) including one nonsynonymous variation, *ORM2* 328G>A (E110R). The frequencies of novel variations were less than 0.06. We also detected several known *ORM* SNPs/variations reported in Africans including Ghanaians.[10,11] The nonsynonymous SNPs, *ORM1* 113G>A (Q38R) and *ORM2* 421G>C (G141R), which were reported in a variety of ethnic populations and designated as *ORM1*S* and *ORM2*H19*, respectively,[8] were also found in our Japanese population at frequencies of 0.171 and 0.044, respectively.

Because the LD analysis indicated the region covering the *ORM* genes as a single LD block, the haplotypes through the entire *ORM* region were determined. Forty-one haplotypes were identified/inferred, and these were classified into four groups based on two nonsynonymous SNPs, *ORM1* 113G>A (Q38R) and *ORM2* 421G>C (G141R). The frequency of haplotype **I*, which is the most common haplotype and corresponds to the reported *"ORM*F1-ORM2*M"*,[14] is 0.823. The current analysis proved that the reported variation *ORM1*S*, 113G>A (Q38R), could be further classified into two groups, **II* and **III*, and the reported *ORM2*H19*, 421G>C, (G141R), could be classified into two groups, **III* and **IV* (Fig. 3).

Association of the *ORM* Genotypes on the Serum AGP Level

Next, we examined the impact of copy number and genetic variations of *ORMs* on serum AGP levels at baseline in patients receiving PTX therapy. The results showed that the duplication of *ORM1* or *ORM2* did not affect AGP levels. The previous report speculated that in the *ORM* duplication formation, wherein a recombination process was assumed, the second *ORM1* gene might be inserted between the 5′-flanking region and intron 2 of the downstream *ORM2*.[14] In that study, in a majority of subjects with *ORM1* duplication, the 5′-flanking region of the second *ORM1* contained the Alu sequence of the 5′-flanking region of *ORM2*,[14] implying that majority of the second *ORM1* might have the regulatory region of *ORM2*. Currently, it is generally recognized that the expression level of *ORM1* is higher than that of *ORM2* (approximately 3:1 as AGP in the plasma)[26–28] and that the hepatic mRNA level of *ORM2* was reported to be very low.[5] These reports imply that the expression level of the second *ORM1* might be downregulated similarly as *ORM2*, and this might partly explain the lack of apparent effect of the duplicated *ORMs* on the AGP level and PK parameters of PTX. In this context, it is of interest that our sequencing of *ORM1* and *ORM2* in the 5′-flanking region revealed substantial differences in the corresponding sites/region of more than 600 bases upstream of the translational initiation site between the two genes. Thus, the regulatory mechanisms for the expression of *ORM1* and *ORM2* might be different.

Regarding the effects of *ORM* variations and haplotypes on the serum AGP levels, we found similar associations results with *ORM1* −559T>A, *ORM2* 421G>C (G141R), or the haplotype **III* harboring nonsynonymous SNPs *ORM1* 113G>A (Q38R) and *ORM2* 421G>C (G141R). Then, we further evaluated the contributions of the *ORM* variations and identify the responsible genetic marker associated with AGP levels by multivariate analysis, considering the nongenetic factors selected in the bivariate analysis. After adjusting for patient background and blood test data, the increasing tendency by *ORM1* −559T>A was still observed with borderline significance (Table 6). On the contrary, no significant correlation with *ORM2* 421G>C (G141R) was found (data not shown). The functional significance of *ORM1* −559T>A and other linked SNPs is unknown, but these variations are not located in the region of a putative glucocorticoid responsive element (GRE) corresponding to GREs of rodent orosomucoid genes. Therefore, functional investigation of this and other linked variations is needed.

Association of the *ORM* Genotypes on the PK of PTX

Regarding the association of *ORM* variations on the PK of PTX, significant increases in the AUC values of *di* OH–PTX and 6α-OH–PTX were observed in patients bearing *ORM1* −559T>A compared with wild-type patients, whereas there was no difference in the AUC of PTX between them. As a close association between AGP levels and AUC values of the PTX metabolites was observed (Fig. 6), these results may be reflected by the higher levels of AGP in patients with *ORM1* −559T>A. On the contrary, the effects of the *ORM* genotypes on the clearance of the PTX were not significant; thereby, the clinical significance of the *ORM* genetic variations for PTX therapy may not be substantial.

Relationship Between AGP Level and PK of PTX

The current study revealed positive correlations between AGP levels and the AUC values of PTX metabolites (Fig. 6). A previous report showed that the PTX binds to AGP stronger than to albumin.[29] The reported PTX amount possibly binding to AGP per mg protein was more than 10 times larger than that binding to albumin. AGP was demonstrated to be incorporated into the liver via the asialoglycoprotein receptor or other undefined receptors that interact with the carbohydrate moiety of AGP, and the drugs binding to AGP are also thought to be transported into the liver via these AGP receptor systems.[30,31] These findings led us to hypothesize that higher AGP levels could increase the uptake of the PTX–AGP complex from the plasma into the liver via the AGP receptors, and this might result in the enhancement of the PTX metabolism. In other words, AGP may function as a carrier of PTX from the blood into the liver. In addition, however, the possibility could not be excluded that PTX metabolites may also bind to AGP or other constituents in the blood and this may lead to the reduced clearance of PTX metabolites.

Regarding influence of serum AGP level on PK/PD of drugs, several reports showed that the associations between the increased plasma AGP level and the lower clearance of drugs and/or lower drug responses for chemotherapies with docetaxel and imatinib.[2,3,32,33] These reports implicated that the

free fraction of the active parent drug in the plasma was lowered by its binding to AGP, and this may reduce its metabolism and delivery into the target tissue. In contrast, our study on PTX revealed that PTX clearance was not correlated with the serum AGP level. This observation could be explained by the fact that cremophor, an additive for the PTX injection that solubilizes PTX by forming micelles, holds PTX not to be delivered to the tissues,[34] and this may minimize the influence of the fluctuation of AGP level on the total concentration of PTX in plasma.

In conclusion, the present study provided detailed information on *ORM* genetic variations and haplotype structures in Japanese and also identified *ORM* variations that were associated with AGP levels and PTX metabolism. In addition, a possible clinical role of AGP as a carrier of drug from the blood into the hepatocytes was suggested. Further studies are needed to clarify the clinical significance of the *ORM* genotypes and AGP for the PK/PD of PTX or other drugs binding to AGP.

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PHASE I STUDIES

Safety and pharmacokinetic study of *nab*-paclitaxel plus carboplatin in chemotherapy-naïve patients with advanced non–small cell lung cancer

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Summary *Background* Nanoparticle albumin-bound paclitaxel (*nab*-paclitaxel) is a Cremophor EL–free formulation of paclitaxel newly designed to avoid solvent-related toxicities. We have evaluated the safety, tolerability, pharmacokinetics, and tumor response profile of weekly *nab*-paclitaxel (100 mg/m^2) infusion together with administration of carboplatin at an area under the curve (AUC) of 6 every 3 weeks in Japanese patients with advanced non–small cell lung cancer (NSCLC). *Methods* *Nab*-paclitaxel (100 mg/m^2) was administered without steroid or antihistamine premedication as a 30-min intravenous infusion once a week in combination with carboplatin at an AUC of 6 on day 1 of repeated 21-day cycles. The pharmacokinetics of both drugs were analyzed, and both adverse events and treatment response were monitored. *Results* Eighteen patients were enrolled in the study. The most frequent treatment-related toxicities of grade 3 or 4 were neutropenia (67%), leukopenia (50%), and anemia (22%). No severe hypersensitivity reactions were observed despite the lack of premedication, and no unexpected or new toxicities were detected. Pharmacokinetics analysis did not reveal any substantial drug-drug interactions. Seven partial responses were observed among the 18 evaluable patients, yielding a treatment response rate of 38.9%. *Conclusions* The combination of *nab*-paclitaxel (100 mg/m^2) administered weekly and carboplatin at an AUC of 6 every 3 weeks was well tolerated in Japanese patients with advanced NSCLC. This combination therapy also showed promising antitumor activity and was not associated with relevant pharmacokinetic interactions.

Keywords *Nab*-paclitaxel · Carboplatin · Non–small cell lung cancer · Pharmacokinetics · Safety

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Introduction

Lung cancer is the leading cause of death related to cancer worldwide, with non–small cell lung cancer (NSCLC) accounting for 85% of lung cancer cases [1]. Platinum-based chemotherapy is the mainstay of first-line treatment for advanced NSCLC on the basis of the moderate improvement in survival and quality of life it confers compared with best supportive care alone [2]. Given the safety and efficacy limitations of current therapeutic options, however, new chemotherapeutic agents and combi-

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nation regimens are needed to further ameliorate symptoms and increase antitumor activity in a manner that is both convenient and safe in patients with advanced NSCLC.

The most commonly used taxane combination regimen for treatment of advanced NSCLC is carboplatin plus solvent-based paclitaxel. Paclitaxel is highly hydrophobic, and first-generation formulations include Cremophor EL (polyoxyethylated castor oil) and an ethanol vehicle to allow parenteral administration [3]. Given that Cremophor EL causes leaching of the plasticizers from standard intravenous tubing and is also associated with hypersensitivity reactions, administration of solvent-based paclitaxel requires a long infusion period (typically 3 h), the use of special non–polyvinyl chloride infusion systems and in-line filtration, and premedication with corticosteroids, diphenhydramine, and an H_2 histamine receptor antagonist to minimize the incidence of potentially life-threatening hypersensitivity [4, 5]. Severe and sometimes fatal hypersensitivity reactions sometimes still occur, however, even after administration of these premedications [6].

Nanoparticle albumin-bound paclitaxel (*nab*-paclitaxel, Abraxane) was developed for delivery of paclitaxel as a suspension of albumin particles in saline, allowing a shorter infusion time and use of a standard infusion set [7]. This new Cremophor EL–free formulation does not require steroid and antihistamine premedication to prevent hypersensitivity reactions. Furthermore, preclinical studies have suggested that this formulation may improve drug delivery into tumors [8]. In phase I trials, *nab*-paclitaxel has been administered safely at doses higher than labeled doses for solvent-based paclitaxel [7]. A phase III trial in patients with advanced breast cancer showed that administration of *nab*-paclitaxel every 3 weeks (q3w) resulted in a significantly higher response rate (33 versus 19%, $P<0.001$) and longer time to tumor progression (5.8 versus 4.2 months, $P<0.006$) compared with q3w solvent-based paclitaxel [9]. A phase II study of *nab*-paclitaxel at 260 mg/m^2 q3w in chemotherapy-naïve patients with advanced NSCLC also revealed single-agent antitumor activity with a response rate of 16% [10]. Furthermore, weekly administration of *nab*-paclitaxel (125 mg/m^2) yielded an increased response rate of 30% in 40 individuals with advanced NSCLC who had not received prior chemotherapy [11]. More recently, a dose-finding phase II study demonstrated that *nab*-paclitaxel administered weekly was associated with less serious adverse events when administered q3w, with significant reductions in the incidence of peripheral neuropathy, myalgia, arthralgia, and alopecia [12]. In the phase II study, weekly administration of *nab*-paclitaxel at 100 mg/m^2 combined with carboplatin (area under the curve [AUC], 6) yielded a response rate of 48% and median progression-free survival of 6.2 months as first-line treatment for advanced NSCLC [12]. Given the promising efficacy and excellent safety of *nab*-paclitaxel, the combination of weekly *nab*-paclitaxel with carboplatin warrants further investigation. To date, however, pharmacokinetic data for such treatment have been limited. The primary objective in the present study was to evaluate the safety of weekly *nab*-paclitaxel (100 mg/m^2) administered in combination with q3w carboplatin at an AUC of 6 in Japanese advanced NSCLC without prior systemic chemotherapy. The secondary objectives were to determine the pharmacokinetics of paclitaxel after *nab*-paclitaxel administration on cycle 1 days 1 (with carboplatin) and 15 (without carboplatin).

Patients and methods

Patients

Eligible patients were 18 years of age or older with histologically or cytologically confirmed NSCLC of stage IIIB or IV. They were required to be naïve with regard to chemotherapy for metastatic disease. The eligibility criteria also included adequate bone marrow, hepatic, and renal function, an Eastern Cooperative Oncology Group performance status of 0 or 1, a life expectancy of >12 weeks, and radiologically documented measurable disease. Individuals were excluded if they had evidence of active brain metastasis or preexisting peripheral neuropathy of grade ≥2 defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0, or if they had received radiotherapy in the previous 4 weeks. Patients with any other clinically serious concurrent illness were also excluded.

The study followed the ethical principles in the Declaration of Helsinki, and the study protocol was approved by the institutional review board of each participating center. All patients received information regarding the nature and purpose of the study, and they provided written informed consent before study-related procedures were performed.

Treatment

The study was conducted to evaluate the safety, tolerability, pharmacokinetics, and tumor response profile of weekly *nab*-paclitaxel at 100 mg/m^2 and q3w carboplatin at an AUC of 6 in Japanese patients with advanced NSCLC. Carboplatin was administered at an AUC of 6 calculated according to the Calvert formula on day 1 of a 21-day cycle. *Nab*-paclitaxel (100 mg/m^2) was administered by a 30-min intravenous infusion on days 1, 8, and 15 of each cycle without steroid or antihistamine premedication to prevent a hypersensitivity reaction. On days of carboplatin dosing, patients received the serotonin/5-hydroxytryptamine receptor 3 (5-HT_3) antagonist as antiemetic therapy. A