Irinotecan is an important drug for the treatment of various tumors including lung, colon, and gastric (Smith et al., 2006). The infused drug is metabolized to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterases, and SN-38 is inactivated by UDP-glucuronosyltransferase (UGT)1A1-mediated glucuronidation. UGT1A9 also mediates this reaction. In a recent study, it was reported that the UGT1A9 IVS1+399 (I399)C>T polymorphism is associated with increased SN-38 glucuronidation both in vitro and in vivo. However, its role in UGT1A9 expression levels and activity is controversial. Thus, we evaluated the role of I399C>T in SN-38 glucuronidation using 177 Japanese cancer patients administered irinotecan. I399C>T was detected at a 0.86 allele frequency. This polymorphism was in strong linkage disequilibrium (LD) with UGT1A9 IVS1-1b (−126_−118T>G, D'=0.99) and UGT1A1*6 (211G>A, 0.86), in moderate LD with UGT1A1*60 (−327T>G, 0.55), but weakly associated with UGT1A1*28 (−54_−39A(TA)TAA>A(TA)TAA, 0.25). Haplotype analysis showed that 98% of the I399C alleles were linked with low-activity haplotypes, either UGT1A1*6, *28, or *60. On the other hand, 85% of the T alleles were linked with the UGT1A1 wild-type haplotype *1. Although I399T-dependent increases in SN-38 glucuronide/SN-38 area under concentration-time curve (AUC) ratio (an in vivo marker for UGT1A activity) and decreases in SN-38 AUC/dose were apparent (P<0.0001), these effects were no longer observed after stratified patients by UGT1A1*6, *28, or *60 haplotype. Thus, at least in Japanese populations, influence of I399C>T on SN-38 glucuronidation is attributable to its close association with either UGT1A1*6, *28, or *60.

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ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; UGT, UDP-glucuronosyltransferase; SNP, single nucleotide polymorphism; SN-38G, SN-38 glucuronide; AUC, area under concentration-time curve; I399, UGT1A9 IVS1+399; LD, linkage disequilibrium.
SN-38 glucuronidation activity by this SNP is significant among subjects without UGT1A1*28. Sandanaraj et al. (2008) have also reported that I399C/C patients showed higher SN-38 AUC than C/T and T/T patients. With the same UGT1A1 diplotypes, patients with I399T/T (and UGT1A9-126,-118T>T) have shown higher SN-38G Cmax with SN-38CT (and T/TG) patients. UGT1A9*1b (UGT1A9-126,-118T>TG) has been shown to have no effect on UGT1A9 expression levels (Girard et al., 2006; Ramirez et al., 2007; Sandanaraj et al., 2008). Thus, two groups did suggest that I399CT allele was associated with higher glucuronidation activity. However, using human liver microsomes, Ramirez et al. (2007) showed that I399CT had no significant effect on both UGT1A9 mRNA levels and glucuronidation activities for two UGT1A9 substrates. Therefore, the roles of I399CT in UGT1A9 activities as well as SN-38 glucuronidation remain inconclusive.

In the present report, we reveal the linkage of I399C>T with UGT1A1, UGT1A7, and UGT1A9 polymorphisms and analyze its association with the SN-38G/SN-38 AUC ratio and SN-38 AUC/dose (per dose) to clarify its role in SN-38 glucuronidation.

Materials and Methods

Patients. One hundred and seventy-seven patients (81 lung, 63 colon, 19 stomach, and 14 other cancer patients) administered irinotecan at the National Cancer Center were enrolled in this study as described previously (Minami et al., 2007). This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants. Eligibility criteria, patient profiles, and irinotecan regimens are summarized in our previous report (Minami et al., 2007). In brief, patients consisted of 135 males and 42 females with a mean age of 60.5 (26–78 years old), and their performance status was 0 (84 patients), 1 (89 patients), or 2 (4 patients). Irinotecan administrations with a mean age of 60.5 (26–78 years old), and their performance status was 0 (84 patients), 1 (89 patients), or 2 (4 patients). Irinotecan administrations were determined previously (Saeki et al., 2006). Hardy-Weinberg equilibrium (p = 0.1 frequencies in Japanese populations (Saeki et al., 2006). When assessed by the ID1 value, I399CT was in complete LD with UGT1A7 387T>G, 391C>A and 392G>A (UGT1A7*2, ID1 = 1.00); in strong LD with UGT1A9-126,-118T>TG (UGT1A9*1b, 0.987), UGT1A7 622T>C (UGT1A7*3, 0.977), and UGT1A1 211G>A (UGT1A1*6, 0.864); and in moderate LD with UGT1A1-3279T>G (UGT1A1*60, 0.554), but weakly associated with UGT1A1-54,-39A(TA)7TAA>A(TA)7TAA (UGT1A1*6, 0.252). In r2 values, the I399CT was in strong LD with UGT1A7*2 (r2 = 0.976) and UGT1A9*1b (0.916), in moderate LD with UGT1A7*3 (0.478), but in weak LD with UGT1A1*6 (0.261) and UGT1A1*60 (0.208), and in little LD with UGT1A1*28 (0.018).

Haplotypic Analysis. Haplotype analysis was performed using the 9 polymorphisms including I399C>T. As shown in Fig. 1, 95% (123/129) of the I399C alleles were linked with the UGT1A9-126,-118T alleles, and 100% (225/225) of the T alleles were linked with the T10 alleles (UGT1A9*1b). The 98% (126/129) of the I399C alleles were completely unchanged activity for SN-38 glucuronidation (Gagne et al., 2002), nor with the *28 allele, was weakly associated with the reduced ratio.

Association Analysis. The associations of I399C>T with irinotecan pharmacokinetic parameters were then analyzed using the estimated haplotypes. First, association with SN-38G/SN-38 AUC ratio, an in vivo parameter of UGT1A activity (Sai et al., 2004; Minami et al., 2007; Sandanaraj et al., 2008), was analyzed. UGT1A7*2 had unchanged activity for SN-38 glucuronidation (Gagne et al., 2002), and neither UGT1A9*1b nor UGT1A7*3 had significant effects on the SN-38G/SN-38 AUC ratio in our previous study (Minami et al., 2007). On the other hand, the UGT1A1*6, *28, and *60 haplotypes were associated with the reduced SN-38G/SN-38 AUC ratios (Minami et al., 2007). Although effects of the haplotype *28 and *6 were more striking, haplotype UGT1A1*60, harboring only the *60 allele without the *28 allele, was weakly associated with the reduced ratio. To remove even this weak effect and clarify the real effect of I399C>T, UGT1A1*60 was also considered as low-activity haplotype in this analysis. Namely, we analyzed the associations of I399C>T with the AUC ratio within the groups stratified by the UGT1A1 haplotypes, UGT1A1*28 (*28b and *28d), *6 (*6a), and *60 (*60a) (combined and shown as UGT1A1*4).
increase in the SN-38G/SN-38 AUC ratio was observed ($p < 0.0001$, Jonckheere-Terpstra test) (Fig. 2A). However, this trend was obviously dependent on biased distributions of UGT1A1 haplotypes; e.g., 96% of the I399C/C patients were homozygotes for UGT1A1*28, *6, or *60; and “UGT1A1*28, *6, or *60”-dependent reduction of SN-38G/SN-38 AUC ratio was found within the I399T/T genotypes ($p < 0.05$). As shown in Fig. 2B, SN-38G/SN-38 AUC ratio was found within the I399T/T genotypes ($p < 0.0001$), biased distributions of the I399C-dependent reduction of SN-38 AUC/dose was detected ($p < 0.0001$), and no significant effect of I399C on SN-38 glucuronidation, a possible enhancing effect has been suggested. Girard et al. (2006) have shown that I399C>T was associated with increased UGT1A9 protein levels and enzyme activity toward an UGT1A9 probe drug propofol using human liver microsomes derived mainly from whites. In contrast, using human liver microsomes from 46 white subjects, Ramírez et al. (2007) have revealed that the I399C>T had no significant effects on UGT1A9 mRNA levels and in vitro glucuronidation activities toward the two UGT1A9 substrates, flavopiridol and mycophenolic acid. Furthermore, another report has demonstrated that I399C>T had no influence on the pharmacokinetic parameters (such as AUC and $C_{\text{max}}$) of mycophenolic acid in 80 Japanese renal transplant recipients (Inoue et al., 2007). Thus, these latter two studies did suggest that the I399C>T polymorphism has no effect on UGT1A9 enzymatic activity. Note that, at least for Japanese populations, no study has reported that I399C>T affects UGT1A9 activity.

**Discussion**

In the present study, LD between I399C>T and UGT1A1, UGT1A7, or UGT1A9 polymorphisms in Japanese populations was shown for the first time. Moreover, the apparent effect of I399C>T on SN-38 glucuronidation in Japanese cancer patients was suggested to result from its close association with UGT1A1*28, *6, or *60.

### Table

<table>
<thead>
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<th>Gene</th>
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<th>UGT1A1</th>
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<td>IVS1+399 C&gt;T</td>
<td>387 T&gt;G</td>
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<td>Allele name</td>
<td>*1b</td>
<td>*2, *3</td>
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**Fig. 1.** Haplotypes assigned by using common UGT1A9, UGT1A7, and UGT1A1 polymorphisms. 1Haplotypes were shown as UGT1A9 haplotypes – UGT1A7 haplotypes – UGT1A1 haplotypes. Major allele, white blocks; minor allele, gray blocks. 2IC*3-6a, *2, *3, *2, *3, *3, *60, *60, *28, *28, *6. 3UGT1A1 alleles, or UGT1A9 alleles. 4Haplotypes-dependent increase was again evident, and the UGT1A1 haplotypes-dependent increase was significant within the I399 C/T and T/T patients ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 2C). Moreover, no significant effect of I399C>T on SN-38 AUC/dose was found when stratified by the UGT1A1 haplotypes ($p > 0.05$ within the −/−, −/+ or +/+ patient group in Fig. 2D).

As for the influence of I399C>T on UGT1A9 activity, conflicting results have been reported. Girard et al. (2006) have shown that I399C>T was associated with increased UGT1A9 protein levels and enzyme activity toward an UGT1A9 probe drug propofol using 48 human liver microsomes derived mainly from whites. In contrast, using human liver microsomes from 46 white subjects, Ramírez et al. (2007) have revealed that the I399C>T had no significant effects on UGT1A9 mRNA levels and in vitro glucuronidation activities toward the two UGT1A9 substrates, flavopiridol and mycophenolic acid. Furthermore, another report has demonstrated that I399C>T had no influence on the pharmacokinetic parameters (such as AUC and $C_{\text{max}}$) of mycophenolic acid in 80 Japanese renal transplant recipients (Inoue et al., 2007). Thus, these latter two studies did suggest that the I399C>T polymorphism has no effect on UGT1A9 enzymatic activity. Note that, at least for Japanese populations, no study has reported that I399C>T affects UGT1A9 activity.

As for the influence of I399C>T on SN-38 glucuronidation, a possible enhancing effect has been suggested. Girard et al. (2006) have shown an increasing effect of I399C>T on SN-38 glucuronidation, and that this SNP did not show any close linkages with the UGT1A1*28 or *60 allele ($r^2 < 0.06$). In addition, Sandanaraj et al. (2008) have reported that in 45 Asians consisting of Chinese (80%), Malay (18%), and others (2%), I399C/C patients had higher SN-38 AUC than C/T and T/T patients. Again, this SNP was not in LD with the UGT1A1*28, *6, or *60 allele ($r^2$ were <0.09). Furthermore, association of I399T with increased SN-38G $C_{\text{max}}$ has been observed even after stratified patients by UGT1A1 genotypes, although the study sample size was small. These findings suggest that the I399T
allele was associated with increased glucuronidation activity for SN-38 without linkages with the UGT1A1 polymorphisms. Our data demonstrate that an increase in SN-38G/SN-38 AUC ratio (i.e., increased glucuronidation activity) was also found with I399C/H11022T; however, after stratified patients by the UGT1A1*6, *28, or *60 haplotypes (haplotype/–, no UGT1A1*28, *6, or *60; –/+; heterozygotes for either UGT1A1*28, *6, or *60; +/+), homozygotes or compound heterozygotes for either UGT1A1*28, *6, or *60. B and D, UGT1A1 –/–, –/+; and +/+ patients were further divided by I399 C/C, C/T, and T/T genotypes. Gene dose effects of I399C>T and the UGT1A1 + haplotype were assessed by the Jonckheere-Terpstra test.

In conclusion of this study, the apparent influence of I399 (UGT1A9 IVS1+399C>T on SN-38 glucuronidation is attributable to its close association with UGT1A1*6, *28, or *60 in the Japanese population. Furthermore, additional genotyping of I399C>T for personalized irinotecan therapy seems to be clinically irrelevant for Japanese populations.

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