Clinical relevance of *DPYD* variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data

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Summary

Background The best-known cause of intolerance to fluoropyrimidines is dihydropyrimidine dehydrogenase (DPD) deficiency, which can result from deleterious polymorphisms in the gene encoding DPD (*DPYD*), including *DPYD**2A and c.2846A>T. Three other variants—*DPYD* c.1679T>G, c.1236G>A/HapB3, and c.1601G>A—have been associated with DPD deficiency, but no definitive evidence for the clinical validity of these variants is available. The primary objective of this systematic review and meta-analysis was to assess the clinical validity of c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity.

Methods We did a systematic review of the literature published before Dec 17, 2014, to identify cohort studies investigating associations between *DPYD* c.1679T>G, c.1236G>A/HapB3, and c.1601G>A and severe (grade ≥3) fluoropyrimidine-associated toxicity in patients treated with fluoropyrimidines (fluorouracil, capecitabine, or tegafur-uracil as single agents, in combination with other anticancer drugs, or with radiotherapy). Individual patient data were retrieved and analysed in a multivariable analysis to obtain an adjusted relative risk (RR). Effect estimates were pooled by use of a random-effects meta-analysis. The threshold for significance was set at a p value of less than 0·0167 (Bonferroni correction).

Findings 7365 patients from eight studies were included in the meta-analysis. *DPYD* c.1679T>G was significantly associated with fluoropyrimidine-associated toxicity (adjusted RR 4·40, 95% CI 2·08–9·30, p<0·0001), as was c.1236G>A/HapB3 (1·59, 1·29–1·97, p<0·0001). The association between c.1601G>A and fluoropyrimidine-associated toxicity was not significant (adjusted RR 1·52, 95% CI 0·86–2·70, p=0·15). Analysis of individual types of toxicity showed consistent associations of c.1679T>G and c.1236G>A/HapB3 with gastrointestinal toxicity (adjusted RR 5·72, 95% CI 1·40–23·33, p=0·015; and 2·04, 1·49–2·78, p<0·0001, respectively) and haematological toxicity (adjusted RR 9·76, 95% CI 3·03–31·48, p=0·00014; and 2·07, 1·17–3·68, p=0·013, respectively), but not with hand-foot syndrome. *DPYD**2A and c.2846A>T were also significantly associated with severe fluoropyrimidine-associated toxicity (adjusted RR 2·85, 95% CI 1·75–4·62, p<0·0001; and 3·02, 2·22–4·10, p<0·0001, respectively).

Interpretation *DPYD* variants c.1679T>G and c.1236G>A/HapB3 are clinically relevant predictors of fluoropyrimidine-associated toxicity. Upfront screening for these variants, in addition to the established variants *DPYD**2A and c.2846A>T, is recommended to improve the safety of patients with cancer treated with fluoropyrimidines.

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Introduction

The fluoropyrimidines capecitabine, fluorouracil, and tegafur are the backbone of treatments for gastrointestinal, breast, and head and neck cancers. Of the patients treated with fluoropyrimidines, 10–30% have severe treatment-related toxicity, which is lethal in 0·5–1% of patients (with treatment-related mortality of up to 5% reported in elderly patients).[1–4] The most well known cause of intolerance to fluoropyrimidines is deficiency of the key enzyme for metabolism of fluorouracil, dihydropyrimidine dehydrogenase (DPD), encoded by the gene *DPYD*. DPD deficiency is detected in 39–61% of patients with severe toxicity, emphasising its importance as a risk factor for severe toxicity.[5] The activity of DPD is regulated at the transcriptional level, including by transcription factors SP1 and SP3, and at the post-transcriptional level, for instance by microRNA 27-a (miR-27a) and microRNA 27-b.[5–8] A substantial proportion of the cases of DPD deficiency are, however, the result of deleterious polymorphisms in *DPYD*, which have therefore received widespread attention as predictors of fluoropyrimidine-associated toxicity.[9–18]

The most well established deleterious *DPYD* variants associated with fluoropyrimidine-associated toxicity are *DPYD**2A (IVS14+1G>A, c.1905+1G>A, or rs3918290) and c.2846A>T (D949V or rs67376798).[19,20] The results of several

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studies and a meta-analysis have shown strong associations between these variants—both with a frequency of heterozygotes of about 1% in white people—and fluoropyrimidine-associated toxicity.[9,10,12,13,21,22] Importantly, screening before treatment for *DPYD**2A, and a 50% reduction in starting dose given to patients who carry the variant allele heterozygously, results in therapeutic fluorouracil exposure and reduces the risk of severe toxicity, showing the clinical utility of upfront *DPYD* screening to prevent severe toxicity. Furthermore, this strategy of *DPYD* genotype-guided dosing in patients carrying *DPYD**2A was shown to be feasible in routine clinical practice and to be cost saving.[23]

Three other *DPYD* variants have been associated with altered DPD activity and fluoropyrimidine-associated toxicity—ie, c.1679T>G, c.1236G>A, and c.1601G>A—but data on clinical validity are inconclusive. Conclusive evidence for clinical validity of *DPYD* variants is crucial before upfront screening and dose adjustments can be recommended as a strategy to improve safety of patients treated with fluoropyrimidines.

The variant c.1679T>G (I560S, *DPYD**13, or rs55886062) has a frequency of heterozygosity of about 0·2% in the white population,[10,12,24–26] and has been associated with reduced DPD activity in in-vitro studies.[27] The Clinical Pharmacogenetics Implementation Consortium has recommended a 50% dose reduction for patients with this variant in heterozygous form.[28] However, because of the low frequency of c.1679T>G, the association between c.1679T>G and fluoropyrimidine-associated toxicity has not been shown definitively in any study.[10,12,14,16,29] More data on the clinical validity of this variant are therefore needed before advising upfront screening. For c.1236G>A (E412E or rs56038477), a synonymous variant that is in complete linkage with the deleterious deep intronic variant c.1129-5923C>G (rs75017182) in haplotype B3 (HapB3),[29,30] an association with fluoropyrimidine-associated toxicity has been shown in several studies,[14,29,30] but the results from other studies did not confirm these associations.[9,13,15,16,31] Data for the effect of c.1236G>A/HapB3 on DPD activity are inconclusive, and it therefore remains to be established whether a dose reduction should be recommended for patients with this variant.[28,30,32] A third variant, c.1601G>A (S534N, *DPYD**4, or rs1801158), has been associated with altered DPD activity[27] and an increased risk of fluoropyrimidine-associated toxicity in one study,[16] but no significant association with toxicity was noted in other studies.[9,11,13,29,31,33]

Unlike the well studied *DPYD* variants *DPYD**2A and c.2846A>T, data for clinical validity of c.1679T>G, c.1236G>A/HapB3, and c.1601G>A are inconsistent and no meta-analytic data are available. Therefore, we did a systematic review and meta-analysis using individual patient data from previous investigations to assess the clinical relevance of c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity. The primary objective in this meta-analysis was to find out whether these *DPYD* variants are associated with severe (grade ≥3) fluoropyrimidine-associated toxicity, according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTC-AE), in patients treated with fluoropyrimidine-based anticancer regimens.

Methods

Search strategy and selection criteria

We did a literature search of PubMed and Embase to identify studies reporting on associations between c.1679T>G, c.1236G>A/HapB3, and c.1601G>A and fluoropyrimidine-associated toxicity, published before Dec 17, 2014. Additionally, an unpublished pharmacogenetic analysis from our own institute, which investigated the association between *DPYD* variants and fluoropyrimidine-associated toxicity in 1606 patients, was also included in the analysis (Meulendijks D, unpublished data). The following search terms were used for the literature search: "(DPYD OR DPD OR dihydropyrimidine dehydrogenase) AND (polymorphism OR Polymorphism, Single Nucleotide[mesh] OR Polymorphism, Genetic[mesh] OR pharmacogenet*[tiab] OR Pharmacogenetics[mesh] OR mutation/genetics OR genotype[mesh] OR polymorphisms OR variant OR variants OR SNP OR c.1236G>A OR E412E OR rs56038477 OR c.1129-5923C>G OR rs75017182 OR c.1601G>A OR S534N OR DPYD*4 OR rs1801158 OR c.1679T>G OR I560S OR rs55886062 OR DPYD*13) AND (toxicity OR adverse OR side-effects OR Antineoplastic Combined Chemotherapy Protocols/adverse effects[mesh])".

All search results were screened by title and abstract, and full-text articles of potential relevance were retrieved and assessed. Reference lists were searched for additional relevant publications. Studies were eligible for inclusion if they met the following criteria: patients were treated with fluoropyrimidines (fluorouracil, capecitabine, or tegafur-uracil; as single agent or in combination with other anticancer drugs or with radiotherapy); patients were genotyped for c.1679T>G, c.1236G>A/HapB3, or c.1601G>A (for c.1236G>A/HapB3, both c.1236G>A and c.1129-5923C>G were a proxy for haplotype B3 and these variants were assumed to be in complete linkage based on published data[14,30] and our own unpublished data); the study had a cohort design (including secondary analyses of clinical trials) so as to allow appropriate estimation of the relative risk (RR); and toxicity was assessed and recorded according to the CTC-AE. If several studies reported on (or part of) the same patient population, patients were included in the analysis only once (ie, the most extensive report was included). Studies were excluded from the primary analysis if any of the following was applicable: the patient population was selected on the basis of their toxicity phenotype or *DPYD* genotype status (if only some of the patients were selected on the basis of toxicity phenotype or *DPYD* status, these patients were excluded from the analysis), the study was reported in a

language other than English, or none of the patients had any of the *DPYD* variants investigated. Review articles were excluded. For completeness, all identified case-control studies investigating the effect of *DPYD* variants on the risk of fluoropyrimidine-associated toxicity were selected for a secondary analysis (appendix).

Data gathering

We aimed to gather all individual patient data from investigators who previously reported on associations between c.1679T>G, c.1236G>A/HapB3, or c.1601G>A and fluoropyrimidine-associated toxicity. The requested data consisted of the maximum toxicity per patient during the period studied by the investigators, patients' characteristics known to be relevant in relation to fluoropyrimidine-associated toxicity for use as covariables (preferably including age, sex, treatment regimen or concomitant chemotherapy, dose of the fluoropyrimidine, and renal function). If individual patient data could not be gathered, toxicity counts were extracted from the report. A descriptive analysis of the quality of the included studies was done independently by two investigators (LMH and DM) with the recommendations from Strengthening the Reporting of Genetic Association studies[34] and Human Genome Epidemiology Network[35] as guidelines. The reported results are based on consensus between the two investigators.

Statistical analysis

A summary of the statistical analysis is provided here (full details are provided in the appendix). The primary endpoint was RR for any severe, CTC-AE grade 3 or greater fluoropyrimidine-associated toxicity in carriers of heterozygous or homozygous variant alleles compared with patients without the variant allele. A two-stage analysis approach was used. First, the endpoint was calculated for each individual study, based on individual patient data whenever available, with modified Poisson regression with adjustment for factors known to be associated with toxicity. Whenever available, the following covariables were included in the multivariable analysis: age, sex, fluoropyrimidine dose, renal function, and treatment regimen. If individual patient data could not be gathered, a crude RR was calculated using a 2×2 table, based on data extracted from the publication, and the crude RR was included in the analysis without correction for covariables. A zero-cell count continuity correction of 0·5 was applied if needed.[36] A dominant genetic model was applied because of the low frequency of homozygous variant genotypes.

In the second stage, RRs from the individual studies were combined by use of DerSimonian-Laird random-effects meta-analysis.[37] A random-effects model was chosen because true differences in effect size between patient populations, as a result of differences in patients' characteristics and treatment regimens, were assumed. Results were reported as RRs with their 95% CI and corresponding p values. Heterogeneity was assessed with Cochrane's *Q* test, with a threshold for the p value of less than 0·1 for significance, and the Higgins and Thompson I^2 statistic was assessed.[38] A Bonferroni correction for multiple testing of the three *DPYD* variants was applied—ie, the threshold for significance for the primary endpoint was set at a p value of less than 0·0167. The same threshold for significance was used for analysis of subtypes of fluoropyrimidine-associated toxicity. The reported p values are unadjusted.

See Online for appendix

The effect of *DPYD* variants on risk of subtypes of fluoropyrimidine-associated toxicity—ie, gastrointestinal toxicity, haematological toxicity, and hand-foot syndrome—was analysed with a one-stage approach based on the retrieved individual patient data, with adjustment for age, sex, treatment regimen, and the study in which the patient was treated. To investigate the robustness of associations between *DPYD* variants and toxicity across patients' characteristics and treatment regimens, prespecified subgroups according to age, sex, and treatment regimen were assessed in the same pooled dataset. Statistical interaction terms between *DPYD* variants and patients' characteristics and treatment regimens were also assessed in this dataset.

Leave-one-out (leave-one-study-out) meta-analysis was done to assess robustness of findings in terms of the primary endpoint. Publication bias was assessed with Begg's funnel plots and Egger's regression test for funnel plot asymmetry. The effect of timeframe in which toxicity was assessed on the primary endpoint was investigated by comparison of the summary estimates from studies that assessed a short timeframe (shorter than the complete treatment duration) with studies that assessed a long timeframe (whole treatment duration) by use of metaregression.

Sensitivity and positive predictive value of the *DPYD* variants to predict severe fluoropyrimidine-associated toxicity were calculated for each individual study and subsequently combined using DerSimonian-Laird random-effects meta-analysis.[37] Frequencies of other established *DPYD* variants (*DPYD**2A and c.2846A>T) in groups of patients depending on c.1679T>G, c.1236G>A/HapB3, and c.1601G>A genotype were calculated whenever data for *DPYD**2A and c.2846A>T were available. Meta-analyses were repeated after excluding patients with either *DPYD**2A or c.2846A>T, to assess the potential effect of these variants on the results of the analysis. Additionally, meta-analysis was done for variants *DPYD**2A and c.2846A>T to compare effect sizes with those obtained for the investigated variants.

All statistical analyses were done in *R* (version 3.1.1). The PRISMA-individual patient data statement was used as a guideline for preparation of the final report.[39]

Role of the funding source

There was no funding source for this study. DM, LMH, and JHMS had full access to the data and final responsibility to submit.

Results

Figure 1 shows the selection process of studies investigating the associations of *DPYD* variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A with severe fluoropyrimidine-associated toxicity. Eight studies met the inclusion criteria (table). These eight studies together included 7365 patients (table). The c.1679T>G variant was measured in five studies (5616 patients), c.1236G>A/HapB3 in six studies (4261 patients), and c.1601G>A in five studies (3900 patients; table). Individual patient data could be gathered from three (60%) of five studies for c.1679T>G (2535 patients), all six (100%) studies of c.1236G>A/HapB3 (4261 patients), and all five (100%) studies of c.1601G>A (3900 patients).

Three studies were prospective cohort studies, three were secondary analyses of randomised controlled trials, and two were retrospective cohort studies (table). Patients were treated in Europe, the USA, and Australia, and ethnic origin, when stated, was predominantly white (table). The median age of patients in the studies ranged between 58 years and 67 years, and slightly more men than women were enrolled in most studies (table). Colorectal cancer was the most common type of tumour and patients most often received combination treatment including oxaliplatin (table). The quality assessment of the included studies is summarised in the appendix. Studies included in the main analysis scored positive on a mean of 8·5 of nine items. In all studies, the investigated endpoint was fluoropyrimidine-associated toxicity, although the toxicities that were scored varied between the studies, as did the timeframe in which toxicity was assessed (which varied between first cycle only and the full treatment duration; appendix). The clinical data provided by the investigators and the covariables included in the multivariable analysis are also summarised in the appendix.

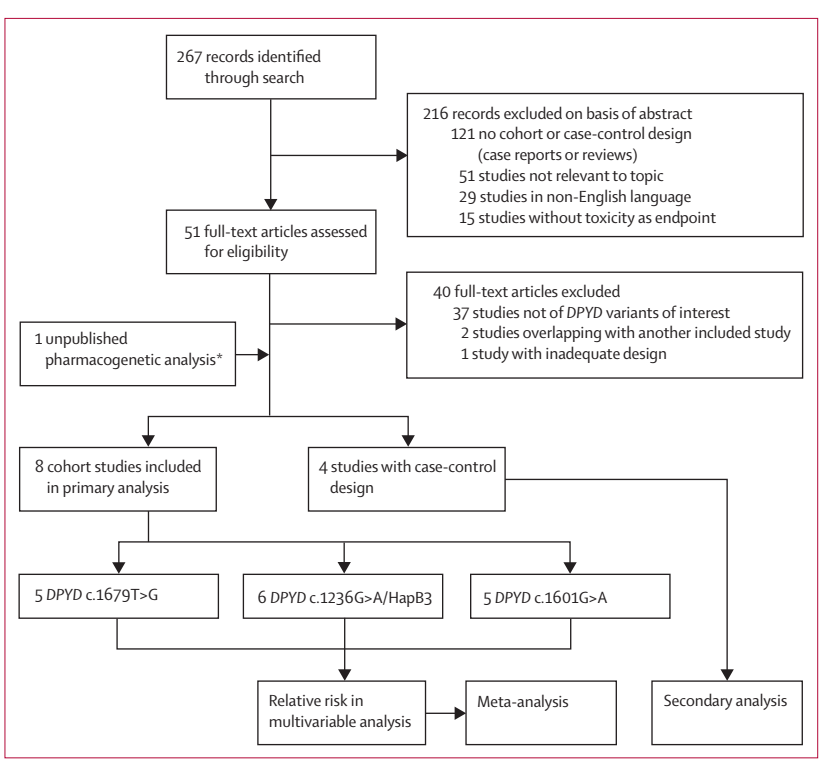

Figure 1: **Flow diagram of study selection**
*A pharmacogenetic analysis was done in our own institute, the details of which will be reported separately.

Figure 2 shows the results of the primary analysis of the associations between *DPYD* variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A, and severe fluoropyrimidine-associated toxicity. Of 5616 patients included in the analysis of *DPYD* c.1679T>G, 11 (0·2%) were heterozygous. There was a significant association between c.1679T>G and global severe fluoropyrimidine-associated toxicity both before adjustment (RR 4·30, 95% CI 2·10–8·80, p<0·0001) and after adjustment for covariables (4·40, 2·08–9·30; p<0·0001; figure 2A).

Evidence of heterogeneity between the studies was substantial, possibly because of the small number of variant allele carriers. I^2 was 85%, and a Q test was significant (Q 26·67, p<0·0001). There was no indication of publication bias (Egger's regression test, p=0·16; appendix). The leave-one-out sensitivity analysis showed that c.1679T>G remained associated with severe toxicity on exclusion of any of the studies (point estimates ranged from 3·20 to 6·01, with p values of less than 0·044; appendix).

Analysis of the subtypes of fluoropyrimidine-associated toxicity showed a significant association between c.1679T>G and severe haematological toxicity (adjusted RR 9·76, 95% CI 3·03–31·48, p=0·00014), and also severe gastrointestinal toxicity was more frequent in individuals with the c.1679T>G variant allele (RR 5·72, 95% CI 1·40–23·33, p=0·015). None of the six individuals with the c.1679T>G variant allele in the pooled dataset had severe hand-foot syndrome, and therefore a RR for severe toxicity could not be calculated.

In the metaregression analysis to investigate the effect of timeframe, the effect of c.1679T>G on risk of severe toxicity seemed similar in studies with long and short timeframes (model coefficient for long *vs* short timeframe –0·76, 95% CI –2·28 to 0·76, p=0·33; appendix).

Of 4261 patients who were included in the analysis of c.1236G>A/HapB3, 174 (4·1%) patients were heterozygous, and three (0·1%) patients were homozygous polymorphic. There was a significant association between c.1236G>A/HapB3 and global severe fluoropyrimidine-associated toxicity (unadjusted RR 1·72, 95% CI 1·22–2·42, p=0·0018; adjusted RR 1·59, 95% CI 1·29–1·97, p<0·0001; figure 2B).

Leave-one-out sensitivity analysis showed that the association was consistent on exclusion of the individual studies (p<0·006; appendix). The point estimate ranged from 1·50 (with exclusion of Froehlich and colleagues' study[14]) to 1·72 (with exclusion of Rosmarin and colleagues' study[13]). There was little evidence for heterogeneity (I^2 23% and Q 6·52, p=0·26) and no

	Study design	Clinical data gathering	Ethnic origin; nationality	Number patients	Age (years; median, range)	Men and women	Tumour type	Patients given capecitabine or fluorouracil	Treatment regimens	Investigated *DPYD* variants	Data used	Hardy-Weinberg equilibrium reported and in equilibrium?
Morel et al, 2006[10]	Cohort	Prospective	100% white; French	487	63 (23–88)	66% and 34%	Not reported (primarily colorectal cancer)	100% fluorouracil	35% fluorouracil + leucovorin or folinic acid, 20% FOLFIRI, 19% FOLFOX, 20% fluorouracil + platinum, and 6% FEC	c.1679T>G c.1601G>A (invariant and therefore not included)	Extracted from report	Not reported
Deenen et al, 2011[11]	RCT	Prospective	Not reported; Dutch	568*	63 (31–83)	61% and 39%	100% colorectal cancer	100% capecitabine	100% CAPOX-bevacizumab (with or without cetuximab)	c.1236G>A/HapB3 c.1601G>A	Individual patient data	Yes
Jennings et al, 2013[15]	Cohort	Retrospective	Not reported; British	253	67 (23–88)	57% and 43%	100% colorectal cancer	63% capecitabine and 37% fluorouracil	23% capecitabine 40% capecitabine + other drug 25% fluorouracil 12% fluorouracil + other drug	c.1236G>A/HapB3	Individual patient data	Yes
Loganayagam et al, 2013[16]	Cohort	Retrospective	85% white, 12% African American or African Caribbean, and 4% Asian; British	430†	62 (20–83)	57% and 43%	85% colorectal cancer	57% capecitabine and 43% fluorouracil	36% CAPOX 22% FOLFOX 18% capecitabine 24% capecitabine or fluorouracil + other	c.1679T>G c.1236G>A/HapB3 c.1601G>A	Individual patient data	Yes
Rosmarin et al, 2014[13]	RCT	Prospective	Not reported; British, Australian, and Austrian	927‡	65 (27–85)	57% and 43%	100% colorectal cancer	100% capecitabine	100% capecitabine with or without bevacizumab	c.1236G>A/HapB3 c.1601G>A	Individual patient data	Yes
Lee et al, 2014[12]	RCT	Prospective	88% white, 7% African American or African Caribbean, and 5% Asian; North American	2594	58 (19–86)	53% and 47%	100% colorectal cancer	100% fluorouracil	100% FOLFOX (with or without cetuximab) or FOLFIRI (with or without cetuximab)	c.1679T>G	Extracted from report	Yes
Froehlich et al, 2015[14]	Cohort	Prospective	99% white, 1% Asian, African American, or African Caribbean; Swiss	500§	62 (18–99)	60% and 40%	55% colorectal cancer, 19% gastro-oesophageal cancer	21% capecitabine and 79% fluorouracil	35% FOLFOX, FOLFIRI, FOLFOXIRI 20% fluorouracil (with or without leucovorin or folinic acid) 19% fluorouracil + platinum 26% capecitabine or fluorouracil + other	c.1679T>G c.1236G>A/HapB3 c.1601G>A¶	Individual patient data	Yes
Meulendijks et al, 2015 (unpublished)	Cohort	Prospective	96% white; Dutch	1606‖	60 (21–89)	45% and 55%	53% colorectal cancer, 23% breast cancer, 14% gastric or gastro-oesophageal cancer	90% capecitabine and 10% fluorouracil	27% capecitabine + radiotherapy 26% capecitabine 24% capecitabine + platinum 13% capecitabine + other 10% fluorouracil-based	c.1679T>G c.1236G>A/HapB3 c.1601G>A	Individual patient data	Yes

RCT=randomised controlled trial. CAPOX=capecitabine plus oxaliplatin. FEC=fluorouracil, epirubicin, and cyclophosphamide. FOLFIRI=fluorouracil, leucovorin, and irinotecan. FOLFOX=fluorouracil, leucovorin, and oxaliplatin. FOLFOXIRI=fluorouracil, leucovorin, oxaliplatin, and irinotecan. *n=568 for c.1236G>A/HapB3; n= 481 for c.1601G>A. †n=425 for c.1236G>A/HapB3; n=430 for c.1601G>A and c.1679T>G. ‡n=909 for c.1236G>A/HapB3; n=888 for c.1601G>A. §500 patients were included prospectively in the analysis (15 patients selected on the basis of toxicity were excluded); data for 111 of 500 patients were reported by Amstutz and colleagues[29] in 2009 and are included only once. ¶Data for c.1601G>A were not reported in original report.[14] ‖n=1606 for c.1236G>A/HapB3, n=1601 for c.1601G>A, and n=1605 for c.1679T>G.

***Table*: Studies included in the primary analysis**

indication of publication bias (Egger's regression test, p=0·99; appendix). In terms of the subtypes of toxicity, c.1236G>A/HapB3 was most strongly associated with gastrointestinal toxicity (adjusted RR 2·04, 95% CI 1·49 to 2·78, p<0·0001) and haematological toxicity (2·07, 1·17 to 3·68, p=0·013). Like c.1679T>G, an association was not found between c.1236G>A/HapB3 and hand-foot syndrome (RR 1·11, 95% CI 0·70 to 1·77, p=0·65). The risk of severe hand-foot syndrome was also not increased in the subgroup of patients treated with capecitabine-based chemotherapy (RR 1·14, 95% CI 0·53 to 2·44; p=0·74). The effect of c.1236G>A/HapB3 on risk of toxicity seemed similar for studies assessing a long timeframe versus a short timeframe (model coefficient for long *vs* short timeframe –0·19, 95% CI –0·64 to 0·26; p=0·41; appendix).

Of 3900 patients included in the analysis of c.1601G>A, 182 (4·7%) patients were heterozygous and two (0·1%) patients were homozygous. The primary analysis showed no significant association between c.1601G>A and global severe fluoropyrimidine-associated toxicity (unadjusted RR 1·69, 95% CI 0·78–3·65, p=0·15; adjusted RR 1·52, 95% CI 0·86–2·70, p=0·15; figure 2C). We noted substantial between-study heterogeneity (I^2 91% and Q 42·48; p<0·0001), and a stronger effect size was noted in the study by Loganayagam and colleagues[16] than in the remaining studies (figure 2C). Leave-one-out sensitivity analysis showed that heterogeneity dropped from 91% to 0% on exclusion of the study by Loganayagam and colleagues (appendix). The calculated RR thereby dropped from 1·52 to 1·20 (p=0·11; figure 2C; appendix). There was no statistical evidence of publication bias (Egger's

A ***DPYD*** **c.1679T>G**

	Total	Mutant, toxicity	Mutant, no toxicity	Wild-type, toxicity	Wild-type, no toxicity	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Morel et al, 2006[10]	487	1	0	43	443	11·30 (8·50–15·03)	11·30 (8·50–15·03)	
Loganayagam et al, 2013[16]	430	1	0	103	326	4·17 (3·52–4·93)	4·78 (3·40–6·73)	
Lee et al, 2014[12]	2594	2	2	857	1733	1·51 (0·57–4·03)	1·51 (0·57–4·03)	
Froehlich et al, 2015[14]	500	1	1	71	427	3·51 (0·86–14·26)	3·10 (0·42–22·9)	
Meulendijks, 2015 (unpublished)	1605	1	2	165	1437	3·24 (0·65–16·14)	2·81 (0·51–15·6)	
Overall	**5616**					**4·30 (2·10–8·80)**	**4·40 (2·08–9·30)**	**<0·0001**

B ***DPYD*** **c.1236G>A/HapB3**

	Total	Mutant, toxicity	Mutant, no toxicity	Wild-type, toxicity	Wild-type, no toxicity	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Deenen, 2011[11]	568	21	7	247	293	1·64 (1·30–2·07)	1·66 (1·30–2·11)	
Jennings, 2013[15]	253	3	7	41	202	1·78 (0·66–4·77)	2·31 (0·85–6·28)	
Loganayagam et al, 2013[16]	425	6	9	96	314	1·71 (0·90–3·25)	1·60 (0·89–2·89)	
Rosmarin, 2014[13]	909	18	25	284	582	1·28 (0·89–1·84)	1·23 (0·86–1·76)	
Froehlich et al, 2015[14]	500	11	12	61	416	3·74 (2·30–6·09)	2·24 (1·46–3·45)	
Meulendijks, 2015 (unpublished)	1606	6	52	160	1388	1·00 (0·46–2·17)	0·99 (0·45–2·14)	
Overall	**4261**					**1·72 (1·22–2·42)**	**1·59 (1·29–1·97)**	**<0·0001**

C ***DPYD*** **c.1601G>A**

	Total	Mutant, toxicity	Mutant, no toxicity	Wild-type, toxicity	Wild-type, no toxicity	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Deenen, 2011[11]	481	14	10	224	233	1·19 (0·84–1·69)	1·13 (0·79–1·60)	
Loganayagam et al, 2013[16]	430	16	0	88	326	4·70 (3·91–5·66)	3·33 (2·68–4·14)	
Rosmarin, 2014[13]	888	18	23	285	562	1·30 (0·91–1·87)	1·29 (0·90–1·83)	
Froehlich et al, 2015[14]	500	4	14	68	414	1·58 (0·65–3·84)	1·48 (0·67–3·27)	
Meulendijks, 2015 (unpublished)	1601	10	74	156	1361	1·16 (0·64–2·11)	1·05 (0·58–1·89)	
Overall	**3900**					**1·69 (0·78–3·65)**	**1·52 (0·86–2·70)**	**0·15**

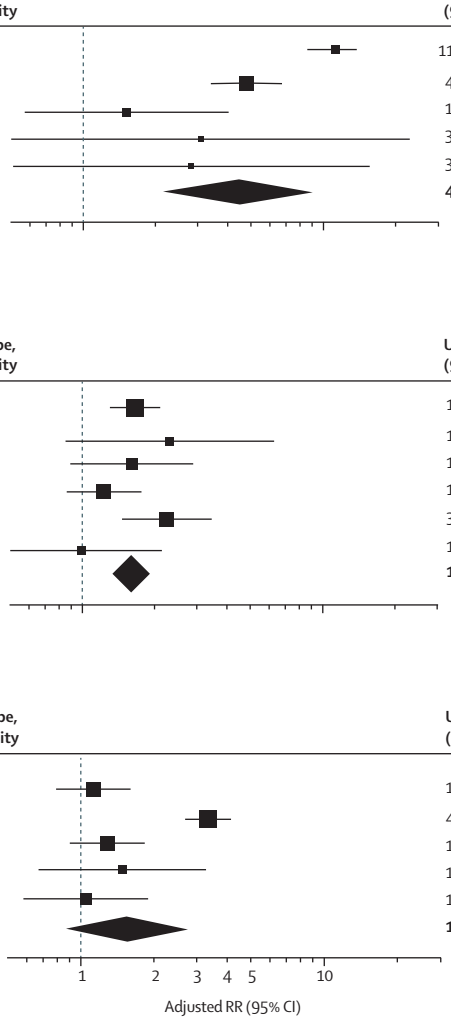

Figure 2: **Meta-analyses of studies investigating associations between *DPYD* variants and severe fluoropyrimidine-associated toxicity**
RR=relative risk.

regression test, p=0·35) but Loganayagam and colleagues' study seemed to be an outlier in the funnel plot (appendix). A mixed-effect meta-analysis incorporating between-study heterogeneity showed no significant effect of c.1601G>A (RR 1·13, 95% CI 0·79–1·60, p=0·50). Two (12·5%) of 16 patients with c.1601G>A in Loganayagam and colleagues' study[16] also had *DPYD**2A or c.2846A>T. Addition of the *DPYD**2A or c.2846A>T genotype to the regression model for Loganayagam and colleagues' study[16] slightly reduced the effect estimate for c.1601G>A, but it remained significant (RR 2·89, 95% CI 2·26–3·71, p<0·0001; appendix). The effect of c.1601G>A on risk of toxicity seemed similar for studies with a long timeframe versus a short timeframe (log RR –0·44, –1·36 to 0·47; p=0·34; appendix).

In the pooled dataset, a statistical interaction term between the study in which patients were treated and the effect of c.1601G>A was highly significant for Loganayagam and colleagues' study (p<0·0001), and on exclusion of the data from this study the association

A *DPYD* c.1679T>G

	Number	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Age (years)				
<60	1103	1·92 (0·35–10·56)	2·38 (0·33–17·5)	0·39
60–70	892	6·65 (5·69–7·77)	5·45 (0·73–40·7)	0·099
>70	540	6·06 (5·01–7·32)	7·34 (0·88–61·2)	0·066
Sex				
Female	1268	1·57 (0·29–8·62)	2·04 (0·28–14·9)	0·48
Male	1267	7·67 (6·65–8·84)	7·98 (1·87–34·1)	0·0051
Treatments				
Fluorouracil in combination regimens	603	3·77 (1·66–8·56)	4·83 (1·17–19·9)	0·029
Fluorouracil in all regimens	753	2·95 (1·09–7·96)	4·06 (0·99–16·6)	0·051
Capecitabine in combination regimens	1244	6·47 (5·68–7·37)	7·49 (1·03–54·6)	0·047
Capecitabine in all regimens	1782	3·72 (0·93–14·96)	5·50 (0·76–39·7)	0·091
Overall	**2535**	**3·45 (1·54–7·73)**	**4·48 (1·42–14·1)**	**0·010**

B *DPYD* c.1236G>A/HapB3

	Number	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Age (years)				
<60	1653	1·87 (1·33–2·64)	1·71 (1·11–2·65)	0·015
60–70	1572	1·63 (1·23–2·16)	1·53 (1·06–2·22)	0·023
>70	1036	1·37 (0·86–2·19)	1·23 (0·69–2·17)	0·48
Sex				
Female	1987	1·45 (1·06–1·99)	1·33 (0·90–1·96)	0·16
Male	2274	1·86 (1·43–2·40)	1·7 (1·23–2·37)	0·0015
Treatments				
Fluorouracil monotherapy	211	3·98 (1·40–11·31)	5·34 (1·11–25·7)	0·037
Fluorouracil in combination regimens	630	2·46 (1·58–3·83)	1·96 (1·17–3·26)	0·010
FOLFOX	305	1·25 (0·34–4·59)	1·43 (0·34–5·99)	0·62
Other fluorouracil combinations	325	2·81 (1·84–4·30)	2·63 (1·37–5·03)	0·0035
All fluorouracil-based regimens	841	2·70 (1·79–4·07)	2·08 (1·28–3·36)	0·030
Capecitabine in monotherapy	1503	1·49 (1·05–2·12)	1·31 (0·85–2·02)	0·022
Capecitabine in combination regimens	1917	1·49 (1·10–2·01)	1·46 (1·00–2·12)	0·051
CAPOX	1129	1·65 (1·23–2·22)	1·59 (1·05–2·42)	0·029
Capecitabine + radiotherapy	438	0·60 (0·09–4·06)	0·60 (0·08–4·35)	0·61
Other capecitabine combinations	350	1·06 (0·44–2·56)	1·24 (0·45–3·42)	0·68
All capecitabine-based regimens	3420	1·46 (1·16–1·84)	1·40 (1·06–1·86)	0·020
Overall	**4261**	**1·66 (1·36–2·03)**	**1·52 (1·18–1·95)**	**0·0010**

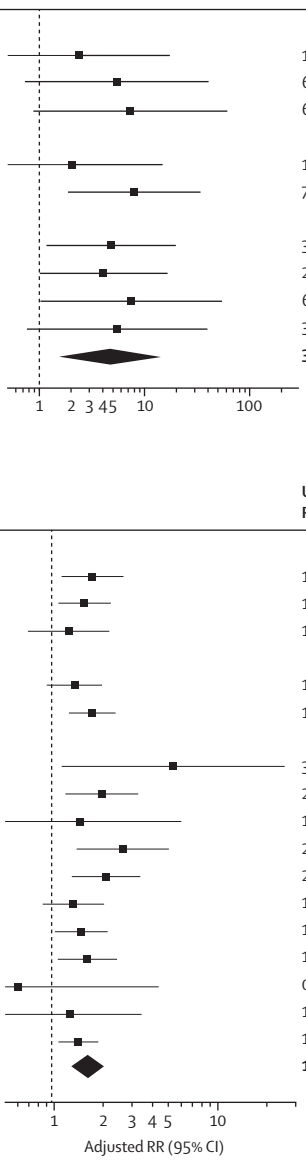

***Figure 3*: Effect of *DPYD* c.1679T>G (A) and c.1236G>A/HapB3 (B) in subgroups of patients**
One patient with c.1679T>G was treated with fluorouracil monotherapy and did not have severe toxicity (not shown in the figure because a RR could not be calculated). Similarly, one patient with c.1679T>G was treated with capecitabine monotherapy and did not have severe toxicity. RR=relative risk.

did not remain significant (p=0·13). Analysis of individual types of toxicity showed a strong association between c.1601G>A and severe gastrointestinal toxicity (RR 2·00, 95% CI 1·45–2·77, p<0·0001) and haematological toxicity (1·94, 1·16–3·27; p=0·12), but not hand-foot syndrome (0·86, 0·50–1·47; p=0·59). However, also in this analysis, there was a strong effect of Loganayagam and colleagues' study[16] and, on exclusion, none of the associations remained significant (RR 1·44, 95% CI 0·96–2·17, p=0·078 for gastrointestinal toxicity; 1·40, 0·86–2·17, p=0·31 for haematological toxicity; and 0·83, 0·48–1·45, p=0·50 for hand-foot syndrome).

We investigated the effects of patients' characteristics and treatment regimens on risk of severe fluoropyrimidine-associated toxicity in patients carrying c.1679T>G or c.1236G>A/HapB3 within the pooled dataset. No significant interaction was noted between c.1679T>G and age or c.1236G>A/HapB3 and age (p=0·38 and p=0·33, respectively) or between sex and c.1679T>G or sex and c.1236G>A/HapB3 (p=0·35 and p=0·33, respectively). Similarly, no significant interactions between the *DPYD* variants and treatment regimens were noted (data not shown). In a further subgroup analysis by patients' characteristics and treatment regimens, using the pooled dataset that included all data received from the investigators, the effect of *DPYD* variants c.1679T>G and c.1236G>A/HapB3 on risk of severe toxicity seemed to be fairly homogeneous (figure 3). Carrier frequencies of *DPYD**2A and c.2846A>T were low among patients with c.1679T>G or c.1236G>A/HapB3 (0% and 0·6%, respectively), and somewhat higher in patients with c.1601G>A (2·7%; appendix). Results of the meta-analysis after exclusion of patients with *DPYD**2A or c.2846A>T showed similar summary estimates for the investigated variants, indicating that the overall effect of *DPYD**2A and c.2846A>T on the outcome of the analysis was small (appendix). *DPYD**2A and c.2846A>T were both significantly associated with severe fluoropyrimidine-associated toxicity in the meta-analysis (RR 2·85, 95% CI 1·75–4·62, p<0·0001; and 3·02, 2·22–4·10, p<0·0001, respectively; figure 4). For *DPYD**2A, the evidence for heterogeneity between the studies was strong: I^2 was 73%, and a Q test was significant (Q 21·8, p=0·0013). The evidence for heterogeneity between studies for c.2846A>T was also strong: I^2 was 80%, and a Q test was significant (Q 34·2, p<0·0001). The findings did not indicate publication bias for *DPYD**2A and c.2846A>T (Egger's regression test, p=0·49 and p=0·51, respectively).

The sensitivity of c.1679T>G in prediction of fluoropyrimidine-associated toxicity was estimated by meta-analysis as 0·3% (95% CI 0·0–0·6), whereas the

A *DPYD**2A

	Total	Mutant, toxicity	Mutant, no toxicity	Wild-type, toxicity	Wild-type, no toxicity	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Morel et al, 2006[10]	487	6	4	38	439	7·53 (4·17–13·60)	7·53 (4·17–13·6)	
Deenen et al, 2011[11]	568	5	2	265	296	1·51 (0·94–2·44)	1·47 (0·92–2·34)	
Jennings et al, 2013[15]	253	2	1	42	208	3·97 (1·70–9·25)	1·98 (0·75–5·24)	
Loganayagam et al, 2013[16]	430	4	0	127	299	3·35 (2·90–3·88)	3·18 (1·16–8·69)	
Lee et al, 2014[12]	2594	22	3	837	1732	2·70 (2·31–3·15)	2·70 (2·31–3·15)	
Rosmarin et al, 2014[13]	905	4	3	289	609	1·78 (0·93–3·40)	1·63 (0·85–3·11)	
Froehlich et al, 2015[14]	500	0	4	72	424	0·69 (0·05–9·59)	0·69 (0·05–9·59)	
Overall	**5737**					**2·87 (2·14–3·86)**	**2·85 (1·75–4·62)**	**<0·0001**

B *DPYD* c.2846A>T

	Total	Mutant, toxicity	Mutant, no toxicity	Wild-type, toxicity	Wild-type, no toxicity	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p value
Morel et al, 2006[10]	487	6	4	38	439	7·53 (4·17–13·60)	7·53 (4·17–13·6)	
Deenen et al, 2011[11]	568	5	3	265	295	1·32 (0·77–2·28)	1·34 (0·80–2·24)	
Jennings et al, 2013[15]	253	2	0	42	209	5·98 (4·53–7·88)	4·81 (3·04–7·62)	
Loganayagam et al, 2013[16]	430	5	0	126	299	3·37 (2·91–3·91)	3·89 (3·08–4·91)	
Lee et al, 2014[12]	2594	22	5	837	1730	2·50 (2·07–3·02)	2·50 (2·07–3·02)	
Rosmarin et al, 2014[13]	881	9	2	279	591	2·55 (1·90–3·43)	2·53 (1·95–3·29)	
Froehlich et al, 2015[14]	500	0	3	72	425	0·86 (0·06–11·58)	0·86 (0·06–11·6)	
Meulendijks et al, 2015 (unpublished)	1605	4	15	162	1424	2·06 (0·85–4·98)	2·16 (1·00–4·68)	
Overall	**7318**					**3·11 (2·25–4·28)**	**3·02 (2·22–4·10)**	**<0·0001**

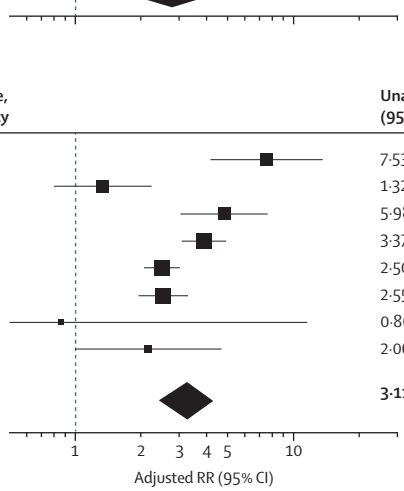

Figure 4: **Meta-analysis of variants *DPYD**2A (A) and c.2846A>T (B)**
RR=relative risk.

sensitivity of c.1236G>A/HapB3 was 6·4% (4·2–8·6). The positive predictive value of c.1679T>G for severe toxicity was 46% (95% CI 5–87), and the positive predictive value of c.1236G>A/HapB3 was 41% (18–64).

In the secondary analysis of the four case-control studies (799 patients; appendix), the summary effect estimates were similar to those from the primary analysis, but associations between the *DPYD* variants and global severe toxicity were not significant (appendix).

Discussion

The results of this analysis show that in addition to patients who are carriers of *DPYD**2A or c.2846A>T, patients who have the *DPYD* c.1679T>G or c.1236G>A/HapB3 variant alleles are at significantly increased risk of severe fluoropyrimidine-associated toxicity, confirming the clinical validity of these *DPYD* variants.

Substantial evidence exists of the clinical validity of *DPYD**2A and c.2846A>T, and current guidelines recommend a dose reduction of fluoropyrimidines in patients with these variants.[21,28] For c.1679T>G, until now only eight patients with this mutation had been described in a clinical setting (now 11 including this analysis).[10,12,14,16] The results of this meta-analysis show that the risk of global severe toxicity was increased about four times in patients with c.1679T>G. Risk of haematological and gastrointestinal toxicities were increased 9·8 and 5·7 times, respectively. Based on the available functional data for c.1679T>G, a heterozygous genotype is expected to result in a 40–50% decrease in DPD activity, similar to the effect of *DPYD**2A.[27,40,41] In view of DPD accounting for 80–90% of fluorouracil metabolism,[42] the 40–50% decrease in DPD activity is expected to result in a 50–100% increase in tissue exposure to fluorouracil. Indeed, systemic fluorouracil exposure was shown to be 50% higher in *DPYD**2A carriers.[41] Based on the available functional data, and the clinical data presented here, we recommend a dose reduction of 50% in patients with c.1679T>G, in line with the recommendation by the Clinical Pharmacogenetics Implementation Consortium.[43]

Clinical validity of c.1236G>A/HapB3 has remained uncertain until now.[9,13–16,29–31] We found that c.1236G>A/HapB3, for which about 4% of the white patients are heterozygous, was significantly associated with risk of severe toxicity. The magnitude of the effect was smaller than that for c.1679T>G, which is what was expected based on the functional consequences of this variant.[30] Because c.1236G>A/HapB3 has a fairly high frequency, it provides fairly high sensitivity to identify patients at risk of severe toxicity. c.1236G>A is in complete linkage with the deleterious polymorphism c.1129-5923C>G in intron 10 (rs75017182), and both variants occur within haplotype B3.[14,30] c.1129-5923C>G results in aberrant pre-mRNA splicing—ie, a 44-bp fragment is inadvertently inserted into mature mRNA, resulting in a premature stop codon.[30] Van Kuilenburg and colleagues[44] showed that although c.1129-5923C>G resulted in the formation of corrupt mRNA in a patient homozygous for c.1236G>A/HapB3, wild-type mRNA could still be detected in this patient. The production of normal mRNA was not completely abolished by c.1129-5923C>G in a homozygous patient, indicating that splicing efficiency to produce wild-type mRNA is reduced but not completely abolished. In agreement with this finding, we previously noted that DPD activity in two patients with c.1236G>A/HapB3 in homozygous form was reduced by about 50%, and not completely impaired (Meulendijks D, unpublished data). A homozygous genotype of *DPYD**2A, by contrast, results in complete DPD deficiency (about 0% activity).[20] These data show that c.1236G>A/HapB3 results in about half the reduction in DPD activity compared with *DPYD**2A (or c.1679T>G). This finding, combined with the presented data for the association between c.1236G>A/HapB3 and fluoropyrimidine-associated toxicity, lends support to an upfront dose reduction of 25% in patients with this variant in heterozygous form, which we expect normalises fluorouracil exposure and risk of fluoropyrimidine-associated toxicity.[43] Few data exist about the safety of fluoropyrimidine treatment in patients homozygous for c.1236G>A/HapB3 and great caution should be used when administering fluoropyrimidines to these patients. We expect that a 50% reduced dose will usually be tolerated because we previously treated three patients homozygous for c.1236G>A/HapB3 safely with low doses of capecitabine (825 mg/m² twice a day, Meulendijks D, unpublished data). Importantly, after *DPYD* genotype-guided dose reduction, subsequent dose-titration upward (starting in cycle two or three) is strongly recommended if deemed safe based on tolerability or therapeutic drug monitoring, to avoid underdosing of patients who might be able to tolerate higher doses.

In the secondary analysis of case-control studies, the effect estimates for c.1236G>A/HapB3 and c.1601G>A were similar to those in the primary analysis. For c.1601G>A, both the primary and the secondary analyses showed no significant association with severe toxicity. Unlike the results of the primary analysis, the association between c.1236G>A/HapB3 and severe toxicity was not significant in the analysis of case-control studies. This non-significance is most likely explained by a much smaller number of patients being included in the secondary analysis (799 *vs* 4261 patients in the primary analysis).

Although the risks of severe gastrointestinal and haematological toxicity were increased in c.1679T>G and c.1236G>A/HapB3 carriers, the risk of hand-foot syndrome was not. This finding could indicate that there is a weaker association between *DPYD* variants and occurrence of hand-foot syndrome, but could also be the result of severe hand-foot syndrome generally

occurring at later cycles of fluoropyrimidine treatment than do severe gastrointestinal and haematological toxicities (cycle three *vs* cycle one or two, respectively; Meulendijks D, unpublished data), and the timeframe in which toxicity was monitored was short for some of the studies. Additionally, treatment modifications for gastrointestinal or haematological toxicity might affect the risk of severe hand-foot syndrome in later cycles.

For c.1601G>A, little evidence exists for an association with toxicity, and strong evidence exists for between-study heterogeneity. The results of most larger studies of patients with c.1601G>A have shown small, non-significant, increases in risk of fluoropyrimidine-associated toxicity. Although c.1601G>A has been detected in patients with DPD deficiency,[26] a functional analysis with an established in-vitro cellular system showed that c.1601G>A was associated with an increase in DPD activity instead of a decrease.[27] The investigators therefore proposed that c.1601G>A could have a protective effect on fluoropyrimidine-associated toxicity. Our results do not suggest, however, a protective effect. The RR (1·52, 95% CI 0·86–2·70) indicates that a protective effect with a RR of less than 0·86 is unlikely. The stronger effect for c.1601G>A in the study by Loganayagam and colleagues[16] could partly—but not completely—be explained by the presence of other *DPYD* variants. Other possible confounding factors related to risk of toxicity, including patient and treatment-related factors, or the concomitant presence of other genetic polymorphisms associated with toxicity, or which interact with *DPYD*, contributed to the large effect size in this study.[6,16] Of interest in this respect are polymorphisms in *MIR27A*, the gene encoding miR-27a, which has been shown to regulate DPD activity in human beings.[7] Amstutz and colleagues[6,7] showed that rs895819, a polymorphism known to increase miR-27a expression and reduce DPD activity, strongly increased patients' risk of fluoropyrimidine-associated toxicity when present in combination with *DPYD* variants. The results of their study showed that in patients who had both a *DPYD* variant and rs895819, incidence of severe fluoropyrimidine-associated toxicity was strongly increased (12 [71%] of 17 patients), whereas in patients who were carriers of a *DPYD* variant but not rs895819, incidence of severe fluoropyrimidine-associated toxicity was average (five [25%] of 20 patients). These findings, which suggest that genotyping of *MIR27A* in conjunction with *DPYD* variants can lead to a substantially higher positive predictive value for identifying patients at risk of severe toxicity, were confirmed in a second cohort of 1592 patients (Meulendijks D, unpublished data). We believe it is therefore likely that the diagnostic accuracy of *DPYD* genotyping could be further improved by combining *DPYD* genotyping with *MIR27A* genotyping. Although definitive evidence of clinical validity is needed before clinical implementation, *MIR27A* genotyping should be included in future studies of the clinical validity and clinical utility of *DPYD* genotype-guided dosing of fluoropyrimidines.

A strength of the current analysis is that we were able to retrieve most of the available individual patient data and analyse the data in a multivariable analysis, thereby adjusting for other relevant factors associated with toxicity. The risk estimates obtained from the analysis with a random-effects model indicate the mean risk ratios that are likely to occur in other patient populations treated with fluoropyrimidines, and the results of this analysis therefore can most likely be extrapolated to other clinical settings. However, the frequency of variants c.1679T>G and c.1236G>A/HapB3 might differ depending on ethnic origin. For instance, c.1236G>A/HapB3 was absent in Japanese and Korean populations, indicating that clinical utility might be lower in non-white populations.[18] Reliable frequency data for c.1679T>G in non-white populations are not available. Further research needs to be done in patient populations of other ethnic origins to establish the clinical value of *DPYD* genotypes as predictors of fluoropyrimidine-associated toxicity in these populations.

The dosing recommendations proposed for c.1679T>G and c.1236G>A/HapB3 are based on a small amount of functional data, in addition to the clinical data reported here. To establish more definitively the optimum starting doses, a comprehensive pharmacokinetic–pharmacodynamic modelling approach in a sufficiently large number of patients is needed.

We investigated the effect of timeframe in which toxicity was assessed on the primary endpoint (appendix). This analysis showed that with both long and short timeframes, an effect of c.1679T>G and c.1236G>A/HapB3 on risk of severe toxicity was notable. Effect estimates for all three *DPYD* variants were non-significantly lower for long timeframes than with short timeframes, most likely as a result of the ability to detect an increased risk of toxicity in variant allele carriers decreases with an increasing proportion of patients in the control group having at least one severe adverse event (this rate will increase with longer treatment). The relative risk will gradually trend towards 1 (no difference) as a result. This effect can, therefore, only result in an underestimation of the effect of the *DPYD* risk variants. The results of the analysis show, however, that the impact of this effect on the overall conclusions was small.

Although our data show that *DPYD* variants can be used to identify patients with DPD deficiency at risk of fluoropyrimidine-associated toxicity, a negative test for specific *DPYD* variants does not guarantee that a patient is DPD proficient. That is, DPD deficiency cannot always be traced back to a (currently known) genetic alteration in *DPYD* associated with reduced enzyme activity. An upfront screening strategy with *DPYD* genotyping alone therefore has little sensitivity to identify patients at risk. An estimated half of patients with DPD deficiency can be identified by screening for the four *DPYD* variants for which clinical validity has

now been established, although a reliable estimate is not available.[17,18] A combined *DPYD* genotyping and DPD phenotyping approach is likely to substantially improve sensitivity of the upfront test.[45] Definitive evidence on clinical validity of phenotyping tests is not yet available, however. The value of DPD phenotyping is being investigated in two ongoing prospective clinical studies (NCT01547923 and NCT02324452). Additional screening approaches might be useful, including *MIR27A* genotyping, as described, or possibly screening of mutations in *TYMS*.[13,46]

One of the common concerns in meta-analysis is the issue of publication bias.[47] However, we assessed this in our study, and there was little indication for an effect of publication bias on the conclusions drawn from this analysis.

In conclusion, our analysis confirms the clinical validity of *DPYD* variants c.1679T>G and c.1236G>A/HapB3, in addition to *DPYD**2A and c.2846A>T, as predictors of fluoropyrimidine-associated toxicity. The magnitude of effect of c.1679T>G is in the same range as that of *DPYD**2A, and a dose reduction of 50% is advised for individuals with variant alleles.[28] The effect of c.1236G>A/HapB3 on risk of toxicity is smaller than for *DPYD**2A or c.1679T>G, in accordance with the functional effect of this variant.[30] A dose reduction of 25% is rational in heterozygous carriers of c.1236G>A/HapB3, occurring in about 4% of white patients, and we recommend adding c.1236G>A/HapB3 to the guideline on dosing recommendations for *DPYD* variants.[28] Clinical validity has now been established for four *DPYD* variants—*DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A/HapB3—and upfront screening for these mutations with dose adaptation in variant allele carriers is advised to improve safety of patients treated with fluoropyrimidines. As upfront screening for one *DPYD* variant has been shown to be feasible and cost saving in routine clinical practice, with improved safety, it is likely that upfront screening for an extended panel of *DPYD* variants will further improve the safety of the large group of patients treated with fluoropyrimidines.

Contributors

DM, LMH, and JHMS were responsible for the initial concept of this analysis. MJD, TKF, UA, CRL, BAJ, AMM, JDS, ZK, PK, MS, UMZ, CP, IT, EG, ABPvK, CJAP, MK, JHB, AC, and JHMS were responsible for acquisition or reporting of individual patient data, or both. LMH did the review of the individual patient data sent by the participating centres. DM and LMH did all statistical analyses under supervision by GSS. DM and LMH led the interpretation of the data and writing of the report. All authors had input into the data interpretation and preparation of the report for publication and approved the final version of the report.

Declaration of interests

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References

1 Hoff PM, Ansari R, Batist G, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* 2001; **19:** 2282–92.

2 Tsalic M, Bar-Sela G, Beny A, Visel B, Haim N. Severe toxicity related to the 5-fluorouracil/leucovorin combination (the Mayo Clinic regimen): a prospective study in colorectal cancer patients. *Am J Clin Oncol* 2003; **26:** 103–06.

3 Bajetta E, Procopio G, Celio L, et al. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. *J Clin Oncol* 2005; **23:** 2155–61.

4 Lévy E, Piedbois P, Buyse M, et al. Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. *J Clin Oncol* 1998; **16:** 3537–41.

5 Van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* 2004; **40:** 939–50.

6 Amstutz U, Offer SM, Sistonen J, Joerger M, Diasio RB, Largiadèr CR. Polymorphisms in MIR27A associated with early-onset toxicity in fluoropyrimidine-based chemotherapy. *Clin Cancer Res* 2015; **21:** 2038–44.

7 Offer SM, Butterfield GL, Jerde CR, Fossum CC, Wegner NJ, Diasio RB. microRNAs miR-27a and miR-27b directly regulate liver dihydropyrimidine dehydrogenase expression through two conserved binding sites. *Mol Cancer Ther* 2014; **13:** 742–51.

8 Zhang X, Li L, Fourie J, Davie JR, Guarcello V, Diasio RB. The role of Sp1 and Sp3 in the constitutive *DPYD* gene expression. *Biochim Biophys Acta* 2006; **1759:** 247–56.

9 Schwab M, Zanger UM, Marx C, et al. Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. *J Clin Oncol* 2008; **26:** 2131–38.

10 Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther* 2006; **5:** 2895–904.

11 Deenen MJ, Tol J, Burylo AM, et al. Relationship between single nucleotide polymorphisms and haplotypes in *DPYD* and toxicity and efficacy of capecitabine in advanced colorectal cancer. *Clin Cancer Res* 2011; **17:** 3455–68.

12 Lee AM, Shi Q, Pavey E, et al. *DPYD* variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). *J Natl Cancer Inst* 2014; **106:** 1–12.

13 Rosmarin D, Palles C, Church D, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. *J Clin Oncol* 2014; **32:** 1031–39.

14 Froehlich TK, Amstutz U, Aebi S, Joerger M, Largiadèr CR. Clinical importance of risk variants in the dihydropyrimidine dehydrogenase gene for the prediction of early-onset fluoropyrimidine toxicity. *Int J Cancer* 2015; **136:** 730–39.

15 Jennings BA, Loke YK, Skinner J, et al. Evaluating predictive pharmacogenetic signatures of adverse events in colorectal cancer patients treated with fluoropyrimidines. *PLoS One* 2013; **8:** e78053.

16 Loganayagam A, Arenas Hernandez M, Corrigan A, et al. Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. *Br J Cancer* 2013; **108:** 2505–15.

17 Van Kuilenburg ABP, Haasjes J, Richel DJ, et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the *DPD* gene. *Clin Cancer Res 2000*; **6:** 4705–12.

18 Amstutz U, Froehlich TK, Largiadèr CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. *Pharmacogenomics* 2011; **12:** 1321–36.

19 Van Kuilenburg ABP, Dobritzsch D, Meinsma R, et al. Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 2002; **364:** 157–63.

20 Vreken P, Van Kuilenburg AB, Meinsma R, et al. A point mutation in an invariant splice donor site leads to exon skipping in two unrelated Dutch patients with dihydropyrimidine dehydrogenase deficiency. *J Inherit Metab Dis* 1996; **19:** 645–54.

21 Terrazzino S, Cargnin S, Del Re M, Danesi R, Canonico PL, Genazzani AA. *DPYD* IVS14+1G>A and 2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. *Pharmacogenomics* 2013; **14:** 1255–72.

22 Van Kuilenburg ABP, Meinsma R, Zoetekouw L, Van Gennip AH. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. *Pharmacogenetics* 2002; **12:** 555–58.

23 Deenen MJ, Meulendijks D, Cats A, et al. Upfront genotyping of *DPYD**2A to individualize fluoropyrimidine therapy: a safety and cost analysis. *J Clin Oncol* (in press).

24 Johnson MR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res* 2002; **8:** 768–74.

25 Loganayagam A, Arenas-Hernandez M, Fairbanks L, Ross P, Sanderson JD, Marinaki AM. The contribution of deleterious *DPYD* gene sequence variants to fluoropyrimidine toxicity in British cancer patients. *Cancer Chemother Pharmacol* 2010; **65:** 403–06.

26 Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant *DPYD* alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* 2000; **10:** 217–23.

27 Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic profiling of *DPYD* variations relevant to 5-fluorouracil sensitivity using real-time cellular analysis and in vitro measurement of enzyme activity. *Cancer Res* 2013; **73:** 1958–68.

28 Caudle KE, Thorn CF, Klein TE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. *Clin Pharmacol Ther* 2013; **94:** 640–45.

29 Amstutz U, Farese S, Aebi S, Largiadèr CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* 2009; **10:** 931–44.

30 Van Kuilenburg ABP, Meijer J, Mul ANPM, et al. Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Hum Genet* 2010; **128:** 529–38.

31 Kleibl Z, Fidlerova J, Kleiblova P, et al. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. *Neoplasma* 2009; **56:** 303–16.

32 Pluim D, Jacobs BAW, Deenen MJ, et al. Improved pharmacodynamic assay for dihydropyrimidine dehydrogenase activity in peripheral blood mononuclear cells. *Bioanalysis* 2015; **7:** 519–29.

33 Gross E, Busse B, Riemenschneider M, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* 2008; **3:** e4003.

34 Little J, Higgins JPT, Ioannidis JPA, et al. STrengthening the REporting of Genetic Association studies (STREGA): an extension of the STROBE Statement. *Ann Intern Med* 2009; **150:** 206–15.

35 Little J, Higgins JP. The HuGENetTM HuGE Review Handbook, Version 1.0. Ottawa, Ontario, Canada: HuGENet Canada Coordinating Centre (2006). http://www.medicine.uottawa.ca/public-health-genomics/web/assets/documents/HuGE_Review_Handbook_V1_0.pdf (accessed March 11, 2015).

36 Cox DR. The continuity correction. *Biometrika* 1970; **57:** 217–19.

37 DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7:** 177–88.

38 Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997; **127:** 820–26.

39 Stewart LA, Clarke M, Rovers M, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Individual Participant Data. *JAMA* 2015; **313:** 1657.

40 Offer SM, Fossum CC, Wegner NJ, et al. Comparative functional analysis of *DPYD* variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Res* 2014; **74:** 2545–54.

41 Van Kuilenburg ABP, Häusler P, Schalhorn A, et al. Evaluation of 5-fluorouracil pharmacokinetics in cancer patients with a c.1905+1G>A mutation in *DPYD* by means of a Bayesian limited sampling strategy. *Clin Pharmacokinet* 2012; **51:** 163–74.

42 Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet* 1989; **16:** 215–37.

43 Henricks LM, Lunenburg CA, Meulendijks D, et al. Translating *DPYD* genotype into DPD phenotype: using the *DPYD* gene activity score. *Pharmacogenomics* 2015; **16:** 1–10.

44 Van Kuilenburg ABP, Meijer J, Mul ANPM, et al. Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihydropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Hum Genet* 2010; **128:** 529–38.

45 Boisdron-Celle M, Remaud G, Traore S, et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Lett* 2007; **249:** 271–82.

46 Meulendijks D, Jacobs BAW, Aliev A, et al. Increased risk of severe fluoropyrimidine-associated toxicity in patients carrying a G>C Substitution in the First 28-bp tandem repeat of the thymidylate synthase 2R Allele. *Int J Cancer* 2015; published online July 18. DOI:10.1002/ijc.29694.

47 Begg CB, Berlin JA. Publication bias: A problem in interpreting medical data. *J R Stat Soc Ser A* 1988; **151:** 419–63.