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Pharmacogenetic analyses of 2,183 patients with advanced colorectal cancer; Potential role for common dihydropyrimidine dehydrogenase variants in toxicity to chemotherapy.

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ABSTRACT

Background

Inherited genetic variants may influence response to, and side effects from, chemotherapy. We sought to generate a comprehensive inherited pharmacogenetic profile for oxaliplatin and 5FU/capecitabine therapy in advanced colorectal cancer (aCRC).

Methods

We analysed over 200 potentially functional, common, inherited variants in genes within the 5-FU, capecitabine, oxaliplatin, and DNA repair pathways, together with 4 rare dihydropyrimidine dehydrogenase (*DPYD*) variants, in 2183 aCRC patients treated with oxaliplatin-fluoropyrimidine chemotherapy with, or without, cetuximab (from MRC COIN and COIN-B trials). Primary endpoints were response, any toxicity and peripheral neuropathy. We had >85% power to detect ORs=1.3 for variants with minor allele frequencies >20%.

Results

Variants in DNA repair genes (Asn279Ser in *EXO1* and Arg399Gln in *XRCC1*) were most associated with response (OR 1.9, 95% CI 1.2-2.9, $P=0.004$, and OR 0.7, 95% CI 0.5-0.9, $P=0.003$, respectively). Common variants in *DPYD* (Cys29Arg and Val732Ile) were most associated with toxicity (OR 0.8, 95% CI 0.7-1.0, $P=0.008$, and OR 1.6, 95% CI 1.1-2.1, $P=0.006$, respectively). Two rare *DPYD* variants were associated with increased toxicity (Asp949Val with neutropenia, nausea and vomiting, diarrhoea and infection; IVS14+1G>A with lethargy, diarrhoea, stomatitis, Hand-Foot Syndrome and infection; all ORs>3). Asp317His in *DCLRE1A* was most

associated with peripheral neuropathy (OR 1.3, 95% CI 1.1-1.6, $P=0.003$). No common variant associations remained significant after Bonferroni correction.

Conclusions

DNA repair genes may play a significant role in the pharmacogenetics of aCRC. Our data suggest that both common and rare *DPYD* variants may be associated with toxicity to fluoropyrimidine-based chemotherapy.

INTRODUCTION

Genetic factors affect response to, and side effects from, chemotherapy and biological therapies used in the treatment of advanced colorectal cancer (aCRC). For example, somatic mutations in *KRAS* and *NRAS* in the epidermal growth factor receptor (EGFR) signalling pathway predict a lack of response to anti-EGFR monoclonal antibodies [1,2]. Germline changes in drug metabolism, transport and target genes have also been implicated in altering response [3,4]. Although several large studies have attempted to identify inherited predictive biomarkers, including the analysis of ten variants in 1188 CRC patients [5,6], 1456 5-FU pathway tagging variants in 968 patients [7], and 34 variants in 520 patients [8], none have comprehensively analysed all of the pharmacological pathways. Indeed, the vast majority of studies performed to-date have used small cohorts of patients and most findings have not been validated in independent analyses.

We have previously sought predictive biomarkers for cetuximab response and side effects by analysing 54 common, inherited EGFR pathway variants in 815 aCRC patients from the COIN [9,10] and COIN-B [11] trials that received cetuximab together with oxaliplatin-fluoropyrimidine chemotherapy [12]. Although we identified five potential biomarkers for response and four for skin rash, none remained significant after correction for multiple testing [12]. Here, we sought predictive biomarkers for oxaliplatin-fluoropyrimidine chemotherapy by analysing over 200 potentially functional common inherited variants in 2183 COIN and COIN-B patients treated with oxaliplatin-fluoropyrimidine chemotherapy with, or without, cetuximab.

METHODS

Patients and treatments

All patients had metastatic or locally advanced colorectal adenocarcinoma and received no previous chemotherapy for advanced disease. All patients gave fully informed consent for this study (approved by REC [04/MRE06/60]). COIN patients were randomised 1:1:1 to receive continuous oxaliplatin and fluoropyrimidine chemotherapy (Arm A), continuous chemotherapy +cetuximab (Arm B), or intermittent chemotherapy (Arm C) (ISRCTN27286448) [9,10]. COIN-B patients were randomised 1:1 to receive intermittent chemotherapy and cetuximab (Arm D) or intermittent chemotherapy and continuous cetuximab (Arm E) (ISRCTN3837568) [11]. For the first 12-weeks, at which point the primary pharmacogenetic analyses were carried out, treatments were identical in all patients apart from the choice of fluoropyrimidine (n=834, 38% received infusional 5FU with oxaliplatin [OxMdG] and n=1349, 62% received capecitabine with oxaliplatin [Xelox]) together with the randomisation of \pm cetuximab (n=815, 37% received cetuximab) (Supplementary Table 1).

Selection of potential pharmacogenetic variants

Potentially functional variants were sought in 62 genes identified from literature reviews as likely to play a role in the metabolic pathways associated with the agents used in COIN and COIN-B - 5FU and capecitabine (28 genes) and oxaliplatin (34 genes). Variants were considered potentially functional if there was previously reported clinical or biological evidence for an effect on response or side effects, if they were nonsynonymous, or if they occurred in the promoter region. We also sought similar variants in 155 DNA repair genes that were likely to play a role in repairing the damage caused by these agents. Variants were mined from dbSNP

(v.129, <http://www.ncbi.nlm.nih.gov/SNP/>) and from exome re-sequencing germline data [13], and those with a minor allele frequency (MAF) >5% (Caucasian population) were considered for genotyping.

Genotyping

Most variants were single nucleotide polymorphisms (SNPs) genotyped using a custom Illumina GoldenGate assay. The 'Assay Design Tool' software (Illumina) was used to anticipate genotyping success. This was based on the designability rank and validation class for a given SNP. When two or more SNPs occurred within 60bp of one another, the SNP selected for submission was chosen based on its designability score, MAF and likelihood of being functional using *in silico* analyses (PolyPhen, <http://genetics.bwh.harvard.edu/pph2/> or align-GVGD, <http://agvgd.iarc.fr/>).

Eight variants were assayed 'in house' because they were not suitable for (n=7), or failed (n=1), GoldenGate genotyping. The c.1-99 28bp repeat in the *TYMS* promoter (rs34743033) and the c.939+450 6bp deletion in the *TYMS* 3' untranslated region (UTR) (rs34489327) were assayed using the primers 5'-GGGTTTCCTAAGACTCTCAG-3' and 5'-CCGAGCCGGCCACAGGCATA-3', and 5'-CATCCAAACCAGAATACAGCAC-3' and 5'-CTTTGAGTTAACTCACTGAGGG-3', respectively, and the c.1-1671 insertion A in the *MMP3* promoter (rs35068180) was assayed using the primers 5'-AGCTGCCACAGCTTCTACAC-3' and 5'-GTATTCTATGGTTCTCCATTC-3'. One of the primers for each pair was fluorescently labelled and PCR products were analysed on an ABI3100 using the GeneScan Analysis Software (ABI). The *GSTT1* and *GSTM1* copy number deletions (Accession numbers CG962889 and CN973733, respectively [HGMD,

www.hgmd.cf.ac.uk]) and the variants Phe212Val in *FCGR3A* (rs396991) and His46 (synonymous) in *ERCC5* (rs1047768) were assayed using Taqman real time quantitative PCR assays (ABI). The G>C variant in the 28bp repeat within the *TYMS* promoter (rs2853542) was assayed by direct sequencing without success.

We assayed for four rare *DPYD* variants (IVS14+1G>A [rs3918290], Asp949Val [rs67376798], Lys259Glu [rs45589337] and Ser534Asn [rs1801158]) using KASPar assays (LGC).

Clinical parameters assessed

The primary efficacy endpoint was 12-week response, defined as complete response or partial response versus stable disease or progressive disease at 12-weeks, and secondary efficacy endpoints were overall survival (OS) and overall response rate (ORR). The primary endpoints for toxicity were: (i) a dose reduction or delay in chemotherapy in the first 12-weeks of treatment due to any toxicity except peripheral neuropathy (PN), and, (ii) grade ≥ 2 PN or dose reduction or delay due to PN *versus* grade < 2 PN despite no oxaliplatin dose modification in the first 12-weeks.

Secondary toxicity endpoints were grade ≥ 2 at any point versus grade < 2 for neutropenia, lethargy, nausea or vomiting, diarrhoea, stomatitis, Hand-Foot Syndrome (HFS), infection (infection with grade ≥ 3 neutropenia *versus* infection with normal absolute neutrophil count or no infection) and PN (COIN Arms A and B) at 24-weeks.

Power considerations

Patients from all arms of COIN and COIN-B had similar efficacy and toxicity outcomes at 12-weeks [9-11], so were combined to increase power (n=2183). Based on 2183 patients, we had >85% power ($P<0.05$) to detect an OR of 1.3, corresponding to a 7% difference in response or toxicity (45% responded and 35% had toxicity) [9-11], for a variant with a MAF>20%, and an OR of 1.6, corresponding to an 11% difference in response, for a variant with a MAF>5%.

Statistical analyses

Genotypes were tested for deviation from the Hardy Weinberg Equilibrium (HWE) using a chi-squared test and those with $P<2.5\times 10^{-4}$ (multiple testing for 202 common variants) were excluded. Pharmacogenetic analyses were carried out using Stata 12.1 with a co-dominant model, and tested using the likelihood-ratio chi-squared statistic. For significant associations ($P<0.05$), subsequent analyses were carried out using logistic regression under the best-fitting allele model, adjusted for cetuximab use and type of fluoropyrimidine. Correction for multiple testing was by Bonferroni. Our methods and reporting conform to REMARK criteria [14].

RESULTS

Two hundred and thirty-six potentially functional, common, coding and promoter-region variants were identified in either 39 genes likely to play a role in the metabolic pathways associated with the chemotherapeutic agents used in COIN and COIN-B, or, in 97 genes that were likely to play a role in repairing the damage caused by these agents (Fig.1). Of these, 226 passed *in silico* locus conversion on the GoldenGate platform and 195 were successfully assayed. Eight variants were assayed 'in house' of which 7 were successfully genotyped. Only genotypes for the

c.1-99 28bp repeat in *TYMS* deviated from the HWE and were excluded. Therefore, in total, 201 common variants were considered in the pharmacogenetic analyses of 2183 unrelated patients with aCRC from the UK national trials COIN (2070 of the 2445 randomised) and COIN-B (113 of the 226 randomised) in which all patients received oxaliplatin and fluoropyrimidine chemotherapy with, or without, cetuximab (Supplementary Table 2).

Primary analyses

Eight variants were associated ($P < 0.05$) with response, eight with toxicity and five with PN, prior to correction for multiple testing (Table 1, Supplementary Table 3); none were significant after Bonferroni correction.

For response, the most significant associations were with variants in DNA repair genes. Seventy-one percent of patients with at least one allele encoding serine at residue 279 in *EXO1* responded, as compared to 57% of patients homozygous for alleles encoding asparagine (OR 1.9, 95% CI 1.2-2.9, $P = 0.004$). Also, 48% of patients homozygous for alleles encoding glutamine at residue 399 in *XRCC1* responded, as compared to 59% of patients with at least one allele encoding arginine (OR 0.7, 95% CI 0.5-0.9, $P = 0.003$).

For toxicity, the most significant associations were Cys29Arg and Val732Ile in *DPYD*. These variants were in low linkage disequilibrium (LD) ($r^2 = 0.0$, $D' = 0.5$) suggesting that they may represent independent associations. Arginine at residue 29 reduced toxicity, particularly diarrhoea and stomatitis (34% of patients with at least one allele encoding arginine had severe toxicity as compared to 38% of patients

homozygous for alleles encoding cysteine, OR 0.8, 95% CI 0.7-1.0, $P=0.008$).

Although reduced toxicity was observed with both fluoropyrimidine regimens, it was only statistically significant with Xelox (OR 0.4, 95% CI 0.2-0.8, $P=0.004$)

(Supplementary Table 4).

Forty-five percent of patients with at least one allele encoding isoleucine at residue 732 had severe toxicity as compared to 36% of patients homozygous for alleles encoding valine (OR 1.6, 95% CI 1.1-2.1, $P=0.006$). Increased toxicity was observed with both fluoropyrimidine regimens, but was statistically significant with OxMdG (OR 2.0, 95% CI 1.1-3.5, $P=0.014$) (Supplementary Table 4). The association was primarily caused by neutropenia (20% versus 14% of patients, OR 1.9, 95% CI 1.2-3.1, $P=0.005$) (Table 2).

For PN, the most significant association was with Asp317His in *DCLRE1A*. Twenty-one percent of patients homozygous for alleles encoding histidine had PN, in comparison to 17% of those with a single allele encoding histidine and 13% of those homozygous for alleles encoding aspartic acid (OR 1.3, 95% CI 1.1-1.6, $P=0.003$) (Table 2).

Extended profiling of *DPYD*

Since two common *DPYD* variants influenced toxicity and previous observations that rare *DPYD* variants also affect toxicity [15], we assayed an extended panel of rare (MAFs <5%) nonsynonymous and splicing *DPYD* variants in all patients using KASPar.

Asp949Val, in 1.4% of patients (30/2116), was associated with increased toxicity (OR 2.2, 95% CI 1.1-4.5, $P=0.038$), specifically neutropenia (OR 3.2, 95% CI 1.2-8.2, $P=0.019$), nausea and vomiting (OR 3.4, 95% CI 1.5-7.3, $P=0.002$), diarrhoea (OR 4.6, 95% CI 2.1-10.1, $P<0.001$) and infection (OR 5.5, 95% CI 1.3-24.2, $P=0.024$) (Table 2). We found significantly increased infection with Xelox (OR 31.9, 95% CI 5.7-178) as compared to OxMdG (OR 1.2, 95% CI 0.1-13.0, $P_{interaction}=0.026$; Supplementary Table 4).

IVS14+1G>A, in 1.1% of patients (23/2105), was associated with increased lethargy (OR 5.3, 95% CI 1.9-14.9, $P=0.002$), diarrhoea (OR 4.4, 95% CI 1.7-11.0, $P=0.002$), stomatitis (OR 4.6, 95% CI 1.7-12.6, $P=0.003$), HFS (OR 3.8, 95% CI 1.2-11.8, $P=0.021$) and infection (OR 19.2, 95% CI 5.0-73.8, $P<0.001$) (Table 2). These were consistent across fluoropyrimidine regimens (Supplementary Table 4).

Secondary analyses

Thirteen variants were associated with ORR ($n=7$) or OS ($n=6$) (Supplementary Table 5). In addition, 11 variants were associated with lethargy, 17 with nausea/vomiting, 13 with diarrhoea, 3 with stomatitis, 11 with HFS, 8 with infection and 8 with PN at 24-weeks (Supplementary Table 6). Upon rigorous correction for multiple testing, none of these associations remained statistically significant.

DISCUSSION

Fluoropyrimidines have several mechanisms of cytotoxicity including disruption of the dioxynucleotide pools from thymidylate synthase inhibition and the direct incorporation of fluoropyrimidines into DNA [16]. Platinums cause bulky adducts to

be introduced into DNA. The consequences of these agents are the mutagenic effects of base analogues or mispairs in DNA, the inhibition of replication and the fragmentation of DNA created in the cell's attempts to repair these lesions. The base excision repair (BER), nucleotide excision repair, mismatch repair (MMR) and double strand break repair systems have all been suggested to modify response [16]. In our study, the most significant associations for response to therapy were with variants in DNA repair genes. EXO1 has exonuclease activity and plays a role in MMR and homologous recombination, and XRCC1 is involved in the repair of single-strand breaks following BER. Interestingly, others have also shown a predictive role for Arg399Gln in *XRCC1* in response to oxaliplatin/5-FU treatment for aCRC [17] and in platinum based therapy of oesophageal cancers [18]. We also found that Asp317His in *DCLRE1A* was associated with PN, an oxaliplatin-associated toxicity of chronic peripheral nerve damage causing sensory ataxia and functional impairment [19]. *DCLRE1A* is involved in the repair of interstrand cross-links [20]. Together, these data support a key role for DNA repair in the pharmacogenetics of cancer therapy.

Given that our study was an exploratory analysis, we provided uncorrected *P*-values; however, we also adjusted these for multiple testing by Bonferroni. Although no associations with common variants remained statistically significant after correction, it is noteworthy that the two common variants most significantly associated with toxicity, were both in *DPYD*. *DPYD* encodes DPD, the key enzyme for the catabolism of 5-FU, and reduced DPD activity is thought to cause severe 5-FU induced toxicities. Previous studies have clearly shown that two rare *DPYD* variants are associated with severe toxicity in patients receiving 5-FU [15,21,22] and our data support these observations. Interestingly, we noted that Asp949Val was associated

with increased infection with Xelox as compared to OxMdG. This difference warrants further investigation and may potentially relate to variants within the folinic acid metabolism pathway not studied *herein*.

As yet, there is no consensus on the role of common *DPYD* variants in contributing to toxicity to therapy, but our data provide supportive evidence for their role.

Cys29Arg (MAF=21%) has previously been associated with reduced toxicity (OR 0.5 for gastrointestinal toxicity, 95% CI 0.2-1.0 [23], and, $P=0.041$ [24]) and our data support a protective role for this variant (OR 0.8, 95% CI 0.7-1.0). Interestingly, this variant shows significantly higher enzymatic activity as compared to wild type DPD when expressed in mammalian cells [25], supporting a model in which hyperactive forms of DPD reduce mean circulating levels of 5-FU by increased drug catabolism [25].

Val732Ile (MAF=4%) has previously been associated with increased fluorouracil-related adverse events (OR 1.7, 95% CI 1.3-2.4) including hematologic adverse events (OR 1.9, 95% CI 1.4-2.6), and neutropenia (OR 1.8, 95% CI 1.3-2.4) in CRC patients who received standard adjuvant FOLFOX4 or FOLFOX4 in combination with cetuximab, and these findings were validated in aCRC patients receiving FOLFOX4 [26]. Furthermore, others have associated Val732Ile with leucopenia (OR 8.2, 95% CI 2.4-27.3) and neutropenia (OR 2.8, 95% CI 1.0-7.5) [23]. Our data also support this variant having an association with toxicity.

In addition to these common coding region variants, a recent study has shown that common tagging variants outside of the *DPYD* coding sequence also affect capecitabine toxicity [7].

CONCLUSIONS

It is now standard practice in many European cancer centres to test for a small number of rare genetic variants in *DPYD* before starting patients on 5FU or capecitabine [27,28]. Upfront genotyping, and dose adjustment, has been shown to be feasible and cost effective by reducing the financial burden of managing preventable toxicities [29]. Whilst this strategy is specific, it is far from being sensitive for predicting excessive toxicities [30]. Partial DPD deficiency which is not picked up by current genetic testing may be caused by the presence of other, more common, genetic variants. Our study provides supportive evidence for two such variants in a very large cohort of patients and adds weight to the body of published data suggesting the genetic profiling of both common and rare *DPYD* variants could now be used to guide accurate dosing of 5FU and capecitabine. This would require validation in a prospective trial and might need to be combined with tests assessing DPD function pre-therapeutically [31], or 5FU pharmacokinetics post-therapeutically [32].

APPENDICIES

Appendix A. Supplementary data

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AUTHOR CONTRIBUTIONS

JPCheadle and TSM obtained funding for this study. The study was designed by JPCheadle, AM, TSM, DF and RSK, and was carried out under the direction of JPCheadle. AM carried out the literature searches and, with JPColley, identified the variants for genotyping. TSM was CI of COIN, HW was CI of COIN-B and, RAA and AM were COIN trial fellows; all provided clinical advice and assistance, and supported the translational research. AMM and RSK managed the COIN and COIN-B trials and facilitated access to the clinical data. DF undertook all of the statistical analyses. AM and JPCheadle interpreted the data with input from DF, RAA and TSM. SI extracted the blood DNA samples and, with RH, prepared them for genotyping at Illumina. VH and JM undertook the in-house genotyping under the direction of JPColley. JPCheadle and AM wrote the paper with input from DF, and all authors provided comments.

CONFLICTS OF INTEREST

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Table 1 - Variants with $P < 0.05$ for the primary endpoints

Endpoint	rs no.	Gene	Variant	Endpoint +/-	AA	AB	BB	X^2 (df), P -value ^a	OR (95% CI), P -value ^b																																																																																																																																																								
12-week response	rs4149909	<i>EXO1</i>	Asn279Ser	+	983	75	0	8.7 (1), 0.003	1.9 (1.2-2.9), 0.004 (d)																																																																																																																																																								
				-	758	31	0			rs25487	<i>XRCC1</i>	Arg399Gln	+	119	490	450	9.6 (2), 0.008	0.7 (0.5-0.9), 0.003 (r)	-	127	357	304	rs144848	<i>BRCA2</i>	Asn372His	+	572	409	78	7.2 (2), 0.027	0.8 (0.7-1.0), 0.008 (a)	-	386	323	80	rs1047768	<i>ERCC5</i>	His46	+	368	456	196	7.2 (2), 0.027	1.4 (1.1-1.8), 0.008 (r)	-	282	362	108	rs17714854	<i>EME1</i>	Phe63Leu	+	1024	34	0	4.7 (1), 0.029	2.0 (1.0-3.8), 0.037 (d)	-	776	13	0	rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)	-	458	281	47	rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465	81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2	97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044
	rs25487	<i>XRCC1</i>	Arg399Gln	+	119	490	450	9.6 (2), 0.008	0.7 (0.5-0.9), 0.003 (r)																																																																																																																																																								
				-	127	357	304			rs144848	<i>BRCA2</i>	Asn372His	+	572	409	78	7.2 (2), 0.027	0.8 (0.7-1.0), 0.008 (a)	-	386	323	80	rs1047768	<i>ERCC5</i>	His46	+	368	456	196	7.2 (2), 0.027	1.4 (1.1-1.8), 0.008 (r)	-	282	362	108	rs17714854	<i>EME1</i>	Phe63Leu	+	1024	34	0	4.7 (1), 0.029	2.0 (1.0-3.8), 0.037 (d)	-	776	13	0	rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)	-	458	281	47	rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2	97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0							
	rs144848	<i>BRCA2</i>	Asn372His	+	572	409	78	7.2 (2), 0.027	0.8 (0.7-1.0), 0.008 (a)																																																																																																																																																								
				-	386	323	80			rs1047768	<i>ERCC5</i>	His46	+	368	456	196	7.2 (2), 0.027	1.4 (1.1-1.8), 0.008 (r)	-	282	362	108	rs17714854	<i>EME1</i>	Phe63Leu	+	1024	34	0	4.7 (1), 0.029	2.0 (1.0-3.8), 0.037 (d)	-	776	13	0	rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)	-	458	281	47	rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2		97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																			
	rs1047768	<i>ERCC5</i>	His46	+	368	456	196	7.2 (2), 0.027	1.4 (1.1-1.8), 0.008 (r)																																																																																																																																																								
				-	282	362	108			rs17714854	<i>EME1</i>	Phe63Leu	+	1024	34	0	4.7 (1), 0.029	2.0 (1.0-3.8), 0.037 (d)	-	776	13	0	rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)	-	458	281	47	rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2		97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																
	rs17714854	<i>EME1</i>	Phe63Leu	+	1024	34	0	4.7 (1), 0.029	2.0 (1.0-3.8), 0.037 (d)																																																																																																																																																								
				-	776	13	0			rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)	-	458	281	47	rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2		97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																													
rs2273535	<i>AURKA</i>	Phe31Ile	+	675	331	51	6.5 (2), 0.039	0.8 (0.7-1.0), 0.011 (d)																																																																																																																																																									
			-	458	281	47			rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)	-	24	241	524	rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465	81		rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2	97		1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																											
rs1805388	<i>LIG4</i>	Thr9Ile	+	23	274	760	6.3 (2), 0.042	0.8 (0.6-1.0), 0.014 (d)																																																																																																																																																									
			-	24	241	524			rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)	-	0	53	736	Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2	97		1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																								
rs2229109	<i>ABCB1</i>	Ser400Asn	+	0	97	961	3.9 (1), 0.049	1.4 (1.0-2.0), 0.052 (d)																																																																																																																																																									
			-	0	53	736			Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015	0.8 (0.7-1.0), 0.008 (a)	-	834	465		81	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2		97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																																					
Any Toxicity (except PN)	rs1801265	<i>DPYD</i> ^c	Cys29Arg	+	506	252	28	8.5 (2), 0.015					0.8 (0.7-1.0), 0.008 (a)																																																																																																																																																				
				-	834	465	81			rs1801160	<i>DPYD</i> ^c	Val732Ile		+	2	79	705	7.4 (2), 0.025	1.6 (1.1-2.1), 0.006 (d)	-	2		97	1282	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																																																		
	rs1801160	<i>DPYD</i> ^c	Val732Ile	+	2	79	705	7.4 (2), 0.025					1.6 (1.1-2.1), 0.006 (d)																																																																																																																																																				
				-	2	97	1282			rs4986850	<i>BRCA1</i> ^d	Asp397Asn		+	2	113	671	7.3 (2), 0.026	0.2 (0.1-1.0), 0.046 (r)	-	17	169	1195	rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																																																																
	rs4986850	<i>BRCA1</i> ^d	Asp397Asn	+	2	113	671	7.3 (2), 0.026					0.2 (0.1-1.0), 0.046 (r)																																																																																																																																																				
				-	17	169	1195		rs1979277	<i>SHMT1</i>	Leu474Phe	+		79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)	-	156	591	633	rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																																																																														
rs1979277	<i>SHMT1</i>	Leu474Phe	+	79	380	327	6.3 (2), 0.043	1.2 (1.0-1.4), 0.053 (d)																																																																																																																																																									
			-	156	591	633			rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)	-	1360	21	0																																																																																																																																												
rs5745459	<i>MSH4</i>	Tyr589Cys	+	764	22	0	4.1(1), 0.044	1.9 (1.0-3.5), 0.043 (d)																																																																																																																																																									
			-	1360	21	0																																																																																																																																																											

	rs12022378	<i>DCLRE1B</i>	His61Tyr	+	31	229	526		
				-	29	391	961	6.2 (2), 0.046	1.9 (1.1-3.2), 0.016 (r)
	rs1799966	<i>BRCA1</i> ^d	Ser430Gly	+	343	374	69		
				-	619	602	160	6.1 (2), 0.047	0.7 (0.5-1.0), 0.044 (r)
	rs1800566	<i>NQO1</i>	Pro187Ser	+	27	213	546		
				-	38	449	894	6.0 (2), 0.049	0.8 (0.7-1.0), 0.042 (d)
	rs3750898	<i>DCLRE1A</i>	Asp317His	+	27	119	149		
				-	105	603	1016	8.6 (2), 0.014	1.3 (1.1-1.6), 0.003 (a)
	rs1800058	<i>ATM</i>	Leu72Phe	+	0	18	277		
				-	0	52	1670	5.5 (1), 0.019	2.0 (1.1-3.4), 0.015 (d)
PN	rs3093921	<i>PARP2</i>	Asp222Gly	+	277	18	0		
				-	1668	56	0	4.9 (1), 0.027	1.9 (1.1-3.3), 0.020 (d)
	rs13181	<i>ERCC2</i>	Lys751Gln	+	107	153	35		
				-	722	752	249	6.7 (2), 0.036	1.3 (1.0-1.6), 0.082 (d)
	rs9352	<i>CHAF1A</i>	Ala923Val	+	68	156	71		
				-	526	828	370	6.1 (2), 0.048	1.4 (1.1-1.9), 0.016 (d)

Results shown using a co-dominant model^a and, odds ratios (ORs) and 95% confidence intervals using the best model that fitted the data^b [models for (d) = dominant, (r) = recessive, and, (a) = additive, alleles]. *P*-values uncorrected for multiple testing; none were significant after Bonferroni correction. For endpoints, + = patients that responded, had any toxicity or PN, - = patients that did not respond or did not have any toxicity or PN. The *DPYD* variants^c Cys29Arg and Val732Ile were in low LD ($r^2=0.0$, $D'=0.5$) and therefore may represent independent associations. The *BRCA1* variants^d Asp397Asn and Ser430Gly variants were in high LD ($r^2=0.2$, $D'=1$) so likely to be associated with the same signal. The common allele (A/B) encodes the wild type amino acid, so for Asn279Ser the A allele encodes Asn, for Arg399Gln the B allele encodes Arg, for Cys29Arg the A allele encodes Cys and for Val732Ile the B allele encodes Val. PN – Peripheral neuropathy.

Table 2 – Profiling of *DPYD* and associations with toxicity

Variant(s) & rs no.	Presence (+) or absence (-) of toxicity	Any 12-week toxicity			Neutropenia			Lethargy			Nausea & vomiting			Diarrhoea			Stomatitis			HFS			Infection		
		AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB	AA	AB	BB
Cys29Arg rs1801265	+	506	252	28	178	92	12	435	218	31	251	127	19	331	164	20	142	63	9	120	58	8	45	20	2
	-	834	465	81	1,061	576	88	804	450	69	988	541	81	908	504	80	1,097	605	91	1,119	610	92	1,041	575	86
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	8.5 (2), 0.015			1.1 (2), 0.57			1.5 (2), 0.46			0.3 (2), 0.87			3.2 (2), 0.20			3.8 (2), 0.15			1.0 (2), 0.60			2.3 (2), 0.32		
Met166Val rs2297595	+	655	122	9	239	41	2	567	114	3	330	67	1	426	83	6	175	37	2	148	37	1	54	13	0
	-	1,134	237	9	1,422	289	14	1,094	216	13	1,331	263	15	1,235	247	10	1,486	293	14	1,513	293	15	1,398	290	0
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	2.0 (2), 0.36			1.3 (2), 0.52			1.3 (2), 0.53			2.0 (2), 0.37			1.2 (2), 0.55			0.0 (2), 0.98			0.9 (2), 0.64			0.0 (1), 0.85		
Lys259Glu rs45589337	+	753	14	0	256	7	0	646	13	0	382	5	0	484	11	0	196	1	0	178	2	0	60	1	0
	-	1,315	29	0	1,654	34	0	1,264	28	0	1,528	36	0	1,426	30	0	1,714	40	0	1,732	39	0	1,634	35	0
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	0.6 (1), 0.43			0.4 (1), 0.53			0.0 (1), 0.94			1.5 (1), 0.22			0.0 (1), 0.91			4.4 (1), 0.036			1.1 (1), 0.29			0.0 (1), 0.92		
Ser534Asn rs1801158	+	725	39	0	255	9	0	639	19	0	375	12	0	473	20	0	191	7	0	173	8	0	61	1	0
	-	1,298	43	0	1,619	64	0	1,235	54	0	1,499	61	0	1,401	53	0	1,683	66	0	1,701	65	0	1,599	65	0
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	4.4 (1), 0.036			0.1 (1), 0.79			2.2 (1), 0.14			0.6 (1), 0.43			0.1 (1), 0.75			0.0 (1), 0.95			0.3 (1), 0.58			1.0 (1), 0.31		
Ile543Val rs1801159	+	491	273	22	165	109	8	435	229	19	253	133	12	339	161	14	140	71	3	124	58	4	45	20	2
	-	902	434	44	1,129	543	53	859	423	42	1,041	519	49	955	491	47	1,154	581	58	1,170	594	57	1,115	536	51
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	2.6 (2), 0.27			5.1 (2), 0.08			0.9 (2), 0.63			0.4 (2), 0.81			0.3 (2), 0.85			2.0 (2), 0.37			0.4 (2), 0.83			0.1 (2), 0.97		
Val732Ile rs1801160	+	2	79	705	1	32	249	0	62	622	1	41	356	1	50	464	0	23	191	0	23	163	1	10	56
	-	2	97	1,282	3	130	1,593	0	100	1,220	3	121	1,486	3	112	1,378	0	139	1,651	0	139	1,679	3	132	1,568
	χ^2 (d.f.), <i>P</i> OR (95% CI), <i>P</i>	7.4 (2), 0.025			7.5 (2), 0.023			1.1 (1), 0.29			2.7 (2), 0.27			1.9 (2), 0.39			2.0 (1), 0.16			2.6 (1), 0.11			3.5 (2), 0.17		
		1.6 (1.1-2.1), 0.006			1.9 (1.2-3.1), 0.005			-			-			-			-			-			-		

	+	750	16	0	255	8	0	651	10	0	377	12	0	480	16	0	196	1	0	180	1	0	58	3	0
	-	1,336	14	0	1,674	19	0	1,278	17	0	1,552	15	0	1,449	11	0	1,733	26	0	1,749	26	0	1,654	19	0
Asp949Val rs67376798	χ^2 (d.f.), <i>P</i>	4.3 (1), 0.038			5.0 (1), 0.026			0.1 (1), 0.77			8.6 (1), 0.003			14.7 (1), <0.001			1.5 (1), 0.22			1.2 (1), 0.28			4.0 (1), 0.046		
	OR (95% CI), <i>P</i>	2.2 (1.1-4.5), 0.038			3.2 (1.2-8.2), 0.019			-			3.4 (1.5-7.3), 0.002			4.6 (2.1-10.1), <0.001			-			-			5.5 (1.3-24.2), 0.024		
	+	753	12	0	258	7	0	644	14	0	380	6	0	482	11	0	190	7	0	174	5	0	56	6	0
IVS14+1 G>A rs3918290	-	1,329	11	0	1,669	12	0	1,283	5	0	1,547	13	0	1,445	8	0	1,737	12	0	1,753	14	0	1,654	9	0
	χ^2 (d.f.), <i>P</i>	2.2 (1), 0.13			3.5 (1), 0.06			11.6 (1), <0.001			1.7 (1), 0.19			9.6 (1), 0.002			7.3 (1), 0.007			4.5 (1), 0.034			15.6 (1), <0.001		
	OR (95% CI), <i>P</i>	-			-			5.3 (1.9-14.9), 0.002			-			4.4 (1.7-11.0), 0.002			4.6 (1.7-12.6), 0.003			3.8 (1.2- 11.8), 0.021			19.2 (5.0-73.8), <0.001		

Results shown using a co-dominant model and, for those that were significant (*shaded & underneath*), odds ratios and 95% confidence intervals using the best model that fitted the data (all were dominant apart from Cys29Arg which was additive). *P*-values uncorrected for multiple testing. Neither Lys259Glu nor Ser534Asn significantly increased 5FU-related toxicities (Ser534Asn was associated with skin rash). The common allele (A/B) encodes the wild type amino acid.

LEGEND TO FIGURE

CONSORT diagram of the study design and analyses. Shown are the numbers of variants analysed from genes that were likely to play a role in the metabolic or DNA damage repair pathways associated with the agents used in COIN and COIN-B, together with the numbers of patients studied, and the primary and secondary endpoints. MAF, minor allele frequency; pts, patients; PN, peripheral neuropathy; OS, overall survival; ORR, overall response rate.