Association and prediction of severe 5-fluorouracil toxicity with dihydropyrimidine dehydrogenase gene polymorphisms: A meta-analysis

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Abstract. The aim of the present study was to evaluate the association and prediction of dihydropyrimidine dehydrogenase gene (*DPYD*) polymorphisms and the risk of 5-fluorouracil (5-FU) severe toxicity in cancer patients. A meta-analysis of the published literature was conducted to summarize evidence for *DPYD* gene polymorphisms associated with an increased risk of severe 5-FU toxicity in patients with cancer from an Asian population. Relevant literature was identified using the PubMed and Cochrane databases on April 11, 2014. Combined risk ratios and 95% confidence intervals (CIs) were calculated in a fixed-effects model. A total of 5 clinical studies were retrieved in the meta-analysis, including 764 cancer patients with *DPYD* gene polymorphisms who received 5-FU-based chemotherapy. Overall, *DPYD* gene polymorphisms were associated with the increased risk of 5-FU severe toxicity [risk ratio=2.54 (2.15-3.00); 95% CI, 19.46-84.57; P=0.0001]. In conclusion, the present meta-analysis suggested that polymorphisms of several *DPYD* gene polymorphisms are associated with an increased risk of severe toxic response to 5-FU.

Introduction

Adverse drug reactions to 5-fluorouracil (5-FU)-based chemotherapy have been reported to correlate with dihydropyrimidine dehydrogenase gene (*DPYD*) variations in numerous countries. Three genetic variants of thymidylate synthase (TS; encoded by the *TYMS* gene) and 2 variants of methylenetetrahydrofolate reductase (encoded by the *MTHFR* gene) are also proposed to be associated with an increased risk of toxicity following 5-FU administration (1). 5-FU has been reported to cause 0.5-1% mortality (2,3) Therefore, studies have focused on the identification of biomarkers or predictors of 5-FU toxicity (4,5).

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5-FU metabolism involves numerous enzyme reactions and intermediates, however, dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme of the pyrimidine base catabolism, which may increase the half-life of 5-FU-based chemotherapy drugs, thereby increasing the risk of dose-dependent severe toxicity in cancer patients (6). Numerous genetic polymorphisms and rare polymorphisms in FU metabolism have been reported to influence the risk of toxicity, following the first report of severe DPYD deficiency with life- threatening 5-FU toxicity (7-11).

DPD activity varies widely among different human populations; 3-5% of the general population experiences low or partial DPD deficiency (12-14). Patients with low DPD activity are correlated to a higher risk of developing severe or even lethal toxicity when treated with standard doses of 5-FU (15). This finding was supported by the recently published study from China (16). 5-FU toxicities can be prevented and avoided by dose adjustment when detection of functionally distinct gene polymorphisms can be performed. However, the existing published studies are inconsistent in reporting and testing toxicities. No specificity and validation on several polymorphisms in commercial FU toxicity kits has been reported. Therefore, the detection of candidate gene polymorphisms that are truly associated with FU toxicity becomes uncertain. Numerous studies from Western countries have identified that certain polymorphisms are associated with 5-FU-based chemotherapeutic agents. Recently, there were clinical trials published from an Asian population that investigated the association between *DPYD* polymorphisms and 5-FU-based chemotherapy toxicity. The present study performed a systematic review and a meta-analysis synthesizing these data to examine its potential use as a biomarker of 5-FU toxicity.

Materials and methods

Search strategy. A systematic literature search of the PubMed and Cochrane databases was conducted to identify all the clinical studies evaluating types and frequency of *DPYD* polymorphisms and 5-FU toxicity in cancer patients from Asian populations, including Korea, Japan, China and Thailand, between January 1, 1999 and December 31, 2013. The databases were searched using the medical subject headings or text keywords: Dehydropyrimidine dehydrogenase (*DPYD*) polymorphism or variant, 5-FU-toxicity, Korean, Japanese,

Chinese and Thai cancer patients. A manual search was also performed of the references of the selected studies to identify any overlooked literature. Only English language studies were restricted in the search.

Selection criteria. The inclusion criteria were as follows: i) Research focused on the association of *DPYD* variants with the risk of 5-FU-based treatment toxicity; ii) clinical studies; and iii) available genotype and allele data. Studies were excluded as follows: i) Unpublished studies, conference articles, reviews and duplication of publications; ii) data was unavailable for calculating genotype or allele frequencies (AF); and iii) no data of 5-FU-based toxicity.

Data extraction and synthesis. Two investigators independently selected the studies and extracted data. All the disagreements were resolved by consensus. Abstracts were initially screened to exclude clearly ineligible studies and subsequently the full texts of all the remaining studies were reviewed. A standardized data-recording form was used to summarize data regarding included sequence variations of *DPYD*, allelic variants, and the frequency and severity of 5-FU-based toxicity in Asian populations.

Statistical analysis. A quantitative meta-analytical technique was used to pool the data for the relative risk (RR) of 5-FU-based toxicity related to *DPYD* variants. The meta-analysis was performed using the fixed-effects model in Review Manager (Version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). For the calculation of RR, patients with *DPYD* gene polymorphisms showed severe toxicity (≥grade 2) and were compared with those who did not have toxicity in the same trial, and data was extracted directly from the enrolled studies.

Quality assessment. The methodological quality of the included studies was evaluated through ratings on the Newcastle-Ottawa Scale (17). This scale assesses the quality of observational and nonrandomized or cohort studies. The instrument uses a star system to evaluate studies based on 3 criteria: Participant selection, comparability of study groups and assessment of outcome or exposure. Two investigators independently assessed the quality of all the included studies.

Sensitivity analysis. Sensitivity analyses were conducted by changing the fixed or random effects model to estimate the effect on the pooled results. The influence of individual studies on the pooled results was estimated by omitting one study at a time. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Literature search. Fig. 1 depicts the flow diagram of the systematic literature search and selection of clinical studies. The systematic literature search identified 51 abstracts. A total of 43 studies were excluded as they did not examine *DPYD* variants associated with 5-FU-based toxicity. A total of 5 full-length clinical studies were reviewed and matched the inclusion criteria of the present study (16,18-21).

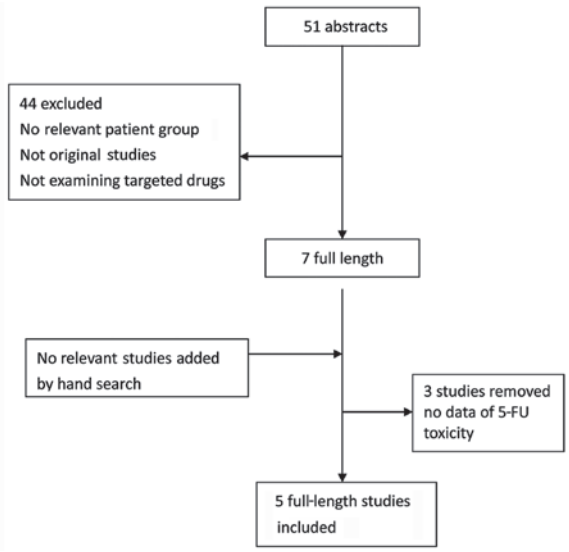

Figure 1. Flow chart of the included studies. 5-FU, 5-fluorouracil.

Characteristics of studies. A total of 764 cancer patients with *DPYD* polymorphisms had received 5-FU-based chemotherapy. Two studies were performed in China, and one each in Japan, Korea and Thailand. A total of 41 single-nucleotide polymorphisms (SNPs) with different AF were detected. Three identical *DPYD* variants were observed in Chinese and Korean cancer patients: 85T>C (*DPYD**9A), 1627A>G (*DPYD**5) and 2194G>A (*DPYD**6). The 1896T>C variant was observed in cancer patients of Korea, Japan and Thailand. The 1774C>T variant was observed in Korean and Thai patients. The majority of the enrolled studies reported that the AF was >1%. The AF of the 85T>C variant (Cys29Arg, *9A) was similar in Japanese (3.7 and 2.9%) and Korean patients (2.5%), but was higher in Chinese patients (7.04%). The 1627A>G variant was present with comparable AF among Thai (37.07%), Chinese (20.8%) and Korean (20.5%) populations. The AF of 2194G>A in the Korean population (1.5%) was similar to the mean AF of 2 studies of the Chinese population (0.7 and 5%). Other novel genotypes, such as 496A>G, 74A>G and 1737 T>C were identified in the Korean and Japanese populations with a low AF. The 1896T>C variants were identified in Japanese and Thai populations at frequencies ranging from 9.8 to 15.52%. All the included studies reported an increased risk of severe toxicity (≥grade 2 or grades 3 and 4) of 5-FU-based chemotherapy that was associated with these common polymorphisms (Table I). The methodological quality of the majority of the studies was moderate (Table II).

Meta-analysis. A meta-analysis of 5 clinical studies showed a significant increased risk of 5-FU-based severe toxicity associated with DPYD gene polymorphisms [risk ratio=2.54 (2.15-3.00); 95% confidence interval, 19.46-84.57; P=0.0001] (Fig. 2).

Sensitivity analysis. The result of sensitivity analysis by changing either the fixed effects model to the random effects model or omitting one study at a time indicated no change of the overall risk. Therefore, the present results were deemed statistically reliable.

Table I. Association between *DPYD* polymorphisms and 5-FU severe toxicity (>grade 2).

Authors (country)	Patients *DPYD*(+) toxicity (n/N)	*DPYD* genetype or variations	Cancer type	Chemotherapy	Toxicity (grade)	RR (95% CI)	P-value	(Refs.)
Cho *et al* 2007 (Korea)	21/67	*5B/*5B, 1737T>C 1*/*5B, *1/*5A 1525-1G>A, 1525-9A>G 1129-15T>C *5B/*9A, 1896T>C 496A>G, 1774C>T 1*/*1	Colorectal	NA	Stomatitis (3,4) Diarrhea (3,4) Neutropenia (3,4)	63.87 (3.94-1,036.57)	0.00001	(18)
Zhang *et al* 2013 (China)	16/20	2194G>A 85T>C 464T>A	Colon	FOLFOX4	Bone marrow (3.4)	2.29 (1.42-3.68)	0.00001	(16)
Zhang *et al* 2012 (China)	162/169	1627A>G 2194G>A 496A>G 274T>A	Gastric	Capecitabineor S1 or 5-FU based combination 5-FU+D/P 5-FU+C/O+D/P & others	Hematology (>2) Gastroenterology (>2)	2.50 (2.09-2.99)	0.00001	(21)
Yamaguchi *et al* 2001 (Japan)	11/69	74A>G 85T>C 1627A>G 812 del T 1714C>G 1896T>C	Gastric Colonretal Esophgeal	MTX-5 FU CDDP-5-FU	Nausea (2) Arrhythmias (2)	15.44 (0.93-255.79)	0.00001	(19)
Sirachainan *et al* 2012 (Thailand)	28/76	1627A>G 967G>A 1774C>T IVS 14+G>A 1011A>T 1236G>A 1896T>C	Breast GI H&N Others	FAC, CMF, 5-FU+leucovorin FOLFOX4 5-FU+CDDP, ECF 5-FU+CBDCA FOLFIRI	Neutropenia (3,4)	30.35 (1.90-484.45)	0.00001	(20)

DPYD, dihydropyrimidine dehydrogenase gene; n, patient number of severe toxicity; N, total number of *DPYD*(+) patients; RR, relative risk; CI, confidence interval; FOLFOX4, oxaliplatin + fluorouracil/leurovorin; MTX, methothrexate; FU, fluorouracil; CDDP, cisplatin; FAC, fluorouracil + adriamycin + cyclophosphamide; CMF, cyclophosphamide + methothrexate + fluorouracil; ECF, epirubicin + cisplatin + 5-flurouracil; CBDCA, carboplatin; FOLFIRI, leucovorin + fluorouracil (5-FU) + irinotecan.

Table II. Assessing the quality of the included studies using the Newcastle-Ottawa Scale.

	Selection				Comparability		Exposure				
Authors and year	1	2	3	4	5	6	7	8	9	Total score=9	(Refs.)
Cho *et al* 2007	★	★	★	★				★	★	6	(19)
Zhang *et al* 2013	★	★	★			★		★	★	6	(16)
Zhang *et al* 2012	★	★	★			★		★	★	6	(22)
Yamaguchi *et al* 2001	★	★	★			★		★	★	6	(20)
Sirachainan *et al* 2012	★	★	★			★		★	★	6	(21)

For case-control studies, 1 indicates cases independently validated; 2, cases are representative of population; 3, hospital controls; 4, controls have no history of cancer; 5, study controls for age; 6, study controls for additional factor (dihydropyrimidine dehydrogenase gene variants); 7, ascertainment of exposure by blinded interview or record; 8, same method of ascertainment used for cases and controls; 9, non-respondents described.

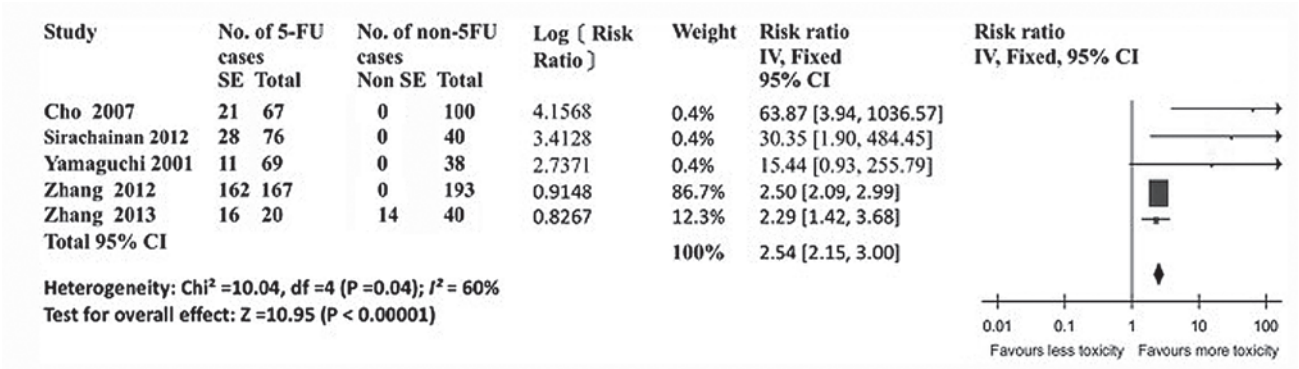

Figure 2. Risk of severe events (SE) (>grade 2) of 5-fluorouracil-based treatment associated with patients with the dihydropyrimidine dehydrogenase gene. IV, inverse variance; df, degree of freedom; CI, confidence interval.

Discussion

The present results indicated that cancer patients with few *DPYD* polymorphisms identified from Asian populations are associated with an increased risk of severe toxicity of 5-FU-based chemotherapy. Of the 41 assessed polymorphisms, only 19 *DPYD* polymorphisms were formally associated with grade 3 or 4 toxicity in the meta-analysis. The associations were present in either 5-FU monotherapy or combination therapy. This finding appears to be inconsistent with a meta-analysis published in 2014 regarding genetic markers of toxicity in the QUASAR2 study, which reported that the associations were only present in 5-FU monotherapy (22). This may be due to differences in ethnicities as certain *DPYD* gene polymorphisms identified in Asian populations are different from those in the Western population; for example, *DPYD*2A* (also known as *DPYD*: IVS14+1G>A, c.1905+1G>A) was not identified in the majority of the patients assessed from the Asian population. The inconsistent findings may be caused by a number of factors, such as geographic variability in the frequencies of rare gene polymorphisms, by sampling effects and possibly by variations in treatment regimens across studies or the individual patient response to toxicity. Individual patient response may be due to the complicated association between *DPYD* genotype and phenotype.

The data from the present meta-analysis showed that *DPYD* 1627A>G with a high AF was identified in cancer patients of China, Korea, Japan and Thailand (AF >20%) and *DPYD* 1896T>C was identified in Korean and Thai patients with AF >14%. The power to detect an association for these polymorphisms was >75%. Therefore, it could be characterized as a common polymorphism in these populations. For other polymorphisms, *DPYD* 85T>C and *DPYD* 2194G>A found in Chinese and Korean patients were low, resulting in ~20% of suboptimal power to detect an association with severe toxicity.

According to the results of certain studies, a mortality rate of 0.5% and grades III-IV toxicity of 20-30% have been reported in patients treated with 5-FU for advanced cancer (17-21). Detection of the genetic polymorphism is thought to be a useful method for the prediction of severe toxicity and treatment outcome. Although the detection of the *DPYD* gene SNP cannot predict all the severe toxicity, ~20% of all early 5-FU-related toxicities could potentially be avoided (1). The identification of the remaining 80% 5-FU toxicity must be reliant on the discovery and analysis of additional *DPYD* polymorphisms that can affect pharmacokinetics. The investigations of genetic markers of 5-FU-based regimens are being performed continuously. A recently published study identified that *TYMS* polymorphisms 5'VNTR2R/3R and 3'untranslated region 6 bp ins-del and *DPYD* 2846T>A and *2A were significantly associated with grade 3 toxicity from the QUASAR2

study (22). In addition, other genes, such as *MTHFR* and *DPYD* haplotypes, may also influence the responses to 5-FU (1,13). There are no adequately published data from Asian populations that investigate in this respect. Therefore, no evidence was available for analysis in the present study.

Limitations in the systematic review included 2 points. Firstly, the number of clinical studies to explore the correlation between the *DPYD* variant and severe toxicity of 5-FU-based chemotherapy were limited, and the present result may be underestimated. Secondly, no studies reported the incidence or risk ratios of severe toxicity of 5-FU-associated with other genes, except *DPYD*, in the Asian population.

In conclusion, the present meta-analysis suggested that several *DPYD* gene polymorphisms are associated with an increased risk of a severe toxic response to 5-FU in the Asian population. Therefore, the role of the *DPYD* gene polymorphisms used in predicting toxicity should be investigated continuously. Large and comprehensive studies are required in the near future prior to considering the use of *DPYD* as genetic markers of toxicity from 5-FU-based regimens in the Asian population.

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