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ORIGINAL REPORT

Genetic Markers of Toxicity From Capecitabine and Other Fluorouracil-Based Regimens: Investigation in the QUASAR2 Study, Systematic Review, and Meta-Analysis

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ABSTRACT

Purpose
Fluourouracil (FU) is a mainstay of chemotherapy, although toxicities are common. Genetic biomarkers have been used to predict these adverse events, but their utility is uncertain.

Patients and Methods
We tested candidate polymorphisms identified from a systematic literature search for associations with capecitabine toxicity in 927 patients with colorectal cancer in the Quick and Simple and Reliable trial (QUASAR2). We then performed meta-analysis of QUASAR2 and 16 published studies (n = 4,855 patients) to examine the polymorphisms in various FU monotherapy and combination therapy regimens.

Results
Global capecitabine toxicity (grades 0/1/2 *v* grades 3/4/5) was associated with the rare, functional *DPYD* alleles 2846T>A and *2A (combined odds ratio, 5.51; $P = .0013$) and with the common *TYMS* polymorphisms 5′VNTR2R/3R and 3′UTR 6bp ins-del (combined odds ratio, 1.31; $P = 9.4 \times 10^{-6}$). There was weaker evidence that these polymorphisms predict toxicity from bolus and infusional FU monotherapy. No good evidence of association with toxicity was found for the remaining polymorphisms, including several currently included in predictive kits. No polymorphisms were associated with toxicity in combination regimens.

Conclusion
A panel of genetic biomarkers for capecitabine monotherapy toxicity would currently comprise only the four *DPYD* and *TYMS* variants above. We estimate this test could provide 26% sensitivity, 86% specificity, and 49% positive predictive value—better than most available commercial kits, but suboptimal for clinical use. The test panel might be extended to include additional, rare *DPYD* variants functionally equivalent to *2A and 2846A, though insufficient evidence supports its use in bolus, infusional, or combination FU. There remains a need to identify further markers of FU toxicity for all regimens.

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INTRODUCTION

Fluorouracil (FU) is the backbone of chemotherapy for colorectal cancer and many other solid tumors. Three methods are used to deliver FU: bolus infusional intravenous administration, and oral capecitabine, a prodrug that undergoes preferential conversion to FU in malignant tissue. Oxaliplatin or irinotecan can be added to FU in combination regimens that include infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX)[1]; capecitabine plus oxaliplatin (XELOX)[2]; and fluorouracil, leucovorin, and irinotecan (FOLFIRI).[3] Depending on the regimen used, 10% to 30% of patients suffer

substantial FU toxicities (grade ≥ 3), typically diarrhea, nausea and vomiting, mucositis/stomatitis, myelosuppression, and hand-foot syndrome (HFS). Overall, FU causes 0.5% to 1.0% mortality (grade 5).[4,5] Consequently, attention has focused on the identification of biomarkers or assays predictive of FU toxicity.[6,7]

FU metabolism involves many enzyme reactions and intermediates (Data Supplement [online only]). Although measurement of enzyme activities could be used for toxicity prediction, these assays may be too cumbersome and expensive for routine, large-scale use. After initial reports linking severe dihydropyrimidine dehydrogenase (DPYD) deficiency with lethal FU use,[8] many genetic polymorphisms and rare variants in FU metabolism genes have been reported to influence the risk of adverse events.[9-11] In theory, by testing a panel of polymorphisms, FU toxicities can be predicted and dose modifications considered. However, the existing published data are limited by inconsistency in reporting and testing toxicities, pooling of patients on different FU schedules, and combined analysis of functionally distinct polymorphisms within the same gene. Several polymorphisms lacking validation may have been included in commercial FU toxicity kits.

Given the uncertainty regarding which genetic variants are truly predictive of adverse events from FU, we have examined associations between candidate polymorphisms and capecitabine toxicity in patients from the Quick and Simple and Reliable trial (QUASAR2). We have then performed a meta-analysis combining these data with those from previously published studies, both of capecitabine and other FU schedules.

PATIENTS AND METHODS

A synopsis of the methods used is presented here. Full details are provided in the Data Supplement.

The QUASAR2 study was the basis of our analysis of genetic markers of capecitabine toxicity. QUASAR2 is a phase III randomized trial of adjuvant capecitabine ± bevacizumab after resection of stage II/III colorectal cancer. We obtained data from 927 patients from the QUASAR2 trial for common FU-related toxicities—diarrhea, nausea and vomiting, mucositis/stomatitis, neutropenia, thrombocytopenia, and HFS. Adverse toxicity events were categorized as high (Common Terminology Criteria for Adverse Events grades 3, 4, or 5 during any treatment cycle) or low (grades 0, 1, or 2). A global toxicity measure was derived based on the presence of any grade 3/4/5 event (high) or absence of any such event (low).

From a systematic literature review (Data Supplement), we identified 36 FU-pathway polymorphisms potentially suitable for analysis (Table 1; Data Supplement). QUASAR2 genotypes were derived from Illumina (San Diego, CA) SNP arrays, individual polymorphism typing assays, or genetic imputation as long as high-quality results were obtained (Data Supplement). Twenty-one polymorphisms were included in the final analysis, after quality control and the exclusion of variants in strong pairwise linkage disequilibrium. They were *CES2*823C>G, *CES2*rs11568314, *CES2*rs11568311, *CES2*rs2241409, *CDA*−451C>T, CDA*2, *UMPS*638G>C, *TYMP*rs470119, *TYMP*S471L, *TYMS*5′VNTR2R/3R, *TYMS*3′UTR 6bp ins-del, *MTHFR*677C>T, *MTHFR*1298A>C, *DPYD*85T>C, *DPYD*496A>G, *DPYD*1236G>A, *DPYD*1601G>A, *DPYD*1627A>G, *DPYD**2A, *DPYD*2194G>A, and *DPYD*2846T>A.

For meta-analysis of genetic predictors of FU toxicity, studies were identified by systematic review.[6,9-35] Sixteen studies fulfilled our inclusion criteria.[9-11,13,18,19,21,23,24,26,28-31,33,35] We did not perform formal, combined analyses across regimens (Data Supplement). For every polymorphism in the meta-analysis (those analyzed for QUASAR2 plus *CES2*6046G>A, *CES2*6320G>A, *CDA*−205C>G, *CDA*rs602950, *CDA*943insC, *CDA*575C>T, *CDA*794G>A, *CDA*771 C>G, *UMPS*1336A>G, *TYMP*A324A, *TYMS*5′VNTR3RG>C, *DPYD*623G>A, *DPYD*1109delTA, *DPYD*1679T>G, and *DPYD*2858G>C), we performed an allelic test of association with global toxicity (grades 0/1/2 *v* 3/4/5) in each set of patients who had received the same regimen. For each FU regimen, meta-analyses assessing the relationship between toxicity (global and individual) and each individual polymorphism were performed using the metan command in STATA (STATA, College Station, TX). SEs and log(risk ratio) from each study were combined using the Mantel-Haenszel method.

For certain variants in *TYMS* and *DPYD,* we performed haplotype and/or set-based tests. The *TYMS*5′VNTR repeat haplotype with the G>CSNP in the second repeat was analyzed by a binary model based on the total number of USF1/USF2 binding sites across both alleles (0 to 2 *v* 3 to 4).[36] The *TYMS*5′VNTR (2R *v* 3R) and 3′UTR polymorphisms, which are in moderate linkage disequilibrium, were analyzed in combination by logistic regression conditioned on study, formal haplotype analysis, and a score test in which toxicity was regressed on the number of *TYMS* toxicity risk alleles (0 to 4) summed from the 3′UTR and 5′VNTR polymorphisms. For *DPYD*, we grouped rare variants with effects on enzyme function (*DPYD**2A and 2846T>A) for analysis.

For our primary investigation of global toxicity, we used a false discovery rate of $q < 0.05$,[37] corresponding to $P < .0065$ for the QUASAR2 analysis, $P < .0033$ for the capecitabine meta-analyses, and $P < .0048$ for the noncapecitabine meta-analyses. We refer to associations that achieve $q < 0.05$ as formally significant and those that achieve $P < .05$ as nominally significant. We also applied these thresholds to assessment of individual toxicities, because these are not independent of global toxicity.

RESULTS

Testing Candidate FU-Toxicity Variants in QUASAR2

Of 927 patients on the QUASAR2 study, 301 developed grade ≥ 3 global toxicity. The most frequent specific grade ≥ 3 toxicity was HFS (n = 206), followed by diarrhea (n = 97), and neutropenia (n = 19). Two patients died as a result of capecitabine-related toxicity; one as a result of respiratory failure second to neutropenia and the other as a result of neutropenic colitis and left ventricular hypertrophy. Three of the 21 polymorphisms were significantly associated with global G3+ toxicity at $q < 0.05$: *TYMS*5′VNTR2R (odds ratio [OR], 1.49; $P = 7.2 \times 10^{-5}$), *TYMS*3′UTR6bp ins (OR, 1.36; $P = .0051$), and *DPYD*2846A (OR, 9.35; $P = .0043$; Table 2). We found no formally significant effect of the other 18 previously reported FU variants on global or specific toxicities (Data Supplement).

The 5′VNTR and 3′UTR*TYMS* polymorphisms are in moderate linkage disequilibrium ($r^2 = 0.17$; $D' = 0.64$). In logistic regression analysis incorporating both variants, only the 5′VNTR polymorphism remained significantly associated with toxicity (Table 2). However, there was modest evidence from the logistic regression analysis that the 3′UTR genotype might have some independent association with toxicity (OR, 1.22; $P = .10$; Table 2), and a regression model with both 5′VNTR and 3′UTR had a slightly better fit to the data than a model with 5′VNTR alone (Aikake information criterion, 1,142 *v* 1,143). To capture the combined signal from the 5′VNTR and 3′UTR polymorphisms, we also tested a quantitative *TYMS* risk score (count, 0 to 4; according to the number of high-risk alleles per patient). The risk score was approximately normally distributed ($P = .76$, Shapiro-Wilk test) and strongly predicted global FU toxicity ($OR_{per\ count}$, 1.33; $P = 1.7 \times 10^{-5}$; Table 2; $OR_{score\ 3\ or\ 4\ v\ score\ 0}$, 2.91; 95% CI, 1.43 to 5.94; $P =$

Table 1. The 36 Previously Studied FU-Toxicity Variants From Systematic Review

Functional Category	Gene Symbol (alias/synonym)	Gene Function	Included Polymorphisms	rsID or hg18 Coordinate	MAF (%)	Past	Kit	Studies
Pro-drug activation	*CES2*	First of three steps in converting capecitabine to FU	823 (830) C/G 5′UTR	rs11075646	8	Y		2
			Intronic SNP	rs11568314	6			1
			Intronic SNP	rs11568311	7			1
			6046G>A; R270H	rs8192924	1			1
			6320 G/A	chr16:65532174	0.8			1
			Intronic SNP	rs2241409	16			1
	CDA (*CDD*)	Second of three steps in converting capecitabine to FU	-451C>T	rs532545	34	Y		1
			-205C>G	rs603412	50			1
			5′UTR SNP rs602950	rs602950	35			1
			943insC	rs3215400	42	Y		2
			CDA*2; 79A>C; K27Q	rs2072671	34			2
			575 C/T	chr1:20817782	40			1
			794 G/A	chr1:20817822	6			1
			771 C/G	chr1:20817978	46			1
	UMPS (*OPRT*)	Conversion of FU to FUMP	638G>C (Gly213Ala)	rs1801019	20			1
			1336A>G (Ile446Val)	rs3772809	0.6			1
	TYMP (*TP*)	Conversion of FU to FUDR	Intronic SNP rs470119	rs470119	39			1
			A324A	rs131804	40			1
			S471L	rs11479	14			1
5-FU target	*TYMS* (*TS*)	Necessary for DNA synthesis; target of FU	5′VNTR 3R G/C SNP	rs2853542	50	Y		10
			5′VNTR 2R/3R	rs45445694	47	Y	Y	18
			3′UTR 1494indel6b	rs16430	31	Y	Y	18
	MTHFR	Lowers levels of folate-derived TYMS cofactor	677C>T; A222V	rs1801133	32	Y	Y	18
			1298A>C; E429A	rs1801131	33	Y	Y	14
Catabolism	*DPYD* (*DPD*)	First catabolic step of activated drug (up to 80%, mostly in liver)	*9A; 85T>C; C29R	rs1801265	23	Y	Y	6
			496A>G; M166V	rs2297595	9		Y	4
			623G>A; R208Q	chr1:97937552	ND			1
			1109delTA	chr1:97831380	ND			1
			1236G>A; E412E	rs56038477	2			3
			*4A; 1601G>A; S534N	rs1801158	2		Y	3
			*5; 1627A>G; I543V	rs1801159	20			4
			*13; 1679T>G; I560S	rs55886062	0.1		Y	1
			*2A; IVS14+1G>A	rs3918290	0.4	Y	Y	9
			*6; 2194G>A; V732I	rs1801160	3			3
			2846T>A; D949V	rs67376798	0.6	Y	Y	6
			2858G>C; C953S	chr1:97320523	ND			1

NOTE. Polymorphisms have been described in various ways and these names are all shown, together with their dbSNP ID (rs number) or, where absent from dbSNP, by chromosomal location in genome build hg18. Past refers to previously published associations at $P < .1$ for increased FU toxicity. Kit refers to inclusion in a commercially available kit for predicting FU toxicity. Studies refer to the number of eligible, published studies that have analyzed this polymorphism for an association with FU toxicity (excluding QUASAR2).

Abbreviations: dbSNP ID, database of SNPs identifier; DPYD, dihydropyrimidine dehydrogenase; FU, fluourouracil; MAF, minor allele frequency; ND, not determined; QUASAR2, Quick and Simple and Reliable 2 trial; Y, yes.

.0032), providing a slightly improved fit (Aikake information criterion, 1,140) to the data.

We then analyzed the individual toxicities underlying the significant associations with global toxicity. The *TYMS* polymorphisms (score test) seemed to have similar effects on HFS (OR, 1.30; $P = .00052$) and diarrhea (OR, 1.24; $P = .038$), but the former toxicity was more common and hence contributed more to the global measure (Table 2). In contrast, the effects of *DPYD*2846A seemed more marked for diarrhea (OR, 3.14; $P = .093$) than for HFS (OR, 1.31; $P = .69$; Table 2).

Meta-Analysis of FU-Toxicity Variants

Effect of variants on toxicity from capecitabine monotherapy. Fifteen variants were analyzed for associations with global capecitabine toxicity (Data Supplement). The four studies additional to QUASAR2 comprised up to 382 patients. For *TYMS* and *DPYD2A*, the conclusions from the QUASAR2 analysis were maintained in the meta-analysis (Table 2; Fig 1). We found no good evidence of an association between any other polymorphism and G3+ toxicity (Data Supplement).

Effect of variants on toxicity from infusional FU monotherapy. Fifteen variants were analyzed (Data Supplement), of which seven were present in single studies only. Only *TYMS* 5′VNTR2R met the formal significance threshold for association with global G3+ toxicity in the meta-analysis (OR, 1.45; 95% CI, 1.13 to 1.85; $P = .0035$; Data Supplement). In an analysis adjusted for the 3′UTR6bp ins-del variant (Data Supplement), the 5′VNTR polymorphism remained associated with toxicity (OR, 1.53; 95% CI, 1.14 to 2.04; $P = .0040$). The *TYMS* risk score was only nominally associated with toxicity ($OR_{per\ count}$, 1.22; 95% CI, 1.02 to 1.45; $P = .031$).

Table 2. Associations Between Selected *DPYD* and *TYMS* Variants and Capecitabine-Related Toxicity

Polymorphism and Toxicity	QUASAR2 Analyses					All Capecitabine Analyses						
	No. of Patients	TAF	OR	95% CI	*P*	No. of Studies	No. of Patients	OR	95% CI	*P*	*P*-het	
*TYMS*5′VNTR2R/3R (*2-repeat allele*)												
Global	918	0.47	1.48	1.22 to 1.80	.000079	5	1,300	1.36	1.15 to 1.60	.00028	.17	Meta
Diarrhea	918	0.47	1.29	0.96 to 1.74	.093	5	1,309	1.12	0.87 to 1.45	.38	.29	
HFS	916	0.47	1.44	1.15 to 1.79	.0013	5	1,306	1.33	1.10 to 1.60	.0029	.23	
*TYMS*3′UTR6bpins-del (*6bp-insertion allele*)												
Global	474	0.69	1.67	1.23 to 2.22	.00084	4	738	1.35	1.07 to 1.70	.012	.024	Meta
Diarrhea	474	0.69	1.49	0.94 to 2.38	.085	4	745	1.11	0.79 to 1.58	.54	.007	
HFS	473	0.69	1.47	1.06 to 2.08	.021	4	743	1.43	1.09 to 1.87	.0091	.34	
5′VNTR adjusted for 3′UTR												
Global	474	0.47	1.24	0.93 to 1.67	.15	2	602	1.27	0.98 to 1.64	.068	—	Pooled
Diarrhea	474	0.47	1.08	0.70 to 1.67	.72	2	602	1.11	0.76 to 1.61	.59	—	
HFS	474	0.47	1.26	0.91 to 1.75	.17	2	602	1.20	0.90 to 1.58	.21	—	
3′UTR adjusted for 5′VNTR												
Global	474	0.69	1.56	1.11 to 2.18	.010	2	602	1.42	1.06 to 1.89	.017	—	Pooled
Diarrhea	474	0.69	1.47	0.88 to 2.45	.14	2	602	1.19	0.78 to 1.81	.43	—	
HFS	474	0.69	1.37	0.94 to 1.98	.10	2	602	1.40	1.02 to 1.93	.038	—	
TYMS score test (*No. of high-risk alleles*)												
Global	474	0.58	1.38	1.16 to 1.64	.00031	2	602	1.33	1.15 to 1.55	.00018	.46	Meta
Diarrhea	474	0.58	1.24	0.96 to 1.61	.096	2	602	1.14	0.92 to 1.42	.24	.20	
HFS	474	0.58	1.31	1.08 to 1.59	.0063	2	602	1.29	1.09 to 1.52	.0030	.73	
*DPYD**2A [*exon skipping allele (A)*]												
Global	905	0.004	2.78	0.62 to 12.5	.18	2	1,035	3.02	0.78 to 11.7	.11	.83	Meta
Diarrhea	905	0.004	1.41	0.17 to 11.8	.75	2	1,035	3.14	0.71 to 13.8	.13	.18	
HFS	903	0.004	2.67	0.59 to 12.0	.20	2	1,033	1.98	0.52 to 7.54	.32	.46	
*DPYD*2846T>A (*A allele*)												
Global	881	0.006	9.35	2.01 to 43.4	.0043							
Diarrhea	881	0.006	3.14	0.82 to 11.9	.093							
HFS	879	0.006	1.31	0.35 to 4.96	.69							
DPYD combined allelic model (*2846A or *2AA allele*)												
Global	863	0.005	5.51	1.95 to 15.5	.0013							
Diarrhea	863	0.005	2.48	0.81 to 7.60	.11							
HFS	861	0.005	1.76	0.66 to 4.71	.26							

NOTE. Fixed-effect meta-analysis and pooled logistic regression analysis results stratified by study are shown for ≥ grade 3 *v* grade 0-2 toxicity. Test alleles are shown in italics.

Abbreviations: DPYD, dihydropyrimidine dehydrogenase; HFS, hand-and-foot syndrome; meta, meta-analysis; OR, odds ratio; *P*-het, *P* value for heterogeneity test; S, No. of studies; TAF, frequency of the putative toxicity-associated allele.

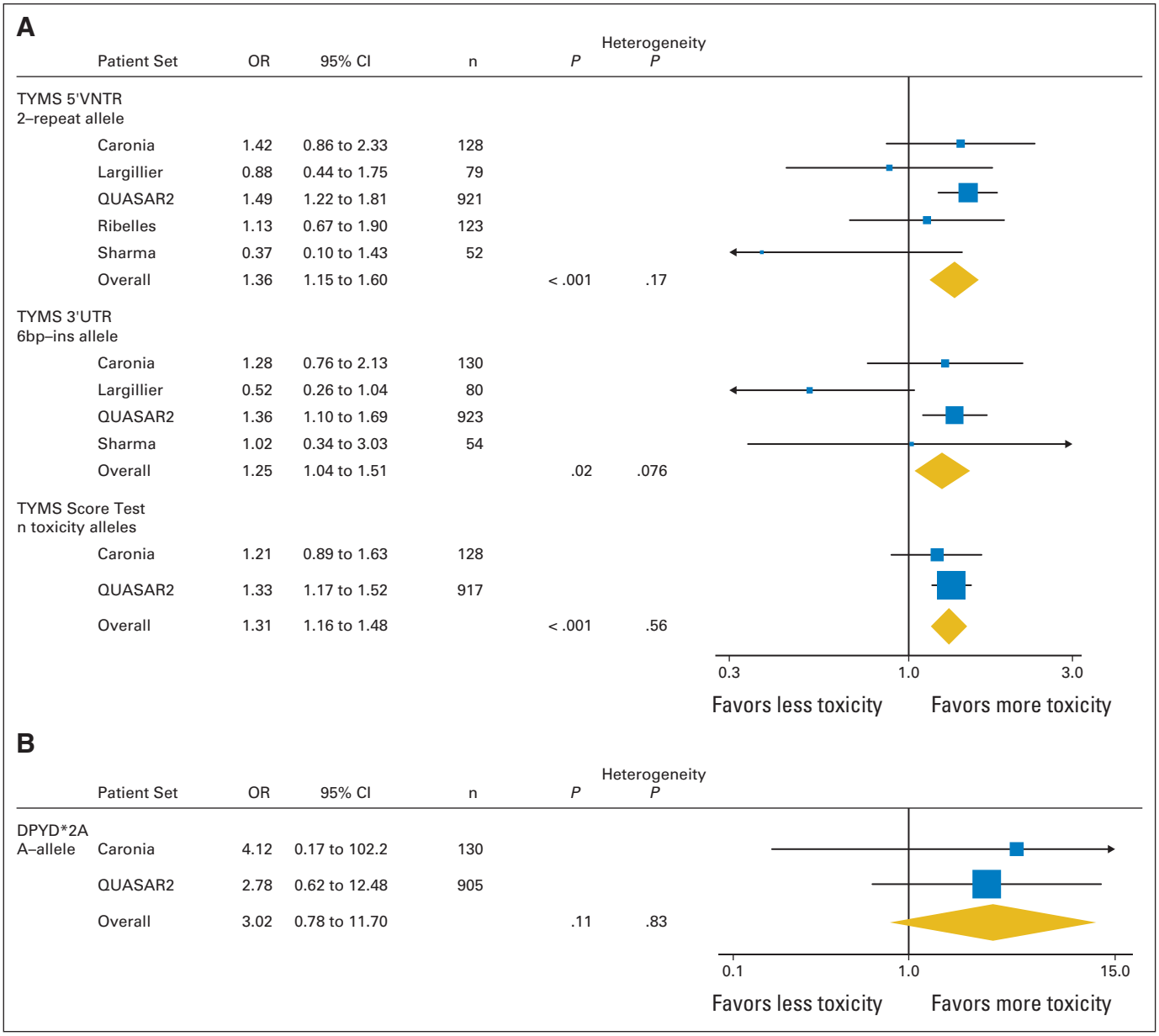

Fig 1. Forest plots of meta-analyses of selected (A) *TYMS* and (B) *DPYD* polymorphisms associated with global capecitabine toxicity. The analyses shown are for global grade ≥ 3 *v* grade 0 to 2 toxicities under a fixed-effects model. *DPYD*2846 is not shown because data were only available for the Quick and Simple and Reliable (QUASAR2) study. Horizontal lines show the 95% CIs. The size of the square is directly proportional to the amount of information contributed by the trial. The diamonds represent overall odds ratio (OR) for the included studies, with the center denoting the OR and the extremities the 95% CI.

Analysis of individual adverse events suggested that the increased toxicity with the *TYMS*5′VNTR2R allele was primarily owing to diarrhea (OR, 1.45; 95% CI, 1.01 to 2.08; $P = .042$).

Although it did not reach the formal significance level for association, a substantial increased risk of global G3+ toxicity was suggested for the *DPYD**2A polymorphism (OR, 6.71; 95% CI, 1.66 to 27.1; $P = .0075$), mainly because of diarrhea (OR, 7.71; 95% CI, 1.61 to 36.9; $P = .011$). In a single-study analysis, the *DPYD*2846A allele showed a trend to greater G3+ toxicity, though this did not reach significance (OR, 3.09; 95% CI, 0.28 to 34.4; $P = .36$). None of the other FU-toxicity variants analyzed showed significant associations with infusional FU toxicity.

Effect of variants on toxicity from bolus FU monotherapy. The only polymorphism significantly associated with global G3+ toxicity as a result of bolus FU was the *TYMS*3′UTR6bp ins allele (OR, 1.98; 95% CI, 1.15 to 3.40; $P = .00038$), principally because of mucositis (OR, 2.03; 95% CI, 1.34 to 3.08; $P = .00086$; Data Supplement). However, this association was not significant after adjusting for 5′VNTR alleles (Data Supplement). The *TYMS* risk score was a weaker predictor (OR, 1.35; 95% CI, 1.06 to 1.71; $P = .014$).

Although the *DPYD**2A variant did not meet the formal level of significance for association with global G3+ toxicity (OR, 3.84; 95% CI, 0.95 to 15.6; $P = .059$), a substantial and significant increase in G3+ neutropenia was evident in patients who carried

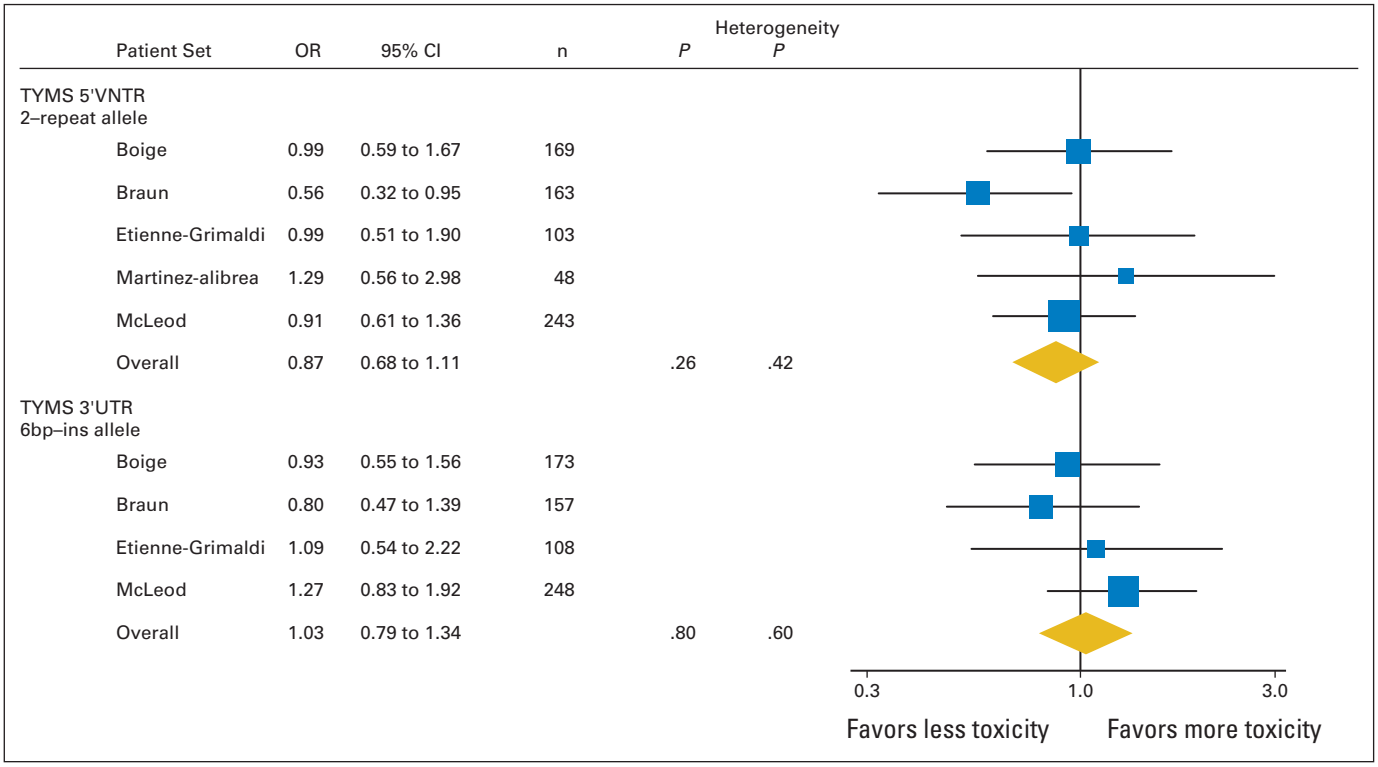

Fig 2. Forest plot of *TYMS* polymorphisms meta-analyzed in infusional fluorouracil, leucovorin, and oxaliplatin patients. Horizontal lines show the 95% CIs. The size of the square is directly proportional to the amount of information contributed by the trial. The diamonds represent overall odds ratios (OR) for the included studies, with the center denoting the OR and the extremities the 95% CI.

this variant (OR, 12.9; 95% CI, 3.13 to 53.3; $P = .0004$). As for infusional FU, patients who carried the *DPYD*2846A allele had trends to all types of toxicity. No other variant was significantly associated with bolus FU toxicity.

Combined Analysis of Rare DPYD *Alleles With Evidence of Effects on Enzyme Function*

For alleles within a single gene that have equivalent functional effects causally related to toxicity, it is justifiable to combine these into one functional class for predictive testing. For *DPYD*, some rare variants have been proposed to cause *DPYD* deficiency syndrome (Online Mendelian Inheritance in Man No. 274270).[38,39] Of these, a few have been shown to reduce DPYD activity in vitro,[40] whereas others have lesser functional evidence from in vivo reports.[41,42] Among variants found in our patient sets, we found good published evidence of functionality for *DPYD*2846A and *2A,[38,39] but not for *9A (85T>C) or Ile370Val (1108A>G), despite these having previously been reported as causing *DPYD* deficiency (Data Supplement). We therefore performed an analysis of *DPYD*2846T>A and *2A rare alleles as a group (presence of either variant *v* no either variant). We found a formally significant association with global toxicity for capecitabine (OR, 5.51; 95% CI, 1.95 to 15.51; $P = .0013$; data from QUASAR2 alone; Table 2) and nominally significant associations in the analyses for infusional ($P = .042$) and bolus ($P = .0068$) monotherapies (Data Supplement). All of these associations were stronger than when either of the variants was considered alone. We noted that of the two patients who died from capecitabine-related toxicity in QUASAR2, one carried *DPYD*2846A and the other, *2A.

Prediction of Toxicity in FU Combination Therapy Regimens

None of the polymorphisms analyzed was associated with global or any specific toxicity in the combination therapy regimens (FOLFOX; CAPOX [capecitabine and oxaliplatin]; FOLFIRI; irinotecan, leucovorin, and fluorouracil [IFL or FLIRI]; Data Supplement). We note that *DPYD**2A was invariant and *DPYD*2846T>A was not analyzed in the available datasets. Figure 2 shows the results from meta-analysis of the two main *TYMS* polymorphisms in studies using FOLFOX, the largest combination therapy data set.

Performance of Panels of Polymorphisms for Predicting FU Toxicity

There are currently three commercially available kits for predicting FU toxicity (Data Supplement). These kits contain a total of 17 polymorphisms that fall into three categories: evidence of toxicity prediction in our analysis (n = 4), present in our analysis but without good evidence of predictive ability (n = 5), or absent from our analysis (n = 8). Of the variants that are absent from our analysis, five are rare *DPYD* variants with evidence of harmful effects on enzyme function [1679(*13), 1897(*3), 295-298del(*7), 703(*8), and 2983(*10); Data Supplement].[38,39]

In QUASAR2, we assessed the prediction of global toxicity by each kit, following the instructions as closely as possible, and using a binary classification of risk (no/low *v* moderate/intermediate/high). Owing to the inclusion of some common polymorphisms, two kits classified almost all patients as at-raised-risk of toxicity. One kit, however, provided better discrimination, with an area under the

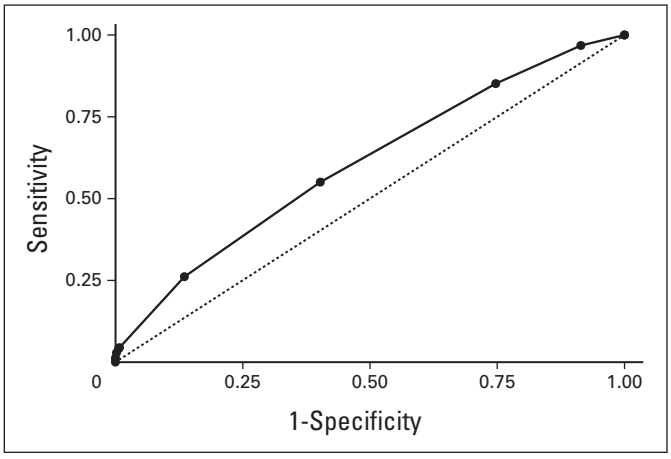

Fig 3. Receiver operating characteristic (ROC) analysis of the *TYMS* score test and *DPYD* group test for predicting global capecitabine toxicity in the Quick and Simple and Reliable (QUASAR2) trial capecitabine patients. Two sensitivity/specificity cut points are marked. Cut points at the bottom-left of the plot corresponds to the maximum proportion of patients correctly classified, with a sensitivity of 4.4%, specificity of 99%, positive predictive value of 73% (PPV; 95% CI, 45% to 91%), and negative predictive value of 68% (NPV; 95% CI, 64% to 71%), largely owing to rare *DPYD* variants. The other cut point (64% correctly classified) affects more patients as a result of utilizing *TYMS* genotypes and corresponds to a sensitivity of 26%, specificity of 86%, PPV of 49% (95% CI, 40% to 58%), and NPV of 70% (95% CI, 66% to 74%).

concentration-time curve of 0.56, 31% sensitivity, 82% specificity, 46% positive predictive value, and 70% negative predictive value (Data Supplement).

We then assessed whether we could improve on the performance of the kits using our *DPYD* combined rare functional alleles test and the *TYMS* score test (Fig 3; Data Supplement). Although no fully independent data set was available for cross-validation, we minimized bias by applying effect size estimates from Caronia et al[33] to QUASAR2 in a logistic regression model. Area under the concentration-time curve was 0.61. At our preferred ln(OR) cutoff of 0.762, sensitivity was 26%, specificity was 86%, positive predictive value was 49%, and negative predictive value was 70%.

DISCUSSION

We have provided the most comprehensive analysis to date of FU toxicity pharmacogenetics. We found that few genetic variants had convincing evidence of an association with toxicity. Of 36 previously assessed polymorphisms, only four—*TYMS* 5′VNTR 2R/3R, *TYMS* 3′UTR 6bpins-del, *DPYD* 2846TA, and *DPYD* *2A—were formally associated with global G3+ toxicity in our analysis. Even so, associations were only present in FU monotherapy regimens. The best evidence came from capecitabine monotherapy in the adjuvant setting although, even here, *TYMS*3′UTR6bp ins-del showed evidence of interstudy heterogeneity and we therefore relied on the larger capecitabine studies for our conclusions regarding this polymorphism. Studies of bolus and infusional FU generally supported the *TYMS* and *DPYD* data, although formally significant associations were less common. We found that formal cross-regimen analysis was not justifiable.

The *TYMS* risk alleles are common in the northern European population. We found the two *TYMS* polymorphisms to be partially independent toxicity predictors and both seem to provide useful information. Despite some inconsistent evidence that the *TYMS* alleles affect mRNA expression levels,[36,43] they have not been shown to cause clinically significant differences in TYMS activity or thymidine incorporation into nucleic acids. Because the identity of the functional *TYMS* variation that causes toxicity is unknown, we have proposed the use of an ad hoc test in which each individual has a score of 0 to 4 according to the number of high-risk alleles they carry at the 5′VNTR and 3′UTR polymorphisms. The score test was a good predictor of global toxicity for capecitabine (OR, 1.33 per allele), with weaker evidence for infusional and bolus FU monotherapy.

For *DPYD*, the two variants associated with toxicity are rare, but for patients with *2A or 2846A, the risk is relatively high (OR, 5.51). We have proposed a group test in which, on the basis of enzyme function, patients carrying either *DPYD*2846A or *DPYD**2A are classed as being variant or wildtype. It is likely that other rare *DPYD* variants with functional effects equivalent to 2846A or *2A could be included in this test (Data Supplement).

Evidence of an association with toxicity was weak for the remaining polymorphisms. Some of these (*DPYD*1627A>G, *DPYD*85T>C, *DPYD*496A>G, *TYMS*5′VNTRG>C, *MTHFR*677C>T, *MTHFR*1298A>C, *CDA*−451C>T, *CES2*823C>G, and the *TYMP* polymorphisms) have common alleles (MAF > 8%). Power to detect an association for these SNPs was approximately 75% to 100%, assuming an odds ratio of 1.5 per allele, and all but modest effects could therefore be excluded where sample sizes were relatively large. For other polymorphisms (eg, *DPYD*1601G>A, *DPYD*1236G>A, *DPYD*2194G>A, *CDA*943insC, and most *CES2* polymorphisms), minor allele frequencies were low or sample sizes small, leading to suboptimal power (approximately 20% to 40%) to detect an association. The case for these as markers of toxicity remains unproven.

Several factors limited our ability to identify polymorphisms associated with FU toxicity. First, the different incidences of individual toxicity phenotypes among FU-based regimens required that we stratify the meta-analyses by FU regimen. This conservative approach decreased power, but prevented us from falsely combining data for toxicity events resulting from different sources. This method also required a larger number of tests, though most were not independent and we corrected for false discovery. Second, in the meta-analysis, there was a little evidence of publication bias; eight of 28 studies failed to provide ORs, and the absence of individual patient data meant that covariate-adjusted analyses were not generally possible. Third, there was no large capecitabine study to validate QUASAR2. Fourth, studies used different genotyping methods, although there was only good evidence of deviation from Hardy-Weinberg equilibrium in two *TYMS* 3′UTR data sets, which were subsequently excluded.

In conclusion, we have found that four specific germline *TYMS* and *DPYD* variants predict capecitabine toxicity. Although our analysis suggests that the polymorphisms may be predictive of toxicity in other FU monotherapy regimens, the data are currently less clear and these regimens are used uncommonly. We found no good evidence of polymorphisms that predict toxicity in patients on FU combination therapies, although no data were available for rare *DPYD* variants in this context. The lack of an association between either of the *TYMS* polymorphisms and toxicity in combination regimens is interesting and might reflect reduced FU dosage in these regimens, overlapping toxicities between drugs, confounding of FU toxicity by other more serious and/or early-onset toxicities, or suboptimal patient set sizes.

Our findings strongly suggest the exclusion of several unwarranted polymorphisms from the currently available FU toxicity tests, leading to better performance at lower cost. Even then, a genetic test comprising the validated polymorphisms—two *TYMS* variants and functional *DPYD* variants—provides only modest predictive power. For genetic tests to be used in clinical practice, there is a need to identify and characterize additional FU toxicity variants. If such variants were added to the panel of polymorphisms identified in our study, a genetic test might well provide the ability to closely monitor patients who are at increased risk of toxicity or to increase FU dosage in those who are at low risk of toxicity.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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