Dose-Dependent Association between UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Low Doses Also Increase Risk

Zhe-Yi Hu1, Qi Yu2, Qi Pei3, and Cheng Guo2

Abstract

Purpose: A previous meta-analysis showed that the association between the UGT1A1*28 genotype and irinotecan-induced neutropenia was influenced by irinotecan dose and that the risk of neutropenia was similar at low doses for patients with all genotypes. However, the sample sizes for the low- and high-dose groups were small. Because additional studies have now been reported, an updated and refined meta-analysis is needed.

Experimental Design: Meta-analyses were done to assess the relationship between UGT1A1*28 and neutropenia. The association between UGT1A1*28 and the relative extent of glucuronidation (REG) of SN-38 was also examined. The studies included were stratified into different dose groups.

Results: A total of 1,998 patients were included for the analysis of neutropenia and 581 patients were included for REG. The risk of neutropenia at low doses was significantly higher among patients with a UGT1A1*28/*28 genotype than among those carrying the UGT1A1*1 allele(s) [relative risk (RR), 2.43; 95% confidence interval, 1.34-4.39; \( P = 0.003 \)]. In addition, the RR of neutropenia at low doses was comparable with that at medium doses (2.43 versus 2.00). The RR of neutropenia at high doses was significantly higher than that at low and medium doses (7.22 versus 2.04). We found the weighted mean difference of REG (UGT1A1*1/*1 or UGT1A1*1/*28 versus UGT1A1*28/*28) increased with increasing dose of irinotecan.

Conclusions: In conclusion, the UGT1A1*28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but also at low doses. The dose-dependent manner of SN-38 glucuronidation explained why the association between UGT1A1*28 and neutropenia was dose dependent. Clin Cancer Res; 16(15); 3832–42. ©2010 AACR.

Irinotecan is approved for use in combination with 5-fluorouracil and leucovorin for first-line treatment of metastatic colorectal cancer (1). Irinotecan efficacy is dependent on activation by carboxyesterases to form the active metabolite SN-38. The major route of SN-38 elimination is via glucuronidation by hepatic UGT1A enzymes (2).

UGT1A1*28 is a common allele with seven TA repeats in the TATA box of the UGT1A1 promoter compared with the wild-type allele, which has six TA repeats. UGT1A1*28 has been shown to be associated with decreased SN-38 glucuronidation in humans (3–11). The association between the UGT1A1*28 genotype and irinotecan-induced neutropenia has been extensively studied (3–5, 12–23). Seven of these studies found that UGT1A1*28/*28 patients had an elevated risk of neutropenia compared with those carrying UGT1A1*1 allele(s) (refs. 4, 12, 13, 17, 19–21).

In 2005, the U.S. Food and Drug Administration (FDA) recommended that Pfizer amend the package insert of irinotecan to warn of the elevated risk of neutropenia for UGT1A1*28/*28 patients. A subsequent meta-analysis showed that the association between UGT1A1*28 and neutropenia was influenced by the dose of irinotecan and that the risk of neutropenia was similar at lower doses for patients with all genotypes (1). It was noteworthy that the sample sizes for the low- and high-dose groups were small (229 patients and 81 patients). In addition, the studies included in this meta-analysis seemed to be weighted by sample size. This weighted method is not the recommended method in the Cochrane Handbook for Systematic Reviews of Interventions version 5.0.2 (http://www.cochrane-handbook.org/). Because additional

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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studies have now been reported, an updated and refined meta-analysis is needed. Moreover, the pharmacokinetic mechanism for the dose-dependent association between UGT1A1*28 and neutropenia is currently unclear. In this study, we carried out an updated and refined meta-analysis to study the association between the UGT1A1*28 genotype and irinotecan-induced neutropenia. To explore the pharmacokinetic mechanism for the dose-dependent association between UGT1A1*28 and neutropenia, the association between UGT1A1*28 and the relative extent of glucuronidation (REG) of SN-38 was also examined.

**Materials and Methods**

**Search strategy and selection criteria**

Two investigators (ZYH, QY) independently searched Medline, PubMed, and Embase (from 1980 until April 15, 2010) databases using the terms "irinotecan," "UGT1A1," and "neutropenia (or pharmacokinetics)". Furthermore, we reviewed citations in the retrieved articles to search for additional relevant studies. Data derived from abstracts were also used.

* A priori we defined strict criteria for inclusion of studies. Studies were included if (a) they could be defined as clinical trials, (b) the exposure of interest was the UGT1A1*28 genotype, (c) the outcome of interest was irinotecan-induced neutropenia (grade III-IV) or REG of SN-38, and (d) the numbers of patients with and without neutropenia were provided (or could be calculated by relevant data). We excluded studies that were not published in English, studies that included <20 patients, studies that included <4 UGT1A1*28/*28 patients (a criterion not considered when we study the association between UGT1A1*28 and REG), studies that reported hematologic toxicity instead of more specific neutropenia, and studies that included children patients.

**Data extraction**

The following information was abstracted from included publications: study design, year of publication, race, irinotecan dose, number of patients with and without neutropenia (grade III-IV) in each genotype group (UGT1A1*1/*1, UGT1A1*1/*28, and UGT1A1*28/*28), REG values in each genotype group (converted to the units of ng × h/mL versus ng × h/mL), mutation detection method, plasma sampling scheme, analytical method, and potential confounders.

Two irinotecan-containing regimens were given to patients in the N9741 study (21), and in our analyses, we analyzed the patients treated with each regimen as two separate samples. Two irinotecan regimens (250 mg/m² and 350 mg/m²) were administered to patients in another study (13). In this study, only three UGT1A1*28/*28 patients were included in the 350 mg/m²-dose group. Hence, this group was excluded from our analysis according to the inclusion criterion. The patients treated with different regimens were analyzed as one sample if separate data were not available. If the patients in a trial received different irinotecan doses and only combined toxicity-related data (or REG data) were available, we calculated the average dose. In one study (16), the numbers of chemotherapy cycles with neutropenia in each genotype group were provided instead of the number of patients. We calculated the number of patients as $C × tN/tC$, where $C$ is the number of chemotherapy cycles with neutropenia in each genotype group, $tC$ is the total number of chemotherapy cycles with neutropenia, and $tN$ is the total number of patients with neutropenia. REG values were reported as medians and ranges instead of means and SDs in three studies (5, 6, 9); we imputed the means and SDs as described by Hozo et al. (24). The REG values were obtained by visual measurement of the figures in two studies (10, 11). The REG values obtained from UGT1A1*1/*28 patients and UGT1A1*1/*1 patients were combined by the formula suggested in the Cochrane Handbook for Systematic Reviews of Interventions version 5.0.2 (http://www.cochrane-handbook.org/).

**Assessment of study quality**

The use of a quality scoring system in meta-analyses of observational studies is controversial. Methodologic components of study designs, rather than aggregate scores themselves, may be important (25). Here, we did not assign a single grade or scores to represent the quality of a study. Instead, we focused on certain items that are reflective of methodologic and reporting quality of the studies as delineated in the published guidelines for reporting of pharmacogenetic studies (26). In addition, we paid attention to other quality criteria that were specific to our study. These issues of concern were source of population, mutation detection method, analytical method, plasma sampling scheme, type of cancer, chemotherapy regimens, and grade criteria for neutropenia (see Supplementary Tables S1 and S2).
Statistical analysis

With regard to the association between UGT1A1*28 and neutropenia, the effect measures of interest were relative risks (RR). The effect measures of interest were mean differences (MD) for the association between UGT1A1*28 and REG of SN-38. Statistical heterogeneity among studies was evaluated using the $\chi^2$ test, $P$ values, and $I^2$ statistics (27). We considered both the presence of significant heterogeneity at the 10% level of significance and values of $I^2 > 56\%$ as an indicator of significant heterogeneity.

A fixed effects model was used to obtain summary RR (Mantel-Haenszel method) or weighted mean difference (WMD; inverse variance method). We evaluated potential publication bias statistically with the methods described by Begg and Mazumdar (28) and Egger (29).

The studies included were stratified into different dose groups. Irinotecan dose levels were pooled into the following three subgroups: low (<150 mg/m$^2$), medium (150-250 mg/m$^2$), and high (≥250 mg/m$^2$) doses on the basis of the three most commonly used dosage regimens (1). We used meta-regression analyses to investigate the effect of irinotecan dose on the association between UGT1A1*28 and neutropenia (or REG). Hardy-Weinberg equilibrium (HWE) was tested using $\chi^2$ test. We conducted a sensitivity analysis in which one or two studies were removed, and the rest were analyzed to evaluate whether there was a statistically significant effect on the results. All statistical tests were two-sided. Meta-analysis was done with Stata version 10.1 (Stata Corp.).

Results

Study characteristics and methodologic quality (association between UGT1A1*28 and neutropenia)

A total of 68 potentially relevant studies were evaluated (Fig. 1 shows the numbers of studies evaluated at each stage). Fifteen clinical trials including 1,998 patients were identified. Table 1 details the studies' characteristics. Three
included studies were published as abstracts (21–23). Patients were predominantly Caucasians in 11 trials. Four trials did not clearly report the race of the participants (16, 20, 22, 23). The patients in these four trials, however, might have been Caucasians because these trials were conducted in Europe or America and the reported frequency of the UGT1A1*28 allele was similar to that of Caucasians.

No deviation from HWE was detected in any of the identified studies (P > 0.05, χ² test).

Quality-assessment tables are shown in full in Supplementary Table S1. Study sample sizes were small (median, 89; range, 20-628). Of the 15 trials, 4 (27%) described a sample size calculation and a priori defined the power to detect effect sizes. Eleven trials (73%) were investigated prospectively. Genotyping procedures were described by five trials (33%). In one trial (11%), the patients were suffering from the same type of cancer. Single chemotherapy regimen was employed in five trials (33%). Five trials (56%) determined the concentrations of SN-38 glucuronide (SN-38G) directly (without hydrolysis of SN-38G) and the other two trials (22%) did not report it clearly. In six trials (67%), the plasma samples from each patient seemed enough for the accurate determination of the pharmacokinetic parameters.

The results of the meta-analysis are summarized in Table 3.

**Table 3. Characteristics of trials included in the meta-analysis of the association between UGT1A1*28 and neutropenia**

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>No. of patients</th>
<th>Age (median or mean)</th>
<th>Source of population</th>
<th>Mutation detection methods</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iyer 2002 (3)</td>
<td>Prospective</td>
<td>20</td>
<td>Unknown</td>
<td>Single center</td>
<td>SPR</td>
<td>C</td>
</tr>
<tr>
<td>Innocenti 2004 (4)</td>
<td>Prospective</td>
<td>59</td>
<td>60</td>
<td>Single center</td>
<td>SPR</td>
<td>Mainly C</td>
</tr>
<tr>
<td>Toffoli 2006 (5)</td>
<td>Prospective</td>
<td>250</td>
<td>61</td>
<td>Multicenter</td>
<td>PYRS</td>
<td>C</td>
</tr>
<tr>
<td>Cote 2007 (12)</td>
<td>Prospective</td>
<td>89</td>
<td>Unknown</td>
<td>Multicenter</td>
<td>SPR</td>
<td>C</td>
</tr>
<tr>
<td>Kweekel 2008 (13)</td>
<td>Retrospective</td>
<td>138</td>
<td>62</td>
<td>Multicenter</td>
<td>PYRS</td>
<td>C</td>
</tr>
<tr>
<td>Ferraldeschi 2009 (14)</td>
<td>Prospective</td>
<td>92</td>
<td>63</td>
<td>Single center</td>
<td>SPR</td>
<td>C</td>
</tr>
<tr>
<td>Marcello 2006 (15)</td>
<td>Prospective</td>
<td>95</td>
<td>68</td>
<td>Unknown</td>
<td>SPR</td>
<td>C</td>
</tr>
<tr>
<td>Massacesi 2006 (16)</td>
<td>Prospective</td>
<td>56</td>
<td>64</td>
<td>Multicenter</td>
<td>Sequencing</td>
<td>Unknown</td>
</tr>
<tr>
<td>Rouits 2004 (17)</td>
<td>Retrospective</td>
<td>73</td>
<td>62</td>
<td>Single center</td>
<td>PYRS</td>
<td>C</td>
</tr>
<tr>
<td>Carlini 2005 (18)</td>
<td>Prospective</td>
<td>62</td>
<td>61</td>
<td>Multicenter</td>
<td>SPR</td>
<td>Mainly C</td>
</tr>
<tr>
<td>Glimelius 2010 (19)</td>
<td>Retrospective</td>
<td>136</td>
<td>62</td>
<td>Multicenter</td>
<td>SPR</td>
<td>C</td>
</tr>
<tr>
<td>Pilott 2006 (20)</td>
<td>Retrospective</td>
<td>34</td>
<td>60</td>
<td>Single center</td>
<td>PYRS</td>
<td>Unknown</td>
</tr>
<tr>
<td>McLeod 2006* (21)</td>
<td>Prospective</td>
<td>103 or 109</td>
<td>Unknown</td>
<td>Multicenter</td>
<td>Unknown</td>
<td>Mainly C</td>
</tr>
<tr>
<td>Roth 2008† (22)</td>
<td>Prospective</td>
<td>628</td>
<td>Unknown</td>
<td>Multicenter</td>
<td>SPR</td>
<td>Unknown</td>
</tr>
<tr>
<td>Tan 2008† (23)</td>
<td>Prospective</td>
<td>54</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

(Continued on the following page)
When all the dose groups are combined, however, publication bias may exist (Egger's test, \( P = 0.043 \); Table 3). This may be caused by the three high-dose studies with high RR estimates.

Overall analyses suggest an increased risk of neutropenia among UGT1A1*28/*28 patients as compared with those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (RR, 2.20; 95% CI, 1.82-2.66; \( P < 0.001 \); Fig. 2A). Heterogeneity was not statistically significant across all studies (\( I^2 = 16.3\% \), \( P = 0.267 \)). The value of \( I^2 \) suggested the existence of weak heterogeneity across all studies, although without statistical significance. Hence, we did meta-regression to determine whether irinotecan dose was a significant source of heterogeneity across studies. Not unexpectedly, meta-regression showed that irinotecan dose was a significant source of heterogeneity (\( P = 0.006 \); Fig. 3A1).

Analyses were further stratified by irinotecan doses. Unexpectedly, the risk of neutropenia at low doses was significantly higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR, 2.00; 95% CI, 1.62-2.47; \( P < 0.001 \); Fig. 2A). With regard to the high-dose subgroup, the risk of neutropenia was much higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR, 7.22; 95% CI, 3.10-16.78; \( P < 0.001 \); Fig. 2A). The RR of neutropenia at high doses was significantly higher than that at low and medium doses (RR, 7.22; 95% CI, 3.10-16.78 versus RR, 2.04; 95% CI, 1.67-2.49; Table 3).

Figure 3A1 shows the relationship between the RRs of neutropenia and irinotecan doses. There was only a slight increase in RRs of neutropenia when irinotecan doses increased from 80 to 250 mg/m\(^2\). However, the RRs of neutropenia increased dramatically when doses increased from 250 to 350 mg/m\(^2\).

It should be pointed out that the N9741 trial included only grade IV toxicity (21). The study by Roth et al. (22) was given the most weight (54%) in our meta-analysis. However, exclusion of both studies from the meta-analysis did not change the general result. For example, the risk of neutropenia at low doses was still higher among UGT1A1*28/*28 patients than among those with at least one UGT1A1*1 allele (RR, 2.61; 95% CI, 1.39-4.91; \( P = 0.003 \)). In addition, the RR of neutropenia at low doses was comparable with that at medium doses (2.61 versus 2.14).
Association between UGT1A1*28 and neutropenia (UGT1A1*1/*28 versus UGT1A1*1/*1)

Fourteen trials compared the risk of neutropenia between patients with a UGT1A1*1/*28 genotype and those with a wild-type genotype (3–5, 12–19, 21–23). One study was excluded from analysis because none of the patients suffered from neutropenia (3). Egger’s test showed evidence of publication bias (Table 3). When we excluded the study by Roth et al. (22), the signs of publication bias disappeared (Egger’s test, \( P = 0.183 \) for low- and medium-dose group; \( P = 0.980 \) for medium-dose group). It is noteworthy that the study by Roth et al. (22) was published as an abstract.

The pooled RR was 1.43 (95% CI, 1.16-1.77; \( P = 0.001 \)) for all studies (Table 3). No statistical heterogeneity was detected (\( I^2 = 0 \), \( P = 0.529 \)). Meta-regression showed that irinotecan dose was not a significant source of heterogeneity (\( P = 0.626 \); Fig. 3B1).

In a sensitivity analysis excluding two studies (N9741 trial; ref. 21) and the study by Roth et al. (22), the results were unchanged. For example, the RR of neutropenia still did not show significant difference among three subgroups. Irinotecan doses had no influence on RR of neutropenia (\( P = 0.454 \)).

Table 2. Characteristics of trials included in the meta-analysis of the association between UGT1A1*28 and relative extent of glucuronidation of SN-38

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>No. of patients</th>
<th>Age (median or mean)</th>
<th>Race</th>
<th>Type of tumors</th>
<th>Sampling scheme</th>
<th>Irinotecan dose (mg/m²)/schedule</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iyer 2002 (3)</td>
<td>Prospective</td>
<td>20</td>
<td>Unknown</td>
<td>C</td>
<td>Solid tumors</td>
<td>15 time points, up to 24 h</td>
<td>300 every 3 wk</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Innocenti 2004 (4)</td>
<td>Prospective</td>
<td>61</td>
<td>60</td>
<td>Mainly C</td>
<td>Solid tumors</td>
<td>14 time points, up to 25.5 h</td>
<td>350 every 3 wk</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Toffoli 2006 (5)</td>
<td>Prospective</td>
<td>71</td>
<td>61</td>
<td>C</td>
<td>mCRC</td>
<td>16 time points, up to 50 h</td>
<td>180 biweekly</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>de Jong 2007 (6)</td>
<td>Retrospective</td>
<td>134</td>
<td>55</td>
<td>C</td>
<td>Solid tumors</td>
<td>Multiple time points, up to 20 time points</td>
<td>350 every 3 wk</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Mathijssen 2003 (7)</td>
<td>Unknown</td>
<td>53</td>
<td>53</td>
<td>Mainly C</td>
<td>Solid tumors</td>
<td>14 time points, up to 48 h</td>
<td>200 to 350 every 3 wk</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Mathijssen 2004 (8)</td>
<td>Prospective</td>
<td>30</td>
<td>55</td>
<td>C</td>
<td>Solid tumors</td>
<td>15 time points, up to 55 h</td>
<td>350 every 3 wk</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Paoluzzi 2004 (9)</td>
<td>Unknown</td>
<td>86</td>
<td>54</td>
<td>C</td>
<td>Solid tumors</td>
<td>Unknown</td>
<td>350 every 3 wk*</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Minami 2007 (10)</td>
<td>Unknown</td>
<td>85</td>
<td>61</td>
<td>A</td>
<td>Solid tumors</td>
<td>7 time points, up to 24 h</td>
<td>60-150/wk or biweekly</td>
<td>HPLC-Flu</td>
</tr>
<tr>
<td>Sai 2004 (11)</td>
<td>Unknown</td>
<td>41</td>
<td>62</td>
<td>A</td>
<td>Solid tumors</td>
<td>7 time points, up to 24 h</td>
<td>60-150/wk or biweekly</td>
<td>HPLC-Flu</td>
</tr>
</tbody>
</table>

NOTE: Solid tumors indicate multiple solid tumor types. Abbreviations: A, Asian; HPLC-Flu, high-performance liquid chromatography with fluorescence detection.

*Patients were treated with irinotecan once every 3 weeks at a fixed dose of 600 mg. A fixed dose of 600 mg was similar to a dose of 350 mg/m².

Association between UGT1A1*28 and REG (UGT1A1*1/*1 or UGT1A1*1/*28 versus UGT1A1*28/*28)

Nine included trials compared REG of SN-38 between patients carrying UGT1A1*1 allele(s) and those with a UGT1A1*28/*28 genotype (3–11). There was no evidence of publication bias according to either Begg’s test or Egger’s test (Table 3).

Overall analyses suggest an increased REG of SN-38 among patients carrying UGT1A1*1 allele(s) as compared with those with a UGT1A1*28/*28 genotype (WMD, 2.44; 95% CI, 1.73-3.14; \( P < 0.001 \); Fig. 2B). No statistical heterogeneity was detected (\( I^2 = 0 \), \( P = 0.642 \)). Analyses were further stratified by dose. WMD of REG at low and medium doses (1.62; 95% CI, 0.57-2.68; \( P = 0.002 \)) was lower than that at high doses (3.08; 95% CI, 2.14-4.02; \( P < 0.001 \); Fig. 2B). Heterogeneity between subgroups was significant (\( P = 0.043 \)). Figure 3A2 shows a nonsignificant positive correlation between WMD of REG and irinotecan doses (\( P = 0.124 \)).

Association between UGT1A1*28 and REG (UGT1A1*1/*1 versus UGT1A1*1/*28)

Nine included trials compared REG of SN-38 between patients with a UGT1A1*1/*1 genotype and those with a UGT1A1*28/*28 genotype (3–11). There was no...
evidence of publication bias according to either Begg’s test or Egger’s test (Table 3).
Analyses were stratified by dose. WMD of REG showed no significant difference between the low/medium-dose group (1.85; 95% CI, 1.00-2.70; *P* < 0.001) and the high-dose group (1.03; 95% CI, −0.09 to 2.16; *P* = 0.072; Table 3). Heterogeneity between subgroups was not significant (*P* = 0.225). There was no correlation at all between WMD of REG and irinotecan doses (Fig. 3B2).

**Discussion**

The primary finding of this study is that the UGT1A1*28/*28 genotype was associated with an increased risk of

<table>
<thead>
<tr>
<th>Table 3. Summary of meta-analysis</th>
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<tbody>
<tr>
<td><strong>Comparison or outcome</strong></td>
</tr>
<tr>
<td>Neutropenia</td>
</tr>
<tr>
<td>Neutropenia (*28/*28 vs. *1/*1 or *1/*28)</td>
</tr>
<tr>
<td>Neutropenia (*28/*28 vs. *1/*1 or *1/*28)</td>
</tr>
<tr>
<td>Neutropenia (*28/*28 vs. *1/*1 or *1/*28)</td>
</tr>
<tr>
<td>Neutropenia (*28/*28 vs. *1/*1 or *1/*28)</td>
</tr>
<tr>
<td>Neutropenia (*28/*28 vs. *1/*1 or *1/*28)</td>
</tr>
<tr>
<td>REG (*1/*1 or *1/*28 vs. *28/*28)</td>
</tr>
<tr>
<td>REG (*1/*1 or *1/*28 vs. *28/*28)</td>
</tr>
<tr>
<td>REG (*1/*1 or *1/*28 vs. *28/*28)</td>
</tr>
<tr>
<td>Neutropenia (*1/*28 vs. *1/*1)</td>
</tr>
<tr>
<td>Neutropenia (*1/*28 vs. *1/*1)</td>
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<td>Neutropenia (*1/*28 vs. *1/*1)</td>
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<td>Neutropenia (*1/*28 vs. *1/*1)</td>
</tr>
<tr>
<td>Neutropenia (*1/*28 vs. *1/*1)</td>
</tr>
<tr>
<td>REG (*1/*1 vs. *1/*28)</td>
</tr>
<tr>
<td>REG (*1/*1 vs. *1/*28)</td>
</tr>
<tr>
<td>REG (*1/*1 vs. *1/*28)</td>
</tr>
</tbody>
</table>

NOTE: Ne, Begg’s and Egger’s tests were not done if <8 studies were included in the analyzed subgroup.
*The risk of toxicity was significantly higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype.
†The REG was significantly higher among patients with a UGT1A1*1/*1 genotype than among those with a UGT1A1*28/*28 genotype.
‡The risk of toxicity was significantly higher among patients with a UGT1A1*1/*28 genotype than among those with a UGT1A1*1/*1 genotype.
§REG was significantly higher among patients with a UGT1A1*1/*1 genotype than among those with a UGT1A1*1/*28 genotype.
neutropenia not only at medium or high doses of irinotecan but at low doses as well. The RRs of neutropenia among UGT1A1*28/*28 patients were comparable at low and medium doses. The secondary finding is that there was no correlation between the RRs of neutropenia among patients heterozygous for UGT1A1*28 (as compared with wild-type genotype) and irinotecan doses. The last finding is that WMD of REG (UGT1A1*1/*1 or UGT1A1*1/*28 versus UGT1A1*28/*28).
UGT1A1*28/*28) increased with increasing dose of irinotecan. In contrast, when comparing REG between patients with a UGT1A1*1/*1 genotype and those with a UGT1A1*1/*28 genotype, WMD of REG did not change with increasing dose of irinotecan. This finding sheds light on the mechanism of the dose-dependent association between UGT1A1*28 and neutropenia.

On the basis of the findings of four initial studies (4, 15, 17, 30), the FDA made the recommendation that patients with a UGT1A1*28/*28 genotype should receive a lower starting dose of irinotecan. Then came conflicting results from a meta-analysis (821 patients) by Hoskins et al. (1), suggesting the association between UGT1A1*28 and neutropenia was dose dependent; UGT1A1*28 had no effect in patients treated with a low dose of irinotecan (<150 mg/m²). Our current meta-analysis based on a large sample size (1,998 patients) indicates that UGT1A1*28/*28 patients are at an increased risk of neutropenia not only if they are being treated with medium (RR, 2.00) and high doses (RR, 7.22) of irinotecan but also if they are being treated with low doses (RR, 2.43; 80-145 mg/m²).

Diarrhea is another important side effect related to irinotecan administration. Recently, we found that UGT1A1*28/*28 patients were at an increased risk of diarrhea at medium (RR, 1.79; 95% CI, 1.08-2.97) or high doses (RR, 2.32; 95% CI, 1.25-4.28) of irinotecan, but not at low doses (RR, 0.65; 95% CI, 0.27-1.58; ref. 31). As a result, when selecting the dose of irinotecan for UGT1A1*28/*28 patients, this information should be considered together with the results of the current meta-analysis.

The implications for clinical practice should be considered. When regimens with a high dose of irinotecan are being considered, dose reduction is advisable for UGT1A1*28/*28 patients (neutropenia RR, 7.22; diarrhea RR, 2.32). When regimens with a medium dose of irinotecan are being considered, dose reduction is also recommended for UGT1A1*28/*28 patients (neutropenia RR, 2.00; diarrhea RR, 1.79). However, dose reduction is optional for UGT1A1*28/*28 patients (neutropenia RR, 2.43) treated at low irinotecan doses. In this regard, we suggest that UGT1A1*28/*28 patients with other predictors of irinotecan-induced toxicity be given a reduced dose.

**Fig. 3.** A1, RR of irinotecan-induced neutropenia (UGT1A1*28/*28 versus UGT1A1*1/*1 or UGT1A1*1/*28) against dose of irinotecan; A2, weighted mean difference (WMD) of REG (UGT1A1*1/*1 or UGT1A1*1/*28 versus UGT1A1*28/*28) against dose of irinotecan. B1, RR of irinotecan-induced neutropenia (UGT1A1*1/*28 versus UGT1A1*1/*1) against dose of irinotecan; B2, WMD of REG (UGT1A1*1/*1 versus UGT1A1*1/*28) against dose of irinotecan. Size of circle is proportional to the study-specific statistical weight. Marked “Excluded” indicates the excluded study by Iyer et al. (ref. 3; zero incidence of neutropenia).
of irinotecan. Other predictors of irinotecan-induced toxicity could be nongenetic factors (neutrophil baseline levels or sex) or genetic factors (UGT1A1*93, ABCC1 IVS11 −48C>T, or SLCO1B1*1b; ref. 32).

Recently, Toffoli et al. (33) evidenced that the recommended dose of 180 mg/m² for irinotecan in FOLFIRI is considerably lower than the dose that can be tolerated by the non-UGT1A1*28/*28 patients. This result was supported by our data that the incidence of neutropenia (%) was not increased with increasing dose of irinotecan in non-UGT1A1*28/*28 patients (Supplementary Fig. S1).

The limitations of this meta-analysis need to be considered. Firstly, the possibility of information and selection biases cannot be completely excluded because some of the included studies were retrospective. Secondly, although no statistical heterogeneity was observed among the analyzed studies, there were many sources of heterogeneity among the analyzed studies, such as study design, source of population, dose administered, chemotherapy regimens, mutation detection methods, toxicity grade criteria, sampling scheme, type of tumor, and stage of disease. Thirdly, three included trials were published as abstracts. Fourthly, in our analysis, grade III-IV neutropenia data were available for most studies, whereas only grade IV neutropenia information could be extracted from two studies (20, 21). Fifthly, for the study of the association between UGT1A1*28 and REG of SN-38, two included trials were conducted on Asian patients. Ethnic difference was not considered here.

To assess the potential for publication bias to have influenced the results of our meta-analysis, we calculated the failsafe numbers using a weighted method (35). A failsafe number could be defined as the number of nonsignificant, unpublished studies that would be needed to reduce a statistically significant observed result to nonsignificance (34, 35). The failsafe number was 14 for the low-dose group, 148 for the medium-dose group, and 21 for the high-dose group (at α = 0.05). It seems unlikely that such numbers of studies with null findings exist and have not been published.

In the present meta-analysis, we excluded two trials conducted on children according to the exclusion criterion (36, 37). The children in these two trials were administered with very low doses of irinotecan (30 and 50 mg/m²). Pooled RR from these two trials (118 patients) showed that the risk of neutropenia was similar between UGT1A1*28/*28 patients and those carrying UGT1A1*1 allele(s) (RR, 0.63; 95% CI, 0.14-2.72; P = 0.532). However, it is still too early to draw a reliable conclusion based on two trials including only 118 patients. As a result, further studies are required in this area.

In conclusion, the UGT1A1*28/*28 genotype was associated with an increased risk of neutropenia not only at medium or high doses of irinotecan but also at low doses. The dose-dependent manner of SN-38 glucuronidation explained why the association between UGT1A1*28 and neutropenia was dose dependent.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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