Examination of multiple UGT1A and DPYD polymorphisms has limited ability to predict the toxicity and efficacy of metastatic colorectal cancer treated with irinotecan-based chemotherapy: a retrospective analysis

Dan Liu†, Jian Li†, Jing Gao, Yanyan Li, Rui Yang and Lin Shen*

Abstract

Background: To evaluate a new UGT1A and DPYD polymorphism panel to better predict irinotecan-induced toxicity and the clinical response in Chinese patients with metastatic colorectal cancer (mCRC).

Methods: The genotypes of UGT1A (UGT1A1*6, UGT1A1*27, UGT1A1*28, UGT1A7*2, UGT1A7*3, UGT1A7*4 and UGT1A9*22) and DPYD (DPYD*5, DPYD c.1896 T > C, and DPYD*2A) were examined by direct sequencing in 661 mCRC patients receiving irinotecan-based chemotherapy. The influences of UGT1A and DPYD polymorphisms on severe irinotecan-induced toxicities and clinical outcomes were assessed.

Results: In the cohort studied here, the incidence of UGT1A1*6, UGT1A1*28, UGT1A7*2, UGT1A7*3, UGT1A9*22, DPYD*5, and DPYD c.1896 T > C variants were 34.8%, 24.2%, 34.3%, 39.4%, 81.8%, 48.4% and 20.4%, respectively. UGT1A1*27 and DPYD*2A had low frequencies and UGT1A7*4 was not found. A total of 59 patients (8.9%) suffered severe diarrhea and 136 patients (20.6%) suffered severe neutropenia. UGT1A1*28 heterozygotes (OR = 2.263, 95%CI 1.395–3.670), UGT1A1*28 homozygotes (OR = 5.910, 95%CI 1.138–30.672) and UGT1A1*6 homozygotes (OR = 4.737, 95%CI 1.946–11.533) were independent risk factors for severe neutropenia. UGT1A polymorphisms were not found to relate to severe diarrhea. DPYD*5 was determined to be an independent risk factor for severe diarrhea (OR = 2.143, 95%CI 1.136–4.041). Neither DPYD*5 nor DPYD c.1896 T > C was found to relate to severe neutropenia. In the first-line irinotecan-based treatment, UGT1A1*28 and DPYD*5 contributed to higher response rates (P = 0.043 and P = 0.019, respectively), while DPYD*5 was found to associate with better progression-free survival (P = 0.015). UGT1A1*27 contributed to worse overall survival (P < 0.001).

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Conclusion: Results still showed UGT1A1*6 and UGT1A1*28 to be partially associated with irinotecan-induced toxicity and clinical response. An examination of more UGT1A loci, except for UGT1A1*6 and UGT1A1*28, was not helpful to improve the predictive value of irinotecan-based toxicity and efficacy. An examination of DPYD*5 assisted in the prediction of severe diarrhea.

Keywords: Irinotecan, UGT1A polymorphisms, DPYD polymorphisms, Metastatic colorectal cancer, Toxicity, Clinical response

Background

Irinotecan is currently one of most important drugs in the management of metastatic colorectal cancer (mCRC) [1, 2]. Although the response rate and overall survival are greatly improved with the drug, about 30–50% of patients suffer severe toxicity, which particularly causes neutropenia and diarrhea [2]. UGT1A polymorphisms, especially UGT1A1*6 and UGT1A1*28, were previously noted to predict irinotecan-induced toxicity, but the results were inconsistent [3, 4]. Based on a previous study performed in our center [5], UGT1A1*6 and UGT1A1*28 were found to be related solely to irinotecan-induced severe neutropenia, and not to diarrhea, as most studies in Asia indicated [4, 6]. The predictive sensitivity and specificity were relatively low, only 37.6% and 61.6%, respectively. Although the combined examination of multiple UGT1A loci improved the predictive sensitivity and specificity to irinotecan-induced toxicity, it still focused on predictability for severe neutropenia [7]. The results were based on studies with small samples. In actual clinical practice, severe diarrhea was more closely associated with mortality than neutropenia [8], but there is still no definite biomarker that can predict severe diarrhea in Asian patients [9, 10]. Moreover, irinotecan is commonly used in combination with fluorouracil, which also induces severe neutropenia and diarrhea. DPYD polymorphisms, which are associated with fluorouracil levels in vivo, are associated with the occurrence of fluorouracil-induced toxicity [11, 12]. In this way, it is necessary to find ways to improve the predictability of combined UGT1A with an examination of DPYD polymorphisms. This is the first large sample analysis of a combined examination of both UGT1A and DPYD polymorphisms to predict irinotecan-based chemotherapy-induced toxicity and the clinical response in Chinese patients.

This study was designed to evaluate the combinations of UGT1A and DPYD polymorphisms in predicting the occurrence of treatment-induced toxicity, clinical response and survival in China. Because of regional ethnic diversity, the genotype distribution differs in various parts of China. Based on the genotype frequency distribution in Chinese and other Asian patients from previous studies [13–16], the genotypes of 661 patients have been examined at 9 loci: UGT1A1*6, UGT1A1*27 (c.686C > A), UGT1A1*28, UGT1A1*2 (c.387T > G), UGT1A7*3 (c.387T > G, c.622T > C), UGT1A7*4 (c.622T > C), UGT1A9*22 (−418T9 > T10), DPYD*5 (c.1627A > G), DPYD*2A (c.1905 + 1G > A), and DPYD c.1896T > C. The relationship of each genotype to the risk of treatment-induced toxicities, response rate and overall survival are explored here. These findings may be used to establish a new panel, that would be more efficient in predicting treatment-induced toxicity or efficacy in China.

Methods

Patients

A total of 2783 colorectal cancer patients who received chemotherapy at Peking University Cancer Hospital between January 2007 and June 2016 were screened for this retrospective study. Patients eligible for the study met the following criteria: histologically confirmed adenocarcinoma of the colorectum, stage IV disease, they received at least 2 cycles of irinotecan-based chemotherapy unless intolerable toxicity or disease progression occurred, they had peripheral blood samples taken, and complete clinical information was available for toxicity and efficacy evaluation. Patients were excluded from the study based on the following criteria: they received irinotecan-based chemotherapy for adjuvant treatment, and they did not have toxicity and efficacy information available for evaluation. The screening process is shown in Fig. 1.

All patients provided written informed consent for their peripheral blood to be used in this research. This study was approved by the Medical Ethics Committee of Peking University Cancer Hospital and was performed according to the principles of the Declaration of Helsinki.

Treatment and drug administration

Before patients received the irinotecan-based chemotherapy, routine blood tests of hepatic and renal function and performance status evaluation of each patient were performed and considered to be essential. The regimens in this study included irinotecanolone or combined with target treatment (n = 71, irinotecan dosage, 180 mg/m²), irinotecan combined with fluorouracil (5-Fu, Capecitabine, S-1 or tegafur) or plus target treatment (n = 554, irinotecan dosage, 180 mg/m²) and FOLFOXIRI (n = 36, irinotecan dosage, 150 mg/m²). Each patient received at least...
2 cycles of irinotecan-based chemotherapy, unless the patients suffered disease progression or intolerable toxicity. Routine blood tests and an evaluation of adverse events were performed after each administration of irinotecan or before the initiation of the next chemotherapy.

**Toxicity and response assessment**

Toxicity was evaluated based on the medical records according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, Version 4.0 (NCI-CTC 4.0 criteria, http://ctep.cancer.gov/reporting/ctc.html; accessed in October 2015). Grade 3 or 4 neutropenia and diarrhea were defined as severe toxicity.

The response rate was evaluated every 2–3 cycles or whenever the patient’s condition changed by imaging evaluation (CT or MRI) according to the response evaluation criteria in solid tumors (RECIST) [17]. All of the survival data were obtained from medical records and telephone follow-up. The last follow-up of recurrence and survival information was August 1, 2016. Progression-free survival (PFS) was identified as the time from the start of chemotherapy to disease progression, the last follow-up, or death of any cause. Overall survival (OS) was defined as the time from the start of irinotecan-based chemotherapy to death.

**Genomic DNA extraction and genotyping of UGT1A and DPYD**

Two-milliliter peripheral blood samples were acquired from metastasis colorectal cancer patients before receiving treatment and stored at −80 °C. The genomic DNA samples were extracted from these blood samples using QIAamp Blood Kit (Qiagen, Hilden, Germany). The fragments of *UGT1A* (*UGT1A1*^*6*, *UGT1A1*^*27*, *UGT1A1*^*28*, *UGT1A7*^*2*, *UGT1A7*^*3*, *UGT1A7*^*4* and *UGT1A9*^*22*) and *DPYD* (*DPYD*^*5*, *DPYD c.1896 T > C* and *DPYD*^*2A*) were amplified by polymerase chain reaction (PCR). All primers are shown in Table 1. Each 20 ul PCR reaction mixture consisted of 2 ul of 10 × LA PCR buffer II, 2 ul of 10 mmol/L dNTPs, 0.15 ul of LA Taq (DRR200A, Takara), 100–150 ng of genomic DNA, and 0.5 ul of each primer (10 umol/L). The PCR conditions of *UGT1A1*^*27* and *DPYD*^*5* were 95 °C for 5 min, 45 cycles of 95 °C for 10 s, 56 °C for 45 s and 72 °C for 20s, and a final extension at 72 °C for 10 min, and a final 4 °C for 10 min. The PCR products were indented by 2% agarose gel.

**Table 1** The primers of UGT1A/DPYD variants genotypes

| Mutation Type | PCR Primer (5’-3’) | Product length |
|---------------|--------------------|----------------|
| *UGT1A1*^*6*  | Primer-F: ACGCCTCG TTGTACATCAGAG 217 bp | Primer-R: CCTTGTT GTGCAGTAAGTGG |
| *UGT1A1*^*27* | Primer-F: ACTTACTGCACAACAAGGAGCT 484 bp | Primer-R: CACACCTGGGATAGTGGATTTTG |
| *UGT1A1*^*28* | Primer-F: AGCCAGTTCAACTGTTGTTGC 208 bp | Primer-R: TTTGCT CCTGCCAGAGGTTC |
| *UGT1A7*^*2/3/4* | Primer-F: TTTGCCGATGCTCCTTGACAG 415 bp | [28] Primer-R: GCTATTTCTAACCTTTTTGAAAAATAGGG |
| *UGT1A9*^*22* | Primer-F: ACTTAACATTGCAGCAGCGACACGG 556 bp | Primer-R: ATGGGCCAAAGCCTTTGAACCT |
| *DPYD*^*5*    | Primer-F: ATTCAGTTCACTGCTCAGTCAGAC 370 bp | Primer-R: GAGAAAGTTTTGGTGAGGGCA |
| *DPYD c.1896 T > C* | Primer-F: TGGACAAAGCTCCTTCTGGAATTA 231 bp | Primer-R: CGCAGAAAGCAACTTTATAGGC |
| *DPYD*^*2A*   | Primer-F: TGACAAAGCGTCTTTTGCAGACT 231 bp | Primer-R: CAGCAAAAGCAACTGGCAGAT |
electrophoresis and sequenced using an Invitrogen 3730XL genetic analyzer. The sequencing results were analyzed using Chromas software.

Statistical analysis
Differences between UGT1A and DPYD variants and severe irinotecan-induced toxicity were analyzed using the chi-square and Fisher’s exact tests. The association of genotypes with risk of severe irinotecan induced adverse events was assessed using logistic models. The Backward method of multivariate analysis model was used to avoid possible interactions. Survival curves were analyzed using the Kaplan-Meier method and compared by the log-rank test. All analyses were carried out using SPSS version 22.0 (SPSS Inc., Chicago, IL, US). The predictive powers of genotypes were recorded using Odds Ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were two-sided tests and P values <0.05 were considered to be statistically significant.

Results
There were 661 mCRC patients who were finally enrolled in this study (all of the clinical data and the patients’ genotypes of UGT1A and DPYD are shown in the Additional file 1). Of the study population, 406 patients (61.4%) were male and 255 patients (38.6%) were female, and the median age was 56 years old (interquartile range [IQR] 47, 63). There were 98 patients (14.8%) who received irinotecan-based regimens as the first-line treatment and 563 patients (85.2%) who received irinotecan-based regimens as the second-line treatment or further. There were 71 patients (10.7%) who received single irinotecan-based chemotherapy and 590 patients (89.3%) who received irinotecan plus fluorouracil-based chemotherapy. All patients were eligible for toxicity evaluation and 634 patients were eligible for response evaluation. The incidence of severe diarrhea and neutropenia was 8.9% (n = 59) and 20.6% (n = 136), respectively. During the follow-up, 512 patients had disease progression and 346 patients were dead.

Among all of the patients, 49 of 71 patients who received single irinotecan-based chemotherapy had all of the UGT1A polymorphism loci examined, while 496 of 590 patients who received irinotecan plus fluorouracil-based chemotherapy had all of the UGT1A and DPYD polymorphism loci examined. The remaining 116 patients (including 22 patients who received irinotecan plus fluorouracil-based chemotherapy) only finished an examination of UGT1A1*6 and UGT1A1*28, because of examination failure and sample depletion. The genotypes are shown in Table 2.

Analysis of chemotherapy-induced toxicities
In this retrospective study, sex, age, primary tumor location [18], chemotherapy regimens, line of treatment, and UGT1A and DPYD polymorphisms were included in the analysis (Table 3). Two loci, UGT1A7*4 and DPYD*2A, were excluded, due to their low frequency. The severe neutropenia incidence was 24.7% in females, and 18.0% in males, with P value of 0.056 in multivariate analysis. There were 30.6% of patients who suffered severe neutropenia in the first-line treatment, while 18.8% of patients suffering severe neutropenia in the second-line treatment or further, with a P value 0.009 in multivariate analysis. DPYD*5 was the independent predictive factor of severe diarrhea (OR = 2.143, 95%CI 1.136–4.041). UGT1A1*28 heterozygotes (OR = 2.263, 95%CI 1.395–
## Table 3 Univariate and multivariate analysis of chemotherapy induced toxicity

| Factors                                      | Severe diarrhea | Severe neutropenia |
|----------------------------------------------|-----------------|--------------------|
|                                              | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|                                              | N/Total(%) | P value | OR(95%CI) | P value | N/Total(%) | P value | OR(95%CI) | P value |
| Sex                                          |            |          |          |          |            |          |          |          |
| Male                                         | 39/406(9.6%) | 0.439    | NA⁷      | NA      | 73/406(18.0%) |          |          |          |
| Female                                       | 20/255(7.8%) |          |          |          | 63/253(24.7%) | 0.037    | 1.538(0.989–2.393) | 0.056 |
| Age                                          |            |          |          |          |            |          |          |          |
| ≤65y                                         | 44/545(8.1%) |          |          |          | 119/545(21.8%) |          |          |          |
| >65y                                         | 15/116(12.9%) | 0.096    | NA      | NA      | 17/116(14.7%) | 0.082    | NA      | NA      |
| Primary tumor location⁵                     |            |          |          |          |            |          |          |          |
| Left-side colorectum                        | 48/487(9.9%) |          |          |          | 97/487(19.9%) |          |          |          |
| Right-side colon                            | 11/174(6.3%) | 0.160    | NA      | NA      | 39/174(22.4%) | 0.485    | NA      | NA      |
| Chemotherapy regimens                       |            |          |          |          |            |          |          |          |
| Single IRI based regimens                   | 8/71(11.3%) |          |          |          | 9/71(12.7%) |          |          |          |
| IRIc + fluorouracil based regimens          | 51/590(8.6%) | 0.464    | NA      | NA      | 127/590(21.5%) | 0.081    | NA      | NA      |
| Line of treatment                           |            |          |          |          |            |          |          |          |
| First line treatment                        | 9/98(9.2%) |          |          |          | 30/98(30.6%) |          |          |          |
| ≥Second line treatment                      | 50/563(8.9%) | 0.923    | NA      | NA      | 106/563(18.8%) | 0.008    | 0.504(0.301–0.845) | 0.009 |
| UGT1A1*6                                    |            |          |          |          |            |          |          |          |
| G/G                                         | 42/431(9.7%) |          |          |          | 79/431(18.3%) |          |          |          |
| G/A                                         | 14/198(7.1%) |          |          |          | 43/198(21.7%) | 1.376(0.854–2.215) | 0.189 |
| A/A                                         | 3/32(9.4%) | 0.548    | NA      | NA      | 14/32(43.8%) | 0.002    | 4.737(1.946–11.533) | 0.001 |
| UGT1A1*27                                   |            |          |          |          |            |          |          |          |
| C/A                                         | 0/10(0.0%) |          |          |          | 109/535(20.4%) |          |          |          |
| C/C                                         | 53/535(9.9%) | 0.609    | NA      | NA      | 2/10(20.0%) | 0.977    | NA      | NA      |
| UGT1A1*28                                   |            |          |          |          |            |          |          |          |
| TA6/TA6                                     | 40/501(8.0%) |          |          |          | 90/501(18.0%) |          |          |          |
| TA6/TA7                                     | 18/152(11.8%) |          |          |          | 42/152(27.6%) | 2.263(1.395–3.670) | 0.001 |
| TA7/TA7                                     | 1/8(12.5%) | 0.323    | NA      | NA      | 4/8(50.0%) | 0.004    | 5.910(1.138–30.682) | 0.034 |
| UGT1A7                                      |            |          |          |          |            |          |          |          |
| UGT1A7*1/*1,1/*2,2/*2                       | 31/330(9.4%) |          |          |          | 54/330(16.4%) |          |          |          |
| UGT1A7*1/*3,2/*3,3/*3                       | 22/215(10.2%) | 0.747    | NA      | NA      | 57/215(26.5%) | 0.004    | NA      | NA      |
| UGT1A9*22                                   |            |          |          |          |            |          |          |          |
| T9/T9                                       | 10/99(10.1%) |          |          |          | 26/99(26.3%) |          |          |          |
| T9/T10,T11,T10                             | 43/446(9.6%) | 0.889    | NA      | NA      | 85/446(19.1%) | 0.107    | NA      | NA      |
| DPYD*5                                      |            |          |          |          |            |          |          |          |
| A/A                                         | 16/256(6.3%) |          |          |          | 53/256(20.7%) |          |          |          |
| A/G                                         | 30/240(12.5%) | 0.016    | 2.143(1.136–4.041) | 0.019 | 51/240(21.3%) | 0.881    | NA      | NA      |
| DPYD c.1896 T > C                           |            |          |          |          |            |          |          |          |
| T/T                                         | 32/395(8.1%) |          |          |          | 84/395(21.3%) |          |          |          |
| T/T/T/C                                     | 14/101(13.9%) | 0.075    | NA      | NA      | 20/101(19.8%) | 0.747    | NA      | NA      |

⁷: Non-acquired; ⁵: Left-side colorectum included splenic flexure, descending colon, sigmoid colon and rectum; Right-side colon included cecum, ascending and transverse colon [18]; c: Irinotecan
3.670), UGT1A*28 homozygotes (OR = 5.910, 95%CI 1.138–30.682) and UGT1A*6 homozygotes (OR = 4.737, 95%CI 1.946–11.533) were the independent predictive factors of severe neutropenia.

Out of all of the patients who received irinotecan-based chemotherapy, those who have more mutational alleles of UGT1A*6 and UGT1A*28 were found to be more likely to suffer severe toxicity (P = 0.001), especially severe neutropenia (P < 0.001). The predictive sensitivity and specificity of UGT1A polymorphisms were 32.4% and 53.1%, respectively. Of the patients who received irinotecan plus fluorouracil-based chemotherapy, we analyzed the severe toxicity risk based on UGT1A*6/*28 and DPYD*5 panels. More mutational alleles of UGT1A*6/*28 and DPYD*5 were also revealed to have increased incidence of severe neutropenia (P = 0.008). And patients with ≥3 mutation alleles had higher risk of suffering severe diarrhea, with the incidence of 15.9%, but without significant P value. The predictive sensitivity and specificity of UGT1A*6/*28 and DPYD*5 panels were 33.1% and 85.3%, respectively (Table 4).

### Analysis of chemotherapy clinical response

The clinical response of irinotecan-based chemotherapy varied across different lines of treatment. In the first-line treatment group of patients, 5 patients were not available to evaluate efficacy due to stopping chemotherapy for intolerable toxicity. Only 4 patients received single irinotecan-based chemotherapy as a first-line treatment, because of old age or bad performance. The objective response rate (ORR) was 32.3% (30/93). For the second-line treatment or further, 22 patients could not evaluate efficacy due to stopping chemotherapy for intolerable toxicity. The objective rate was 12.2% (66/541). Of the patients who received irinotecan plus fluorouracil-based chemotherapy as the first-line treatment, UGT1A*28 and DPYD*5 contributed to a higher ORR. Neither clinical factors (including sex, age, and primary tumor location) nor UGT1A/DPYD polymorphisms were related to the disease control rate (DCR) in any line of treatment (Table 5).

### Analysis of irinotecan-induced progression-free survival and overall survival

Of the patients who received the first-line irinotecan-based chemotherapy, the median PFS was 7.00 months (IQR 3.30, 11.80). DPYD*5 mutation contributed to better PFS than wild type (4.90 months vs. 8.50 months, P = 0.015, Fig. 2a). Patients with the UGT1A1*27 mutation showed a shorter OS than the wild-type patients (5.17 vs. 23.17, P < 0.001, Fig. 2b). In the second-line treatment or further, the median PFS was 5.57 months (IQR 2.63, 11.23). Neither UGT1A nor DPYD polymorphisms showed any significant relationship with PFS or OS (all P values > 0.05).

### Discussion

In this cohort, the incidence of severe diarrhea and neutropenia was 8.9% and 20.8%. These were consistent with the previously reported results at the same center [5]. Clinical factors (including sex, age, primary tumor location, and chemotherapy regimens) did not show a significant relationship with treatment-induced severe diarrhea. Patients who received irinotecan-based chemotherapy as a second-line treatment or further had a lower risk of suffering severe neutropenia. The results are also shown in a previous report [19], which might be explained by more patients with better treatment tolerance receiving the second-line treatment or further. Female patients showed a potentially higher incidence of severe neutropenia, but with no statistical significance; however, in the report of Tsunedomi R

#### Table 4 Correlation of UGT1A polymorphisms with severe toxicity

| Genotype                    | Severe diarrhea N/Total(%) | P-value | Severe neutropenia N/Total(%) | P-value | Severe toxicity N(%) | P-value |
|-----------------------------|---------------------------|---------|-------------------------------|---------|----------------------|---------|
| UGT1A*6/*28 panels (N = 661) |                           |         |                               |         |                      |         |
| Wild type                   | 31/368(8.4%)              | 0.736   | 59/368(16.0%)                 | 0.001   | 84/368(22.8%)        |         |
| Single allele variants      | 23/228(10.1%)             |         | 53/228(23.2%)                 |         | 66/228(28.9%)        |         |
| ≥2 alleles variants         | 5/65(7.7%)                | 0.360   | 24/65(36.9%)                  | 0.001   | 29/65(44.6%)         | 0.001   |
| UGT1A*6/*28 and DPYD*5 panels (N = 496) |                     |         |                               |         |                      |         |
| Wild type                   | 5/114(4.4%)               | 0.147   | 17/114(14.9%)                 | 0.204   | 20/114(17.5%)        |         |
| Single allele variants      | 22/214(10.3%)             |         | 36/214(16.8%)                 |         | 54/214(25.2%)        |         |
| ≥2 alleles variants         | 12/124(9.7%)              |         | 37/124(29.8%)                 |         | 44/124(35.5%)        |         |

*patients with genotype: G/G and TA6/TAs. ³patients with genotype: G/A, and TA6/TAs; or G/G and TA6/TAs. ²patients with genotype: A/A and TA6/TAs; or G/A and TA6/TAs. ¹patients with genotype: A/A and TA6/TAs; or G/A and TA6/TAs. ²patients with genotype: G/G, TA6/TAs and A/A. ³patients with genotype: G/G, TA6/TAs and A/A. ³patients with genotype: G/G, TA6/TAs and A/A. ²patients with genotype: G/G, TA6/TAs and A/A. ³patients with genotype: G/G, TA6/TAs and A/A. ²patients with genotype: G/G, TA6/TAs and A/A. ³patients with genotype: G/G, TA6/TAs and A/A. ²patients with genotype: G/G, TA6/TAs and A/A. ³patients with genotype: G/G, TA6/TAs and A/A.


Table 5 UGT1A/DPYD polymorphisms and clinical response

| Genotype               | Single IRI ± targeted therapy | IRI + fluorouracil ± targeted therapy |
|------------------------|--------------------------------|----------------------------------------|
|                        | First line treatment           | Second line treatment                  |
|                        | ORR   | P value | DCR   | P value | ORR   | P value | DCR   | P value |
|                        |       |         |       |         |       |         |       |         |
| Sex                    |       |         |       |         |       |         |       |         |
| Male                   | 0/1   | (0.0%)  | 1/1   | (100%)  | 0/29  | (0%)    | 18/29 | (62.1%) |
|                        | 17/56 | (304%)  | 49/56 | (87.5%) | 39/295| (13.2%) | 235/295| (79.7%) |
| Female                 | 1/3   | (33.3%) | 1.000 | (67.7%) | 4/34  | (11.8%) | 19/34 | (55.9%) |
|                        | 12/33 | (364%)  | 0.559 | (87.9%) | 23/183| (12.6%) | 0.837 | 141/183| (77.0%) |
| Age                    |       |         |       |         |       |         |       |         |
| ≤65y                   | 0/1   | (0.0%)  | 1.000 | (100%)  | 0/29  | (0%)    | 18/29 | (62.1%) |
|                        | 17/56 | (304%)  | 49/56 | (87.5%) | 39/295| (13.2%) | 235/295| (79.7%) |
| >65y                   | 1/3   | (33.3%) | 1.000 | (67.7%) | 4/34  | (11.8%) | 19/34 | (55.9%) |
|                        | 12/33 | (364%)  | 0.559 | (87.9%) | 23/183| (12.6%) | 0.837 | 141/183| (77.0%) |
| Primary tumor location  |       |         |       |         |       |         |       |         |
| Left-side colorectum   | 1/3   | (33.3%) | 1.000 | (66.7%) | 3/43  | (70%)   | 27/43 | (62.8%) |
|                        | 20/60 | (333%)  | 54/69 | (90.0%) | 47/362| (13.0%) | 289/362| (79.8%) |
| Right-side colon       | 0/1   | (0.0%)  | 1.000 | (100%)  | 0/21  | (0%)    | 9/21  | (42.9%) |
|                        | 1/9   | (11.1%) | 0.216 | (77.8%) | 0.343 | 6/80 | (7.5%) | 0.11 | 59/80 | (73.8%) |
| UGT1A1*6               |       |         |       |         |       |         |       |         |
| G/G                    | 1/4   | (25.0%) | 3/4   | (75.0%) | 3/46  | (65%)   | 28/46 | (60.9%) |
|                        | 17/54 | (315%)  | 49/54 | (90.7%) | 42/310| (13.5%) | 239/310| (77.1%) |
| G/A                    | 0/0   | (0.0%)  | 0/0   | (0.0%)  | 1/15  | (6.7%)  | 7/15  | (46.7%) |
|                        | 9/31  | (290%)  | 0.337 | 25/31 | (80.6%) | 16/145| (11.0%) | 119/145| (82.1%) |
| A/A                    | 0/0   | (0.0%)  | NA    | (NA)    | 0/2   | (0%)    | 2/2   | (100%)  |
|                        | 3/4   | (75%)   | 0.175 | 4/4 | (100%)  | 0.295 | 4/23 | (17.4%) | 0.615 | 18/23 | (78.3%) |
| UGT1A1*27              |       |         |       |         |       |         |       |         |
| C/A                    | 0/0   | (0.0%)  | 0/0   | (0.0%)  | 3/43  | (70%)   | 28/43 | (65.1%) |
|                        | 2/3   | (66.7%) | 2/3   | (66.7%) | 54/387| (14.0%) | 313/387| (80.9%) |
| C/C                    | 1/2   | (500%)  | NA    | (NA)    | 0/0   | (0%)    | NA    | (NA)    |
|                        | 26/84 | (31.0%) | 74/84 | (88.1%) | 0.272 | 0/7 | (0%) | 0.600 | 6/7 | (85.7%) |
| UGT1A1*28              |       |         |       |         |       |         |       |         |
| TA6/TA6                | 1/3   | (33.3%) | 2/3   | (66.7%) | 4/48  | (8.3%)  | 29/48 | (60.4%) |
|                        | 15/59 | (254%)  | 52/59 | (88.1%) | 53/373| (14.2%) | 293/373| (78.6%) |
| TA6/TA7,TA7/TA7        | 0/1   | (0.0%)  | 1.000 | (100%)  | 0/15  | (0%)    | 8/15  | (53.3%) |
|                        | 14/30 | (467%)  | 0.043 | 26/30 | (86.7%) | 0.842 | 9/105 | (8.6%) | 0.129 | 83/105 | (79.0%) |
| UGT1A7                 |       |         |       |         |       |         |       |         |
| UGT1A7*1/*1,1/*2,*2/*2 | 1/2   | (500%)  | 2/2   | (100%)  | 3/32  | (9.4%)  | 20/32 | (62.5%) |
|                        | 12/47 | (255%)  | 42/47 | (894%)  | 35/241| (14.5%) | 194/241| (80.5%) |
| UGT1A7*1/*2/*3,*3/*3   | 0/0   | (0.0%)  | 0.558 | NA     | 0.558 | NA | 0.539 | 0.17 | 0.482 | 0.554 | 0.767 |
Table 5 UGT1A/DPYD polymorphisms and clinical response (Continued)

| Genotype     | 0/0 (0.0%) | 0/11 (72.7%) | 8/11 (39.5%) | 32/38 (84.2%) | 19/153 (12.4%) | 125/153 (81.7%) |
|--------------|-------------|--------------|--------------|---------------|----------------|-----------------|
| **UGT1A9**   |             |              |              |               |                |                 |
| **T9/T9**    | 0/0 (0.0%)  | 0/0 (0.0%)   | 0/8 (0.0%)   | 5/8 (62.5%)   | 6/13 (46.2%)   | 5/71 (7.0%)     |
| **T9/T10,T10/T10** | 1/2 (50.0%) | 2/2 (100.0%) | 3/35 (8.6%)  | 23/35 (65.7%) | 21/72 (29.2%)  | 61/71 (85.9%)   |
| **DPYD**     |             |              |              |               |                |                 |
| **A/A**      | NA          | NA           | NA           | 8/41 (195%)   | 34/41 (82.9%)  | 174/211 (82.5%) |
| **A/G,G/G**  | NA          | NA           | NA           | 19/44 (43.2%) | 40/44 (90.9%)  | 145/183 (79.2%) |
| **DPYD c.1896 T > C** |             |              |              |               |                |                 |
| **T/T**      | NA          | NA           | NA           | 24/69 (34.8%) | 60/69 (87.0%)  | 254/314 (80.9%) |
| **T/T,C**    | NA          | NA           | NA           | 3/16 (18.8%)  | 14/16 (87.5%)  | 65/80 (81.3%)   |

IR: Irinotecan; ORR: Objective response rate; DCR: Disease control rate; Tumor location: Left-side colorectum included splenic flexure, descending colon, sigmoid colon and rectum; Right-side colon included cecum, ascending and transverse colon [18]; NA: Non-acquired
et al., being female was an independent risk factor of severe neutropenia [7].

UGT1A genotype frequency and the effect on treatment-induced toxicity varied across ethnic groups. Early in 2005, UGT1A1*28 was recognized as a risk factor for irinotecan-induced toxicities by the U.S Food and Drug Administration (FDA). In Asia, however, UGT1A1*28 were not applicable to the prediction of irinotecan-induced toxicity because of its low frequency. Several studies have shown that UGT1A7*3 was associated with higher risk of suffering severe neutropenia [7, 25, 26]. Targeted drug treatment might affect the predictability of the toxicity of UGT1A polymorphisms. Regimens with different targeted drugs might also affect evaluation of toxicity. The influence of targeted drugs on the relationship between UGT1A polymorphisms and toxicity should be further studied. Finally, the results showed that UGT1A1*6 and UGT1A1*28 had an association with irinotecan-induced toxicity for Chinese patients. Among the patients who received targeted drugs, only UGT1A7*3 was found to be associated with a higher risk of G3–4 neutropenia incidence. Targeted drug treatment might affect the predictability of the toxicity of UGT1A polymorphisms. Regimens with different targeted drugs might also affect evaluation of toxicity. The influence of targeted drugs on the relationship between UGT1A polymorphisms and toxicity should be further studied. Finally, the results showed that UGT1A1*6 and UGT1A1*28 had an association with irinotecan-induced severe neutropenia. Patients with more mutant variants had a higher risk of suffering severe neutropenia; however, no other significant loci of UGT1A polymorphisms were found to set up a new panel to better indicate irinotecan-induced toxicity.

Fluorouracil is generally combined with irinotecan. DPYD polymorphisms influenced the activity of dihydropyrimidine dehydrogenase (DPD) considerably, which was associated with fluorouracil’s metabolism and ethnic variation.
also appeared in DPYD polymorphisms [14, 16, 30]. In western countries, it has been reported that DPYD*2A mutant variants contribute to a higher risk of severe toxicity [30]; however, DPYD*2A is rarely found. This was consistent with the findings of this study. Only 1 DPYD*2A heterozygote (0.2%) was found in this analysis. The ability of DPYD*2A to indicate fluorouracil-induced toxicity in China could not be assessed. DPYD*5 and DPYD c.1896 T > C had allele frequencies of 28.4% and 10.7%, respectively, which was consistent with previous reports [31]. In this cohort, it was noted that DPYD*5 associated with higher risk of severe diarrhea. However, in Zhang XP et al. and Yamauchi et al.'s study, DPYD*5 related to the incidence of severe neutropenia [31, 32]. In addition, the study of Felicia FS et al. showed that DPYD c.1896 T > C independently predicted severe fluorouracil-induced toxicity, which did not happen in this analysis [16]. A combined examination of UGT1A1*6, UGT1A1*28 and DPYD*5 was found to improve the predictive specificity for toxicity compared with an examination of UGT1A1*6 and UGT1A1*28 (53.1% vs. 85.6%) among patients receiving irinotecan plus fluorouracil-based chemotherapy.

The association between UGT1A and DPYD polymorphisms and clinical outcomes were analyzed, as well as the toxicity. The response rate and survival varied across different treatment lines. Among patients who received irinotecan-based chemotherapy as a first-line treatment, this analysis first noted that UGT1A1*27 contributed to worse OS than wild type variants, although the number of analyzed samples was small. Moreover, UGT1A1*28 contributed to a higher objective response rate, which was consistent with studies reported by Fujita and Toffoli G’s team [25, 33]. While, in Lu CY and colleagues' study, UGT1A1*28 led to bad clinical outcomes [34]. This might be explained by a large number of factors affecting the survival. Single UGT1A gene polymorphisms were found to have only a limited ability to predict survival, and multiple chemotherapy regimens might also be involved. Unlike UGT1A, there have only been limited studies assessing the relationship between DPYD polymorphisms and survival. In this analysis, DPYD*5 mutant variants predicted better PFS in the first-line treatment of irinotecan plus fluorouracil-based regimens, and DPYD polymorphisms were not found to associate with overall survival.

Because this study was a retrospective analysis, bias was unavoidable. The value of this study relies on the large samples’ combined examination for UGT1A and DPYD polymorphisms. Although it was not possible to establish a new panel to improve the predictability of toxicity in this study, the analysis showed that more attention should be paid to homozygote of UGT1A1*6 in the context of irinotecan-induced severe neutropenia, such as constantly monitoring the levels of neutrophile granulocytes and preventive treatment for neutropenia. For this reason, further studies should focus on polymorphisms of other genes related to irinotecan metabolism.

Conclusion
In brief, only UGT1A1*6 and UGT1A1*28 variants were associated with irinotecan-induced neutropenia, but not with diarrhea. A combined examination of UGT1A1*6, UGT1A1*28 and DPYD*5 were found to improve the predictive specificity of toxicity. UGT1A and DPYD polymorphisms were still limited to the prediction of clinical response. A combined examination of more UGT1A polymorphisms will not be helpful in improving predictive value of irinotecan-induced toxicity.

Additional file

Additional file 1: The clinical database and UGT1A/DPYD polymorphisms of all study patients. (XLSX 141 kb)

Abbreviations
CI: Confidence interval; DCR: Disease control rate; IQR: Interquartile range; IRR: Irinotecan; mCRC: Metastatic colorectal cancer; NA: Non-acquired; OR: Odds ratio; ORR: Objective response rate; OS: Overall survival; PFS: Progression free survival; RECIST: Response evaluation criteria in solid tumors

Acknowledgements
We would like to thank LetPub (www. Letpub. com) for its linguistic assistance during the preparation of this manuscript.

Funding
No specific funding was received for this study.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors’ contributions
Conceived and designed the experiments: DL, JL, JG, LS. Performed the experiments and acquired the data: DL, RY, YL. Analyzed and interpreted the data: DL, JG, JL, YL, LS. Drafted the manuscript: DL. Revised the manuscript: JG, JL, LS. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Medical Ethics Committee of Peking University Cancer Hospital and was performed according to the Declaration of Helsinki Principles with the reference number: 2016K773. All patients provided written informed consent for their peripheral blood to be used in research.

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